

UVM ScholarWorks

Estrogen Receptor Beta Is A Negative Regulator Of Mammary Cell Proliferation

Item Type	dissertation;article
Authors	Song, Xiaozheng
Download date	2026-06-18 13:34:51
Link to Item	https://hdl.handle.net/20.500.14849/4202

ESTROGEN RECEPTOR BETA IS A NEGATIVE REGULATOR OF MAMMARY
CELL PROLIFERATION

A Dissertation Presented

by

Xiaozheng Song

to

The Faculty of the Graduate College

of

The University of Vermont

In Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
Specializing in Animal Science

October, 2014

Accepted by the Faculty of the Graduate College, The University of Vermont, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, specializing in Animal Science.

Dissertation Examination Committee:

_____ Advisor
Zhongzong Pan Ph.D.

_____ Co-Advisor
Andre-Denis Wright Ph.D.

Rona Delay, Ph.D.

David Kerr, Ph.D.

_____ Chairperson
Karen M. Lounsbury, Ph. D.

_____ Dean, Graduate College
Cynthia J. Forehand, Ph.D.

Date: May 5, 2014

ABSTRACT

The mammary gland cell growth and differentiation are under the control of both systemic hormones and locally produced growth factors. Among all these important hormones and growth factors, estrogen plays a central role in mammary gland development. The biological function of estrogen is mediated by estrogen receptor α (ER α) and estrogen receptor β (ER β). Both ER α and ER β are expressed in the mammary gland, but with distinct expression patterns. In the mammary gland, ER α has been proved to be the estrogen receptor that mediates the mitogenic function of estrogen. However the function of ER β in mammary cell proliferation is less understood and there remains some controversy. Accumulating evidence indicates that ER β , unlike ER α , is a negative regulator of mammary epithelial cell proliferation.

In this dissertation, ER α and ER β were evaluated for their expression patterns in the mammary gland. In the proestrus phase, ER α was detected in about 20% of mammary epithelial cells; in the diestrus phase, no ER α staining was detected in the mammary gland. ER β was expressed in more than 50% of mammary epithelial cells and ER β staining was detected in some stromal cells in the proestrus phase. In the diestrus phase, ER β staining cells were very limited and the staining intensity was very weak. These data suggest that the expression levels of both ER α and ER β undergo dynamic changes during the estrous cycle. In the ovariectomised (OVX) rats, both ER α and ER β were detected in more than 50% of mammary epithelial cells. Compared with the ovary-intact rats, the mammary gland of the OVX rats showed more cells with ER α expression, but the staining intensity was weaker. Taken together, the expression of ER α and ER β is regulated by estrogen in normal mammary gland, while without estrogen stimulation in the OVX rats, more mammary cells showed ER α expression, but at a lower level in these cells.

The effects of ER α and ER β on mammary cell proliferation were studied by two different approaches, activation of endogenous ER α and ER β via selective agonists, and overexpression of ER α and ER β via lentiviral infection. In the first approach, we used ER α and ER β selective agonists, propylpyrazole-triol (PPT) and diarylpropionitrile (DPN) respectively, to activate endogenous ER α and ER β in the OVX rats. We found that ER β selective agonist DPN counteracts the proliferative effect of ER α selective agonist PPT in the mammary gland. In the second approach, ER α and ER β were ectopically overexpressed in the mammary gland of mature virgin rats by lentivirus infection. We found that ER β overexpression significantly decreased mammary cell proliferation rate in both the proestrus and diestrus phases, indicating that ER β , unlike ER α , is a negative regulator for mammary cell proliferation. Collectively, these data supports that in contrast to ER α , ER β activation or overexpression is able to inhibit mammary cell proliferation.

CITATION

Material from this dissertation has been published in the following form:

Song, X. and Z.-Z. Pan.. (2012). "Estrogen receptor-beta agonist diarylpropionitrile counteracts the estrogenic activity of estrogen receptor-alpha agonist propylpyrazole-triol in the mammary gland of ovariectomized Sprague Dawley rats." *The Journal of Steroid Biochemistry and Molecular Biology* **130**(1–2): 26-35.

ACKNOWLEDGEMENTS

First, I would like to express my deepest appreciation to my advisor Dr. Zhongzong Pan for all the support, encouragement, trust, effort and patience he had given me through my Ph.D. study. Without his detailed guidance and persistent help, I would not have been able to complete my work in this thesis.

I would like to thank the rest of my thesis committee: Dr. Karen M. Lounsbury, Dr. Rona Delay, Dr. David Kerr for their scientific advice, insightful discussions and support over the past few years. A special thanks goes to Dr. Andre-Denis Wright for his support and guidance in the last year.

I would like to thank the lab members I have worked with, Huining and Yili for their great comments and suggestions. I also appreciate all the help and advice I got from other faculty members and graduate students at the Department of Animal Science. I would like to especially thank the TA coordinator Frances Kinghorn and Jeffrey White for helping me to become a good teaching assistant. I also thank Janet Schwarz, the technician of the Microscopy Imaging Center, for teaching me how to use the microtome and microscopes and for technical suggestions.

Finally, I would like to thank my family and friends for their endless love and caring. I especially thank my wife for being a true and great supporter and for standing by me with unconditional love over the past 11 years of my life.

TABLE OF CONTENTS

CITATION.....	ii
ACKNOWLEDGEMENTS.....	iii
LIST OF FIGURES	xii
CHAPTER 1: Introduction	1
Estrogen	1
Estrogen Biosynthesis.....	1
Estrogen Transport and Metabolism.....	3
Estrogen Receptors	4
The Discovery of Estrogen Receptors	4
The Structure of Estrogen Receptors	5
The Expression of Estrogen Receptors.....	7
The Coregulators of Estrogen Receptors	8
Coactivators	8
Corepressors	10

Mechanisms of Estrogen Action	11
Classical Ligand Dependent Pathway	11
Ligand Independent Pathway	11
ERE Independent Pathway	12
Nongenomic Pathway.....	13
Physiological Action of Estrogens.....	14
Estrogen Action on the Ovary	14
Estrogen Action on the Fallopian Tube	15
Estrogen Action on the Uterus.....	16
Estrogen Action on the Cervix	18
Estrogen Action on the Vagina.....	18
Effect of Estrogen on the Menstrual Cycle.....	20
Estrogen Action in the Male Reproductive System.....	21
Estrogen Action in the Brain	23
Estrogen Action in the Cardiovascular System	24
Estrogen Action on Bone.....	25
Estrogen Action on the Lung	26

Estrogen Action in the Colon	27
Estrogen Action in the Mammary Gland	29
Normal Mammary Gland Development	29
Effect of Estrogen in Normal Mammary Gland Development.....	32
Expression of Estrogen Receptors in the Mammary Gland.....	35
Function of ER α in Mammary Gland Development	36
Function of ER β in Mammary Gland Development	41
Function of Estrogen in Breast Cancer	43
ER α and Breast Cancer	45
ER β and Breast Cancer	46
Selective ER Modulators and Breast Cancer	47
Medical Application.....	49
Contraceptive Application	49
Hormone Replacement Therapy	51
Summary	52
References	54

CHAPTER 2: Estrogen receptor-beta agonist diarylpropionitrile counteracts the estrogenic activity of estrogen receptor-alpha agonist propylpyrazole-triol in the mammary gland of ovariectomized Sprague Dawley rats 88

Abstract 88

Introduction 89

Methods 93

Animals 93

Immunofluorescent (IF) and immunohistochemical (IHC) staining 97

RNA isolation and quantitative real-time PCR assay 98

Statistical analysis 99

Results 99

Expression of ER α and ER β in the mammary gland and uterine endometrium of OVX rats 99

DPN counteracts the proliferative effect of PPT in the mammary gland 100

Inhibition of PPT induced amphiregulin expression by DPN in the mammary gland 101

DPN does not inhibit the estrogenic activity of PPT in the uterus 102

Discussion 104

Conclusion	109
Acknowledgements	110
References	111
Figures.....	118
CHAPTER 3: Overexpression of estrogen receptor beta inhibits mammary epithelial cell proliferation in Sprague Dawley rats	129
Abstract	129
Introduction.....	130
Methods.....	134
Lentivirus Production	134
Animal Treatment and Sample Collection	135
Western Blot and Slot Blot.....	136
Immunofluorescent (IF) and Immunohistochemical (IHC) Staining	138
RNA Isolation and Quantitative Real-Time PCR Assay	140
Statistical Analysis.....	141
Results.....	141
Expression of ER α and ER β in the Mammary Gland during Estrous Cycle	141

Overexpression of ER α and ER β in Mammary Gland by Lentiviral Infection	142
Effect of ER α and ER β Overexpression on Rat Mammary Cell Proliferation	143
Effect of ER β on Amphiregulin Expression.....	144
Discussion	145
Acknowledgements.....	150
References.....	152
Figures.....	158
CHAPTER 4: Summary and Discussion	171
Expression of estrogen receptors in the Mammary Gland.....	171
Function of Estrogen Receptors in Mammary Gland Development	173
Function of ER α and ER β on cell proliferation in the uterus, colon, and lung	178
Potential medical use of ER β in hormone replacement therapy and breast cancer treatment	181
Reference	184
COMPREHENSIVE BIBLIOGRAPHY	192

Appendix A.....	233
The effects of ER agonists on colon cell proliferation	233
Appendix B	235
The effects of ER agonists on lung cell proliferation	235

LIST OF FIGURES

Figure 1. Immunostaining of ER α and ER β in the mammary gland and uterine endometrium of ovariectomized (OVX) rats.	118
Figure 2. DPN counteracts the proliferative effect of PPT in the mammary gland of OVX rats.	120
Figure 3. DPN inhibits PPT induced cyclin D1 expression in the mammary gland.	122
Figure 4. DPN inhibits PPT induced amphiregulin expression in the mammary gland.	124
Figure 5. The estrogenic activity of PPT in the uterus is opposed by progesterone but not by DPN.	125
Figure 6. DPN inhibits mammary cell proliferation induced by PPT and progesterone.	127
Figure 7. Amino acid sequence comparisons of human and rat ER α and human and rat ER β	159
Figure 8. Immunostaining of ER α and ER β in the rat mammary gland.	161
Figure 9. Co-immunostaining of ER α and EGFP in the 293FT cell.....	162
Figure 10. Immunostaining of EGFP and immunoblotting of EGFP and ZsGreen in the mammary gland infected by Lentivirus.	163

Figure 11. ER α stimulates and ER β inhibits mammary cell proliferation in the proestrus phase..... 166

Figure 12. ER α stimulates and ER β inhibits mammary cell proliferation in the diestrus phase. 168

Figure 13. ER α increases but ER β slightly decreases amphiregulin expression in the mammary gland. 169

CHAPTER 1: Introduction

Estrogen

Estrogen, a steroid hormone, is a key regulator of growth and differentiation in a broad range of systems and tissues, including the reproductive system, the mammary gland, lung, colon, the central nervous system, and the immune system (Findlay et al. 2001, Hall et al. 2001). There are three major estrogens in the animal body, estrone, estradiol and estriol. The predominant estrogen in the body during the reproductive years is 17β -estradiol. Estrone is the primary form of estrogen after menopause (Pascoe 1996). The estriol is only produced in large amounts during pregnancy (Tulchinsky et al. 1972).

Estrogen Biosynthesis

Estrogen biosynthesis is primarily in ovaries, but also in other tissues at different physiological stages (Nelson et al. 2001). During pregnancy the placenta produces estrogen to stimulate uterine growth to support the growing fetus (Siiteri et al. 1966). Estrogens are also produced in small amounts by other tissues such as the liver, adrenal gland, breast, fat tissue, skin, bone, and brain (Nelson et al. 2001). The biosynthesis of estrogen from acetate through cholesterol requires a series of enzymes. The enzyme aromatase (CYP19), also called estrogen synthetase, is responsible for the key step in the biosynthesis of estrogen. It is a member of the cytochrome P450 superfamily and is able to aromatize androgens into estrogens (Ghosh et al. 2009).

In the ovaries, estrogen is produced by developing follicles and the corpus lutea. According to the 'two cell, two gonadotropin model', estrogen biosynthesis happens in the theca and granulosa cells (Millier et al. 1994). Theca cells synthesize androstenedione from cholesterol. Synthesized androstenedione then moves across the basal membrane into granulosa cells, where it is converted to estrone or estradiol. The production of estrogen in the ovaries is stimulated by two gonadotropin hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Hadley et al. 2006). LH promotes the production of androstenedione from cholesterol, by stimulating the 17α -hydroxylase (CYP17) in the theca cells. FSH stimulates the expression of the cytochrome P450 aromatase in granulosa cells, which catalyze androgens into estrogens (Millier et al. 1994).

Estrogen biosynthesis in peripheral tissues is different from that in the ovary. The fat tissue, skin and other peripheral tissues do not contain a full set of steroidogenic enzymes which convert cholesterol via many steps to estrogen. In these tissues, estrogen production is dependent on the circulating precursors such as androstenedione and the local aromatase activity (Nelson et al. 2001). The adipose tissue and skin are the primary sites of peripheral estrogen biosynthesis in women and men (Hemsell et al. 1974). The aromatase activity increases significantly with age. The conversion rate of androstenedione to estrone is about 2 to 4 fold higher in age 60s than in age 20s (Cleland et al. 1985).

Estrogen Transport and Metabolism

Estrogens, synthesized in the ovaries are transported through blood circulation to target tissues. In the serum, the majority of estrogens are bound to plasma proteins such as sex hormone-binding globulin (SHBG) and albumin, and only 2-3% estrogens are in the free form (Gruber et al. 2002). SHBG proteins have high estrogen binding affinity, and a small amount of estrogens bind to albumin with low affinity (Anderson 1974, Gruber et al. 2002). Although the level of free estrogens is very low in blood circulation, it is responsible for the feedback regulation of estrogen production (Kacsóh 2000).

During menstrual cycles, the serum concentration of 17β -estradiol production varies. In follicular phase, the concentration is between 40 – 200pg/ml. The serum concentration, 250 – 500 pg/ml, reaches the peak in preovulatory phase. The level drops briefly at ovulation, and rises again during the luteal phase for a second peak (100 – 150 pg/ml). At the end of the luteal phase, the level is lowest (40 -50 pg/ml). In postmenopausal women, the serum concentration is lower than 20 pg/ml (Gruber et al. 2002).

The half-life of estrogens is about 13-17 hours. Estrogens are eliminated from the body by metabolic disposition. Most of the oxidative metabolism of estrogens takes place in the liver, several estrogen-metabolizing cytochromes P450 enzymes can also be found in other tissues at low or undetectable levels (Zhu et al. 1998). Estrogens are first metabolized to hormonally inactive (or less active) water-soluble metabolites and then

excreted into urine and/or feces (Zhu et al. 1998). There are several methods to metabolize estrogens. Estrogens can form glucuronides and sulfates, which is catalyzed by glucuronidase and sulfatase respectively (Brooks et al. 1971, Hernandez et al. 1992, Musey et al. 1997). Estrogens can also be metabolized by P450 hydroxylase to form multiple hydroxylated metabolites (Martucci et al. 1993, Zhu et al. 1998). Part of these hydroxylated metabolites can be further catalyzed to form methoxylated estrogens by catechol-O-methyltransferase (Ball et al. 1980). Metabolic hydroxylation and/or conjugation of estrogens make them more water soluble and meanwhile dramatically reduce or completely eliminate their estrogenic activity (Martucci et al. 1993). However, enzymatic O-methylation with fatty acids leads to the formation of lipophilic estrogen metabolites, which have very long half-life and unique estrogenic activities that are not associated with classical estrogen receptors (Larner et al. 1985, Vazquez-Alcantara et al. 1989).

Estrogen Receptors

The Discovery of Estrogen Receptors

The nuclear signaling of estrogen is mediated through two types of estrogen receptors, Estrogen Receptor α (ER α) and Estrogen Receptor β (ER β). ER α was discovered by Elwood Jensen in late 1950s (Jensen 1962) and cloned by Stephen Green in 1986 (Green et al. 1986). In 1996 the second estrogen receptor ER β was identified (Kuiper et al. 1996).

The Structure of Estrogen Receptors

The gene of ER α and ER β are located on different chromosomes, but share similar gene structure. ER α is located on the long arm of chromosome 6, while ER β is located on the long arm of chromosome 14 (Green et al. 1986, Kuiper et al. 1996). Both types of estrogen receptors belong to the nuclear receptor (NR) superfamily of transcription factors (Heldring et al. 2007).

Estrogen receptors have four functional domains, A/B domain, C domain, D domain, and E/F domain. The amino-terminal A/B domain involves ligand-independent activation function (AF1), which interacts with other factors to regulate the transcriptional activation of target genes (McInerney et al. 1996, Onate et al. 1998, Webb et al. 1998). The AF1 domain is highly variable between the two types of estrogen receptors (Mosselman et al. 1996). AF1 domain in ER α is very active in stimulation of target gene expression. However, the activity of AF1 domain in ER β is negligible under the same experiment condition (Cowley et al. 1999). There is evidence indicating that the different responses to the same ligand between ER α and ER β are due to different structures of AF1 in ER α and ER β (McDonnell et al. 1995, Barkhem et al. 1998). The AF1 domain contains specific phosphorylation sites that are responsible for ligand-dependent and independent activities of the estrogen receptors (Arnold et al. 1995, Joel et al. 1998). Serine 104, 106, 118 and 167 in ER α are major phosphorylation sites and are significantly phosphorylated in

response to two major pathways, the MAPK (Mitogen-Activated Protein Kinases) pathway and the AKT pathway (Lannigan 2003). The phosphorylation of Ser residues in the AF1 domain affects the recruitment of coactivators and usually enhances estrogen mediated transcription (Lavinsky et al. 1998, Endoh et al. 1999).

The most conserved C domain between ER α and ER β is the DNA binding domain (DBD). It contains two zinc finger structures that are responsible for receptor dimerization and DNA recognition and binding (Schwabe et al. 1993). ER α and ER β share about 96% similarity in the amino acid sequences of the DBD (Mosselman et al. 1996). Therefore both ER α and ER β can bind to the estrogen response element (ERE) of estrogen responsive genes with similar affinity (Kuiper et al. 1996, Ogawa et al. 1998).

The D domain of estrogen receptors is less characterized. It connects the DNA binding domain and the ligand binding domain, and may also play a role in coregulator binding and nuclear localization (Bhat et al. 1998).

The E/F domain of estrogen receptors is required for ligand binding, also known as the ligand binding domain (LBD). ER α and ER β share about 55% similarity in the amino acid sequences in the E/F domain and their tertiary architectures are very similar (Mosselman et al. 1996). Estrone and estradiol have higher affinity to ER α . However the phytoestrogen genistein has higher affinity to ER β . Most environmental estrogenic chemicals have similar binding affinity to ER α and ER β (Kuiper 1997, Kuiper et al. 1998).

The E/F domain also has other functions such as interaction with heat shock proteins,

receptor dimerization, nuclear localization, and activation function, hence the E/F domain is also named as AF2 domain (Zhang et al. 2003). The function of the AF2 domain is determined by the binding of ligands (Danielian et al. 1992, Darimont et al. 1998). The binding of different ligands induces different conformational changes of the receptor that are important for the recruitment and interaction of coactivators and corepressors (McDonnell et al. 1995, Henttu et al. 1997, Shiau et al. 1998). For example, after estrogens bind to the LBD, the helix 12 in the AF2 domain forms a 3D structure to recruit the coactivators. In contrast, when raloxifene or 4-OH-tamoxifen binds to the LBD, the helix 12 prevents the coactivator binding by covering the coactivator interaction surface (Brzozowski et al. 1997). Phosphorylation in the AF2 region also plays an important role in mediating transcriptional activation. So far the tyrosine 537 is the only phosphorylation site in the AF2 region, which is involved in the recruitment of c-SRC in a ligand-dependent manner (Migliaccio et al. 1996, Migliaccio et al. 2000).

The Expression of Estrogen Receptors

ER α and ER β are expressed in various tissues with different expression patterns. High levels of ER α expression can be detected in the classical estrogen target tissues such as the uterus, mammary gland, placenta, central nervous system, cardiovascular system, and bone (Zhang et al. 2003). The expression of ER α is either low or undetectable in non-classical estrogen target tissues, such as the prostate, testes, pineal gland, thyroid gland,

adrenal gland, gallbladder, and skin (Zhang et al. 2003). However in some of these non-classical estrogen target tissues, however, ER β is highly expressed (Gustafsson 1999). The expression of ER α and ER β is regulated by estrogens in a tissue and/or cell specific manner. In certain tissues or cells, estrogens have opposite effects on the expression of ER α and ER β (Khurana et al. 2000, Vladusic et al. 2000, Tena-Sempere et al. 2001). For instance, in bone mesenchymal stem cells and the adipose tissue, estrogens increase the expression of ER α but decrease the expression of ER β (Anwar et al. 2001, Zhou et al. 2001). In some other tissues or cells, estrogens only affect one ER but not the other. For example, estrogen treatment only downregulates ER β expression in immature rat uterus and stimulates the expression of ER β in the prostate (Khurana et al. 2000, Zhang et al. 2000).

The Coregulators of Estrogen Receptors

Recruitment of coregulators is required for ER-mediated gene transcription activity of both estrogen receptors. Coactivators turn on target gene transcription, whereas corepressors inhibit gene transcription and possibly turn off activated target genes.

Coactivators

Among the coactivators, the steroid receptor coactivator protein, the p300 cyclic AMP response element binding protein, and the thyroid hormone receptor-associated protein have the greatest capacity to increase the transcriptional activity of estrogen receptor (Nilsson et al. 2001).

Steroid receptor coactivator (SRC) family includes three members: SRC-1 (also called p160-1), SRC-2 (also called TIF-2) and SRC-3 (also called AIB1). Three conserved LXXLL motifs are required for the function of these coactivators (Xu et al. 1999). These motifs represent the primary docking site to the AF2 domain of estrogen receptors (Leers et al. 1998). The carboxyl terminal of SRC family members contains two separate transcription activation domains: AD1 and AD2 (Nilsson et al. 2001). AD1 is responsible for the recruitment of CBP/p300 coactivators and acetyltransferases. AD2 is involved in the recruitment of coactivator associated arginine methyltransferase (CARM1) (Chen et al. 1999). The primary function of SRC includes recruitment of chromatin, modification of enzymatic activities, and regulation of target gene expression (Nilsson et al. 2001). The important role of the SRC family members in estrogen receptor signaling has been supported by knockout mouse studies (Xu et al. 1998). Estrogen receptor activation is heavily affected and severe phenotypes have been observed in estrogen target tissues in TIF2 (a member of the SRC-1 family) knockout mice (Gehin et al. 2002).

CBP/p300 are considered as general coactivators involved in multiple signaling pathways. The direct interaction between p300 and estrogen receptors have been reported by many studies. In the presence of estrogen-ER complex, p300 acts synergistically with estrogen receptor to enhance estrogen targeted gene transcription, but in the absence of estrogen-ER, p300 has little effect on gene transcription (Hanstein et al. 1996, Kraus et al.

1998). The pull-down assay found that mutations in the p300 binding site of ER α and ER β and mutations in the A/B domain lead to a reduction in transactivation (Kobayashi et al. 2000).

The thyroid hormone receptor associated protein (TRAP) coactivator complex connects estrogen receptor to the basal transcription machinery (Freedman 1999). The receptor binding subunit (TRAP220) interacts with estrogen receptor through a region containing LXXLL motifs (Yuan et al. 1998). ER α is less efficient than ER β in recruiting the TRAP complex (McKenna et al. 1999).

Corepressors

The corepressors are less characterized compared with the coactivators. There are four major groups of corepressors based on their interaction mechanism with estrogen receptors. The corepressors in the first group contain a classical corepressor interaction motif (CoRNR-box) (Shang et al. 2002). The corepressors in the second group contain an LXXLL motif and are recruited in an estrogen-dependent manner (Chan et al. 1999, Zhang et al. 2000, Palijan et al. 2009). The corepressors in the third group contain an interaction domain outside the LBD (Delage-Mourroux et al. 2000, Mazumdar et al. 2000, Norris et al. 2002, Georgescu et al. 2005). The last type of corepressors may not directly interact with estrogen receptors, but rather may be recruited through transcription complexes (Laherty et al. 1998, Shi et al. 2001, Vo et al. 2001).

Mechanisms of Estrogen Action

The biological effects of estrogens are mediated by estrogen receptors through four pathways: classical ligand dependent pathway, ligand independent pathway, estrogen response element (ERE) independent pathway, and non-genomic pathway (Hall et al. 2001).

Classical Ligand Dependent Pathway

Estrogen as a steroid hormone can freely diffuse across the cell membrane. In the absence of estrogen, estrogen receptors are sequestered in a multiprotein inhibitory complex (Hall et al. 2001). Upon the binding of an estrogen, the receptor undergoes a conformational change and interacts with another ligand-receptor complex to form a homodimer or heterodimer. This dimer moves into the nucleus and binds to the promoter region of a target gene through specific ERE (Pettersson et al. 1997, Nilsson et al. 2001). The dimer does not only bind to the DNA but also recruits a variety of coregulators to the receptor (McKenna et al. 1999). These coregulatory proteins interacting with estrogen receptors are able to alter the chromatin structure and help assemble the RNA polymerase transcriptional machinery (Heldring et al. 2007).

Ligand Independent Pathway

In addition to hormone mediated activation, estrogen receptor function can also be modulated without estrogen binding. The general extracellular signals such as growth factors, cytokines and neurotransmitters can activate intracellular pathways which lead to

phosphorylation and activation of estrogen receptors (Rosenfeld et al. 2006). Epidermal growth factor (EGF) mimics the effect of estrogen action on female reproductive tract and mammary gland (Ignar-Trowbridge et al. 1992, Curtis et al. 1996, Ankrapp et al. 1998). Insulin-like growth factor (IGF) and transforming growth factor- β (TGF- β) are also able to activate estrogen receptor signaling (Aronica et al. 1993, Ma et al. 1994, Newton et al. 1994, Ignar-Trowbridge et al. 1996). Interleukin-2 (IL-2) and dopamine have been reported to modulate estrogen receptor activities, although the mechanism is still under investigation (Power et al. 1991, Smith et al. 1993). Phosphorylation modification of estrogen receptors has been shown to be an important mechanism of ligand-independent activation (Arnold et al. 1995, Joel et al. 1998, Lannigan 2003). The kinases in mitogen activated protein kinase (MAPK), AKT and other signaling pathways are thought to be responsible for these phosphorylation events (Bunone et al. 1996, Joel et al. 1998, Sun et al. 2001). The serine 118 residue in the AF1 domain is phosphorylated by MAPK, which is activated by EGF or IGF treatment (Kato 2001). In contrast to MAPK, cAMP activates estrogen receptors through AF2 domain (El-Tanani et al. 1997).

ERE Independent Pathway

In nonclassical nuclear signaling, the estrogen-ER complex regulates gene transcription without binding directly to the DNA sequence containing ERE, but through interacts with other transcription factors such as Nuclear Factor kappa B (NF κ B),

Specificity Protein 1 (SP1) and Activator Protein-1 (AP-1) (Kushner et al. 2000, Saville et al. 2000, Liu et al. 2005). ER-NF κ B complex prevents NF κ B from binding to DNA and inhibits the expression of cytokine IL-6 (Galien et al. 1997). ER α can activate transcription of the retinoic acid receptor 1 (RAR1) gene via formation of the ER-SP1 complex at the RAR1 promoter region (Sun et al. 1998). The activation of IGF-1 is mediated by ER α through interaction of ER α with c-Fos and Jun at the AP-1 binding site. This activation requires both the AF-1 and AF-2 domain of ER α (Umayahara et al. 1994). Due to structural differences in the AF-1 domain of the receptors, ER β is unable to activate transcription of AP-1 regulated genes (Kushner et al. 2000).

Nongenomic Pathway

The classical nuclear action of estrogen takes minutes or hours to increase or decrease protein synthesis. A number of rapid biological estrogenic effects which happen in seconds cannot be explained by a transcriptional mechanism. These rapid estrogen actions are results of direct estrogenic responses on the cell membrane (Simoncini et al. 2004). On the cell membrane, G protein coupled receptors (GPCRs) such as GPR30 have estrogen binding site. Upon binding with estrogens, GPCRs activate downstream intercellular signal transduction pathways such as the AKT and MAPK signal pathways in many cell types, and generate rapid tissue responses (Collins et al. 1999, Sylvia et al. 2000, Wong et al. 2002, Liu et al. 2009). In endothelial cells, engagement of estrogen receptors

results in rapid endothelial nitric oxide (NO) release through the AKT pathway (Pyo Kim et al. 1999, Chambliss et al. 2002). In pancreatic cells, estrogen regulates the K⁺-ATP channel activity and activates the rapid insulinotropic effect (Ripoll et al. 2008).

Physiological Action of Estrogens

Estrogens are involved in many physiological processes including growth and development of the reproductive system, fat deposition, and estrous/menstrual cycle. Most studies focus on estrogen action on the development of sexual and reproductive systems.

Estrogen Action on the Ovary

Estrogens are mainly produced in the ovary and act on granulosa, theca and luteal cells in the ovary. Estrogens have pleiotropic effects on granulosa cells including promoting proliferation, increasing the ER expression and exerting antiatretic effect (Sherwood et al. 1994, Quirk et al. 2004). In addition, estrogens synergize with gonadotropins in many processes such as promoting ovarian growth, increasing LH and FSH receptor expression and regulating aromatase activity (Hadley et al. 2006).

Most studies support that the ovary is a tissue of estrogen action through the classical ligand dependent pathway (Britt et al. 2002, Jameson et al. 2010). Both estrogen receptors can be detected in the ovary. ER α is mainly expressed in the theca cells and some stromal cells; ER β is predominantly expressed in the granulosa cells of growing follicles

and in some stromal cells as well (Sar et al. 1999, Pelletier et al. 2000, Zhang et al. 2003). However, the ER α knockout and ER β knockout female mice have morphologically normal ovaries before puberty, indicating that neither ER α nor ER β is necessary for the embryonic development of the ovary (Couse et al. 1999, Cheng et al. 2002, Zhang et al. 2003). Although estrogens are not essential for the growth of follicles, estrogen action on granulosa cells and oocytes is required by the orchestration of events that result in a mature oocyte capable of developing into a viable embryo (Jameson et al. 2010).

Estrogen Action on the Fallopian Tube

Two major types of cells exist in the fallopian tube (oviduct): ciliated cells and peg cells. Estrogens act on the ciliated cells to promote growth, proliferation, activity and specializations including ciliogenesis (Shao et al. 2007, Shao et al. 2009). Both ER α and ER β are expressed in the ciliated cells, but ER α serves as the dominant regulator in ciliated cell development (Mowa et al. 2000, Shao et al. 2007). ER β is involved in the regulation of calcium dependent ciliated beating, which helps the ovum to move from the ovary to the uterus in the primate (Shao et al. 2007). In other species, the smooth muscle contractions are more important for the ovum movement, and estrogen and progesterone ratio regulates the activity of the smooth muscle (Jansen 1984).

Only ER α is detected in peg cells. The peg cells are located between the ciliated cells, containing apical granules and producing the tubular fluid. The peg cells undergo a

cycle of hypertrophy and atrophy throughout the estrous cycle. The cell size and secretory activity of the peg cells increases during the follicle phase, and the release of secretory granules occurs in the luteal phase (Nilsson et al. 1969, Jansen 1984). The fluid in fallopian tube supports sperm and oocyte survival, sperm capacitation, and fertilization (Leese 1988). Through stimulation of ER α , estrogens promote the peg cells to produce and release proteins and components of intraluminal tubal fluid (Hewitt et al. 2002, Shao et al. 2007, Buhi et al. 2008).

Estrogen Action on the Uterus

The uterus is one of the major classical estrogen target tissues. It is composed of several heterogeneous cell types: stromal, luminal epithelial, glandular epithelial and smooth muscle cells (Lecce et al. 2001). All of these cells undergo dynamic changes of proliferation, differentiation and apoptosis in response to changes in levels of estrogen and progesterone, and they work together to support embryo development during pregnancy (Martin et al. 1973, Tan et al. 1999). The knockout mouse model has shown the essential role of estrogen in the development of the uterus, but estrogens only stimulate certain types of cells to proliferate in the uterus (Hewitt et al. 2002). In the immature uterus, estrogen induces both the stromal and epithelial cells to proliferate, but the proliferation rate is very low (Martin et al. 1973). In the adult uterus, estrogen only stimulates proliferation of the epithelial cells (Martin et al. 1973). After pregnancy, estrogen first activates and promotes

proliferation of the luminal and glandular epithelial cells, and then ceases the proliferation and stimulates cell differentiation (Dey et al. 1996).

Most of the known effects of estrogen in the uterus are mediated by ER α , which is the predominant type of estrogen receptor expressed in the uterus (Li 1994). Similar to the expression in the mammary gland, the expression of ER α in the uterine epithelial cells decreases after estrogen treatment (Nephew et al. 2000). However, the expression of ER α in stromal cells is upregulated by estrogen (Nephew et al. 2000). ER α is involved in the uterotrophic effects of estrogen, and this has been confirmed by the ER α knockout mice (Lubahn et al. 1993). Cooke and colleagues (1997) showed that ER α mediates the proliferative effect of estrogen on epithelial cells but the epithelial ER α is not sufficient for estrogen-induced epithelial cell proliferation. They also proposed a paracrine model in the uterus that estrogen induction of epithelial cell proliferation is mediated by ER α positive stromal cells (Cooke et al. 1997).

ER β expression is less prominent than that of ER α , but ER β can be detected in epithelial, stromal and glandular cells in the uterus (Matsuzaki et al. 1999, Lecce et al. 2001). In the ER β knockout mice, Zhang and colleagues (2000) found enlarged lumen filled with fluid, increased number of glands, and more proteins detected in uterine secretion. They also found that the level of proliferation marker Ki67 was elevated. These results indicate that ER β acts as the negative regulator of ER α and has an anti-proliferative effect in the uterus.

Estrogen Action on the Cervix

The uterine cervix contains endocervical glands, which are regulated by estrogens. During the proestrus stage, without high level of estrogen stimulation, small glandular cells in the cervix release small amount of acidic fluids which can host spermatozoa. In the estrus stage, high level of estrogens stimulate cell growth, proliferation and secretory activities. The size and number of glandular cells reach the peak right before ovulation occurs (Jones et al. 2006). At this time, mucus production increases up to 10 times more than that in proestrus stage, and the elasticity of mucus, usually referred as spinnbarkeit, is greatly increased (Gorodeski 1996). However, the mechanism of estrogen regulation in the mucus production is not clear. One possible mechanism is that estrogens increase the permeability of cervical epithelium by decreasing the paracellular resistance, leading to increasing mucus production (Gorodeski 1998). Estrogen is also able to modulate transcellular movement of water and ion transport, which might lead to increasing mucus production (Davis et al. 1978, Pragnell et al. 1990).

Estrogen Action on the Vagina

There is growing a number of evidence showing that estrogen is involved in cells proliferation, stratification, and cornification in vaginal epithelium (Hadley et al.). The vaginal epithelium is thin and dry when estrogen level is low, such as before puberty, at menopause or in the OVX mouse (Buchanan et al. 1998). Although ER α is expressed in

vaginal epithelium, estrogen does not stimulate vaginal epithelial cell proliferation through ER α *in vitro*. After the cultured epithelium is transplanted back to vaginal stroma *in vivo*, the epithelial mitogenic response to estrogen is re-established, indicating that estrogen-induced cell proliferation is mediated indirectly through stromal ER α (Cooke et al. 1986).

Estrogen-induced differentiation of vaginal epithelium is more complex and involves differentiation of multiple layers of suprabasal cells (Buchanan et al. 1998). In the presence of estrogen and progesterone, the suprabasal cells stop proliferating and undergo a series of differentiation processes. They move up through epithelium, become larger and undergo structural and morphological changes indicative of cornification, so that the apical layer becomes heavily keratinized (Buchanan et al. 1998).

Mammalian vaginal epithelium undergoes morphological changes during the estrous cycle, which are regulated by the estrogen levels in the plasma (Kim et al. 2004). During early estrus stage, high levels of estrogen promote proliferation of the vaginal epithelium and the vaginal wall gradually thickens (Jones et al. 2006). In the late estrus, along with the decrease in estrogen levels, the superficial cells become keratinized, lose their nuclei and get sloughed off (Kronenberg et al. 1985). In diestrus stage, due to the absence of estrogens and high progesterone levels, mucification of the epithelium takes place (Parekkal et al. 1972, Parakkal 1974).

Effect of Estrogen on the Menstrual Cycle

Menstrual cycle is a complex event which depends on a timely and intricate interaction between hypothalamus, pituitary gland, ovaries and uterus. There are numerous direct and indirect, positive and negative, autocrine and paracrine regulation between main hormones (Pascoe 1996).

Each menstrual cycle can be divided into three main phases: the follicular phase, luteal phase and menstrual phase (Hadley et al 2006). Estrogens are secreted by follicles in the ovaries and stimulate the uterine endometrium to proliferate during the follicular phase. In response to estrogen stimulation, uterine glands begin to enlarge, and the endometrium becomes more richly supplied with blood vessels. The level of estrogen increases throughout the follicular phase and reaches the peak at day 12 or 13. Approximately 24 to 48 hours after the peak, rapidly increased LH leads to ovulation (Jones et al. 2006). Estrogen and other hormones form a complex feedback regulatory network during the follicular phase. At the beginning of the follicular phase when the estrogen level is low, the FSH level rises due to the absence of negative feedback regulation by estrogen on Gonadotropin-releasing hormone (GnRH) which stimulates the secretion of FSH. After estrogen reaches a moderately high level, it negatively regulates GnRH, and the FSH level begins to decrease. The estrogen level reaches the peak the end of follicular phase, which leads to the LH and FSH surge and ovulation (Ferin et al. 1993). After ovulation, the corpus

luteum is formed from the wall of the follicle and begins to secrete estrogen and progesterone. Four days before menstruation, the corpus luteum ceases hormone secretion and the levels of estrogen and progesterone decrease (Jones et al. 2006). Matrix metalloproteinases are activated by estrogen and progesterone and degrade both interstitial matrix and basement membranes, resulting in the degeneration of the endometrium and menstruation (Ferin et al. 1993, Jones et al. 2006).

Estrogen Action in the Male Reproductive System

Estrogen is typically considered as a female hormone, while testosterone is considered as a male hormone. However, both hormones are present in both male and female and estrogen plays an important role in the male reproductive system (Lazari et al. 2009). In males, estrogens are produced in peripheral tissues and testis. Leydig cells and germ cells contain aromatase in adult testis (Hess 2003). Estrogens are present at a very low level in the blood of males (Ganjam et al. 1976). However, the concentration of estrogen is extraordinarily high in semen and rete testis fluids, even higher than the average serum concentration of estrogen in female (Overpeck et al. 1978).

In aromatase knockout mice, loss of estrogen synthesis results in decreased fertility with aging (Fisher et al. 1998, O'donnell et al. 2001). Low concentrations of estrogen have been demonstrated to effectively inhibit germ cell apoptosis (Pentik änen et al. 2000). Estrogen also regulates the storage and phosphorylation of Fos proteins in germ

cells (Cobellis et al. 1999, Cobellis et al. 2002). In addition, estrogens have been reported to regulate differentiation and regeneration of the Leydig cell, sertoli cell function and sertoli-germ cell adhesion (Abney et al. 1991, MacCalman et al. 1997, McKinnell et al. 2001, O'Donnell et al. 2001).

ER α is detected in Leydig and sertoli cells and mediates the regulatory function of estrogen in Leydig cells (O'Donnell et al. 2001, Lucas et al. 2008, Strauss et al. 2009). It has been shown that ER α is not essential in initiating or maintaining spermatogenesis using ER α knockout mice, and the expression of ER α in germ cells is only detected in some studies but not others (Mahato et al. 2000, O'Donnell et al. 2001). ER β is expressed more extensively than ER α in testis. ER β is not only expressed in Leydig-sertoli cells but also in spermatogonia and spermatocytes (Qing et al. 2002). Although ER β knockout male mice have normal testis structure and are fertile, a number of studies suggested that ER β has a potential role in spermatogenesis (Krege et al. 1998, Dupont et al. 2000, Oliveira et al. 2002, Cho et al. 2003). After long term treatment of ICI (a steroidal estrogen antagonist) that have directly effects on ER β , spermatogenesis turns abnormal (Cho et al. 2003).

The highest levels of ER α and ER β are expressed in the efferent ductules of the male reproductive tract, even higher than the uterine tissue, indicating that the efferent ductules are the major target of estrogen action (Hess et al. 1997, Oliveira et al. 2004). The efferent ductules transport sperm from the testis to epididymis. The non-ciliated cells in ductules reabsorb about 90% of the rete testis fluid, therefore the concentration of sperm is

increased (Chan et al. 1995, Hess et al. 2004). Higher concentration of sperm improves their survival and maturation during storage and ensures that a large number of sperm are released per ejaculation (Meisel et al. 1994). The ER α knockout mice and anti-estrogen drug treated mice produce abnormal sperm and morphologically abnormal efferent ductules, as well as significantly reduced concentrations of sperm in the epididymis, suggesting that ER α plays an important role in sperm and efferent ductules development (Hess et al. 1997, Lee et al. 2001).

Both ER α and ER β can be found in epididymis and vas deferens, but the function of estrogen in epididymis and vas deferens is not fully understood (Qing et al. 2002, Shayu et al. 2005, Frenette et al. 2009). Estrogen may be important for sperm concentration and motility in epididymis, and responsible for determining the development of vas deferens (Meistrich et al. 1975, Atanassova et al. 2005, Karlsson 2006, Motrich et al. 2007).

Estrogen Action in the Brain

Besides the reproductive system in female and male, estrogens also target other tissues or organs including the brain, blood vessel, bone, lung and colon.

Estrogens have numerous effects on the brain (McEwen et al. 1999). In the hypothalamic-pituitary-gonadal axis, estrogen acts as a feedback signal to regulate the activity of the hypothalamus and pituitary to control normal reproductive function (Marieb et al. 2007). Estrogens also regulate the concentration of neurotransmitters including

serotonin, dopamine and norepinephrine, which play a key role at brain synapses involved in mood regulation and cognition (McEwen et al. 1999). Estrogens have also been shown to protect the neuronal cells by reducing oxidative damage and promoting neuronal survival (Arimatsu et al. 1986, Mooradian 1993, Green et al. 1997). The loss of estrogen is thought to contribute to the development of the Alzheimer's disease, which represents a result of the neuronal injury (Khachaturian 1985, Birge 2000). Another significant effect of estrogens on the brain is that estrogens can regulate the blood supply in the brain. The brain represents about 2.5% of body weight, but receives 15% of blood and consumes 20% of oxygen. Therefore the blood supply regulated by estrogen is critical for maintaining normal brain function such as cognition and hot flushes, and deficient blood supply caused by lack of estrogen regulation may increase the risk of brain diseases such as stroke, Alzheimer's disease and Parkinson's disease (Henderson 2000, Leon et al. 2011).

Estrogen Action in the Cardiovascular System

Both estrogen receptors are found both in vascular smooth muscle cells and endothelial cells, indicating the important role of estrogen in the cardiovascular system (Karas et al. 1994, Venkov et al. 1996). The primary function of estrogen in the cardiovascular system is to protect blood vessels. Estrogen can affect the lipoprotein metabolism which indirectly protect the cardiovascular system (Stauffer et al. 2000). The effect of estrogen on vessel wall mediates estrogen's protective action directly. On one

hand, estrogen can promote vasodilation by stimulating prostacyclin and nitric oxide synthesis and release (Wen-Chang et al. 1980, Conrad et al. 1993). On the other hand, estrogens have an acute inhibitory effect on vascular smooth muscle contraction, mediated by an rapid effect on Ca^{2+} mobilization and fluxes (Zhang et al. 1994). Furthermore, at the cellular level, estrogens regulate smooth muscle cell migration and proliferation, as well as apoptosis of endothelial cells (Fischer-Dzoga et al. 1983, Spyridopoulos et al. 1997).

Estrogen Action on Bone

Estrogen is an important hormone which regulates bone metabolism in both women and men. Estrogen affects osteocytes, osteoclasts and osteoblasts, which are responsible for the bone formation, remodeling, resorption and maintenance (Kleerekoper 2001). Osteocytes is an important target of estrogen and plays a central role in regulating the activation of bone remodeling and bone resorption. Estrogen deficiency leads to a four time increase in osteocytes apoptosis resulting in bone remodeling. (Tomkinson et al. 1997). Estrogen can increase lifespan of osteoclasts by reducing osteoclast apoptosis, and promote osteoclast differentiation by regulating receptor activator of NF κ B ligand (RANKL) expression (Shevde et al. 2000, Srivastava et al. 2001). Moreover, estrogen affects osteoclasts indirectly by negatively regulate production of many bone-resorbing cytokines which stimulate osteoclast formation and activity (Manolagas et al. 1995, Jilka 1998, Eghbali-Fatourehchi et al. 2003).

Estrogen Action on the Lung

Estrogen and its receptors also play important roles in regulating growth and differentiation of the lung tissue. In the perinatal stage, the development of lung is more rapid in female than male. The neonatal females have higher expiratory flow rates normalized to size than male neonates (Doershuk et al. 1974). In addition, the adult females have a higher mass-specific gas exchange surface area and smaller alveoli than the males (Massaro et al. 1996). These differences between male and female lung development may be due to both the inhibitory effects of androgen and the stimulatory effects of estrogen (Ballard 1989). Estrogens have been proved to stimulate lung cell proliferation in normal lung fibroblast and this proliferated effect can be blocked by fluvestrant (estrogen receptor antagonist, suggesting that estrogen plays an important role in the development of lung epithelium and mesenchyme (Omoto et al. 2001). However, the proliferative effect of estrogen in the lung tissue may increase the risk of lung cancer, suggested by the epidemiological studies on postmenopausal women receiving hormone replacement therapy (HRT) (Adami et al. 1989). This has been supported by other studies as well, suggesting that women have higher risk for developing chronic pulmonary disease and lung cancer than men (Taioli et al. 1994, Zang et al. 1996, Prescott et al. 1997, Stabile et al. 2004).

Studies on the presence of ER α in the lung have always been controversial. There is a study showing that ER α is only expressed in normal lung tissue (Massaro et al. 2007). Other studies reported that ER α is only expressed in malignant lung epithelial cells (Beattie et al. 1985, Stabile et al. 2002, Pezzi et al. 2003, Hershberger et al. 2005, Pietras et al. 2005). However, a number of studies found that little or no ER α is expressed in either normal or cancer tissue of the lung (Matsuda et al. 1993, Ollayos et al. 1994, Brown et al. 1997, Nunno et al. 2000, Patrone et al. 2003, Morani et al. 2008).

Unlike ER α , ER β has been shown to be expressed in normal lung tissue and most non-small cell lung cancer (NSCLC) cell lines (Omoto et al. 2001, Mollerup et al. 2002, Stabile et al. 2002, Stabile et al. 2004). Massaro and colleagues (2007) have reported that the formation of a full complement of alveoli requires the presence of functional ER β (Massaro et al. 2007). Using ER β knockout mice, ER β has also been shown to regulate lung development through platelet derived growth factor A and granulocyte-macrophage colony stimulating factor (Patrone et al. 2003).

Estrogen Action in the Colon

The effects of estrogens on colon have been reported in a number of *in vivo* and *in vitro* studies, but with conflicting results. Most studies showed that estrogens inhibit cell proliferation while others demonstrated that estrogens promote the proliferation of colon cells (Hoff et al. 1980, Xiaomeng et al. 1994, Calle et al. 1995, Newcomb et al. 1995, Di

Domenico et al. 1996, Franceschi et al. 1998, Rossouw et al. 2002, Anderson et al. 2004). The studies using ovariectomized mice showed that estrogens inhibit colon epithelial cell proliferation (Hoff et al. 1979, Hoff et al. 1980, Martineti et al. 2005). The epidemiological studies suggested that the HRT decreases the incidence of colorectal cancer in postmenopausal women, which also suggesting the negative effect of estrogen on colon cell proliferation (Calle et al. 1995, Newcomb et al. 1995, Franceschi et al. 1998, Rossouw et al. 2002, Anderson et al. 2004). However, Arai and colleagues did not find that estrogens inhibit or stimulate colon cell proliferation; rather, they found that the colon cells were poorly responsive to estrogens (Arai et al. 2000).

Conflicting results can also be seen in studies on the expression of ER α . While some of these studies demonstrated the ER α is expressed in normal colon cells, colon cancer cell lines and colon tumor tissue (Waliszewski et al. 1997, Foley et al. 2000, Campbell-Thompson et al. 2001, Weyant et al. 2001), other studies suggested ER α is absent in colon cancer cells (Issa et al. 1994, Fiorelli et al. 1999, Arai et al. 2000).

ER β is the predominant ER in the colon tissue and can be detected in both the normal colon epithelium and colorectal carcinoma (Witte et al. 2001, Martineti et al. 2005). The functions of ER β in the colon are not fully understood yet, but several studies have suggested an inhibitory effect of ER β on colon cell proliferation. ER β reduces expression of cyclin E, a positive proliferation marker, and induces expression of p21, a negative proliferation marker (Sutter et al. 1996). The expression of ER β is significantly decreased

in colon tumors compared with normal colon tissue (Foley et al. 2000, Campbell-Thompson et al. 2001). Overexpression of ER β has an anti-proliferative effect through the ligand independent pathway (Martineti et al. 2005).

Estrogen Action in the Mammary Gland

Normal Mammary Gland Development

Mammary gland growth is related to age and physiological conditions (Visvader et al. 2003). The normal mammary gland development can be divided into several distinct stages, including embryonic, pre-pubertal, pubertal, pregnancy, and lactation.

The mammary gland is derived from the ectoderm and mesoderm layers and develops into rudimentary ductal bud structures. In the embryonic stage, the mammary gland is regulated by several signaling molecules and the epithelia-mesenchymal interaction (Hennighausen et al. 2001). Three pathways, Hedgehog, Notch and Wnt signaling pathway, are mainly involved in the regulation of mammary gland development (Clarke et al. 2003, Willert et al. 2003, Liu et al. 2006, van Amerongen et al. 2009). In addition, several factors have been recognized as key regulators for the initial stage of mammary gland development, including trans-acting T cell specific transcription (GATA), nuclear transcription factor kappa B (NF κ B), fibroblast growth factor (FGF), bone morphogenic protein (BMP) and parathyroid hormone related protein (PTHrP) (Wysolmerski et al. 2001, Kouros-Mehr et al. 2006, Veltmaat et al. 2006, Hens et al. 2007,

Schramek et al. 2011). PTHrP is the first signaling molecule known to be secreted by embryonic mammary epithelial cells and responded by surrounding mammary mesenchymal cells. PTHrP is necessary for the full differentiation of mammary mesenchyme. In the absence of PTHrP, mesenchymal cells condense around the epithelial bud and inhibit the mammary bud growth (Wysolmerski et al. 1998, Dunbar et al. 1999, Foley et al. 2001). Mammary gland development in this stage is independent of estrogen. High doses of estrogen even lead to a number of abnormalities of mammary development, including partial inhibition of the mammary bud, formation of a cavity at the site of the mammary bud, excessive formation of the mesenchyme around the primary mammary duct (Anderson 1978).

At birth, the mammary gland only contains simple rudimentary ducts and keeps almost the same structure until puberty (Sinha et al. 1969). In bovine, the mammary gland grows at the same rate as general body growth (isometric growth) during the first 2-3 months. After 3 months, the growth rate accelerates to about 3.5 times higher than that of general body growth (allometric growth). Then the growth rate returns to isometric growth from 9 months to puberty (Sinha et al. 1969). Unlike the embryonic stage, in prepubertal stage the mammary gland development is hormone dependent. The essential role of estrogen has been proved by ovariectomized cows (Berry et al. 2003). The proliferation of mammary epithelial cells is significantly lower in ovariectomized heifers and the mammary gland size is only one third of normal mammary gland (Berry et al. 2003).

From infancy to right before puberty, the mammary gland has no big difference between the female and male. At the beginning of female puberty, due to large increase in the levels of systemic hormones, the mammary gland undergoes dramatic changes (Pfaff et al. 1994). In human, this process usually takes 3 to 4 years. Estrogen acts synergistically with other hormones such as somatotropin and progesterone to regulate mammary gland growth during this stage (Ruan et al. 1995, Brisken et al. 1998, Silberstein 2001, Mulac-Jericevic et al. 2003). The cyclic changes in estrogen levels during estrous cycle result in duct elongation, branching, thickening and eventually differentiation into lobules and alveoli (Russo et al. 1999).

Full development and differentiation of the mammary gland only takes place during pregnancy and lactation. Mammary gland growth reaches the fastest stage during the later stage of pregnancy. Both estrogen and progesterone levels are elevated and these two hormones synergize to stimulate the development of ducts and lobule alveoli during pregnancy (Glasier et al. 1990). As pregnancy progresses, the entire fat pad is gradually filled with ducts and alveoli (Tucker 1987). Mammary gland growth continues until the peak of lactation. However, the milk secretory cells only develop during pregnancy, and the number of secretory cells does not increase any more during lactation. Therefore the udder contains more secretory cells at the beginning than at the end of lactation (Anderson et al. 1990).

Effect of Estrogen in Normal Mammary Gland Development

The development of mammary glands is regulated by systemic hormones and locally produced growth factors. The most important systemic hormones involved in the development of mammary glands include estrogen, progesterone, growth hormone, prolactin, oxytocin glucocorticoids, thyroid hormones and insulin (Hadley et al.). In addition to the systemic hormones, the mammary gland itself can also produce growth factors which regulate mammary gland development including insulin-like growth factor I (IGF-I), epidermal growth factors (EGF), hepatocyte growth factor (HGF), fibroblast growth factor (FGF), keratinocyte growth factor (KGF), transforming growth factor β (TGF- β) and parathyroid hormone related protein (PTHrP).

Estrogens play a fundamental role in mammary gland development. Locally administered anti-estrogen is able to inhibit mammary cell proliferation and ductal growth in the mammary gland, suggesting that estrogen is a positive regulator (Silberstein et al. 1994). The essential role of estrogen has been further proved by the classical hormone ablation experiment using OVX animals whose ovaries have been removed (Berry et al. 2003). The OVX animals exhibit impaired and limited ductal growth in the mammary gland. The total mammary gland size decreases to 1/3 of the size of control animals. The proliferation rate of mammary epithelial cells in OVX animals is significantly lower than that in wild type animals (Berry et al. 2003). Administration of estrogen along with other

ovarian hormones is able to stimulate and even restore mammary gland growth in OVX animals, indicating that estrogen is essential in mammary gland development (Cheng et al. 2005).

Estrogen can stimulate mammary gland development directly or through other growth factors. Several factors have been demonstrated to mediate indirect effect of estrogen. Estrogen upregulates prolactin gene transcription and promote the secretion of prolactin (Scully et al. 1997). In addition, estrogen inhibits release of dopamine, a negative regulator of prolactin synthesis and secretion, further elevating the level of prolactin (Ojeda et al. 1974, Gudelsky et al. 1981).

Estrogens also induces the expression of progesterone receptors and increase the proliferation of mammary epithelial cells synergistically with progesterone during pregnancy when both hormones are present at high levels, leading to the significant increase in mammary gland growth rate (Clarke et al. 1997).

In the mammary gland, insulin-like-growth factor I (IGF-I) and estrogen can act synergistically to stimulate cell proliferation (Ruan et al. 1995). The expression of IGF-I is regulated by the growth hormone and estrogen. IGF-I is able to activate MAPK and PI3K/AKT pathway which phosphorylate and activate ER α (Kato et al. 1995). Upon binding of estrogen, ER α rapidly induces the IGF-I receptor (IGF-IR) to activate the signaling cascade, forming a rapid estrogen feedback stimulation loop (Kahlert et al. 2000).

Estrogen stimulates production of EGF, transforming growth factor- α (TGF- α) and amphiregulin in the mammary gland (Mallepell et al. 2006, Ciarloni et al. 2007). These growth factors belong to the EGF protein family which plays an important role in regulating cell proliferation and differentiation. Amphiregulin, a required for ductal elongation and side branching in the mammary gland, is an important paracrine mediator of estrogen function (Ciarloni et al. 2007).

EGF signaling pathway, which has been shown to be downstream of ER α signaling, is required by the stimulatory effect of ER on mammary gland development. Estrogen induces rapid phosphorylation of EGF receptors (EGFR) and many EGFR ligands have been described as estrogen target genes (Filardo et al. 2000, Filardo et al. 2002, Britton et al. 2006). Inhibition or selective knockdown of EGFR lead to diminished estrogen-induced MAPK activation and block the stimulatory effect of estrogen on cell growth (Santen et al. 2009).

The crosstalk between ER activity and TGF- β signaling have been reported in a number of studies. TGF- β is a pluripotent cytokine that can arrest cell cycle by inhibiting cyclin-dependent kinase activities and by reducing the expression level of c-myc and inducing cell apoptosis (Donovan et al. 2000, Ewan et al. 2002, Heldin et al. 2009). In contrast, ER α has a strong mitogenic activity which enhances the transcriptional activation of cyclin D and c-myc and prevents apoptosis (Dubik et al. 1987, Musgrove et al. 1994,

Lewis-Wambi et al. 2009). Studies suggested that dysregulation of TGF- β may promote the proliferation of ER α positive cells (Ewan et al. 2005). Estrogen treatment inhibits transcriptional activity of TGF- β through MAD-related protein 3 (Smad3) (Matsuda et al. 2001, Kleuser et al. 2008). Treatment of anti-estrogen promotes the secretion of TGF- β as well as the expression of TGF- β receptor leading to inhibited cell proliferation (Chen et al. 1996).

Expression of Estrogen Receptors in the Mammary Gland

In the mammary gland, both ER α and ER β are expressed, but with distinct expression patterns. ER α is expressed in luminal epithelial cells but not in stromal or myoepithelial cells (Saji et al. 2000). ER β is expressed not only in luminal epithelial cells but also in some stromal cells and myoepithelial cells (Palmieri et al. 2002). In the mammary gland, the expression level of ER α is generally much higher than ER β . However the dominant type of estrogen receptor in stromal cells is ER β (Jensen et al. 2001, Pettersson et al. 2001, Shyamala et al. 2002).

The expression pattern and level of estrogen receptors undergo dynamic changes in different stages of mammary gland development and during the estrous cycle. Approximately 70% of epithelial cells express ER β throughout all developmental phases (Schams et al. 2003). In contrast, the percentage of ER α positive cells varies in different development stages. In human mammary gland, ER α can be found in 20% of epithelial

cells in the follicular phase of the estrous cycle (Clarke et al. 1997). In rodent, ER α protein level changes while ER α mRNA level remains steady during the estrous cycle (Cheng et al. 2005). The highest ER α expression is in the proestrus phase of the estrous cycle and the expression level declines as estrogen levels increase (Cheng et al. 2005). The percentage of ER α positive cells increases during puberty stage and gradually decreases during pregnancy. It rises again during lactation, and drops to a basal level in post-lactation stage (Lemmen et al. 1999, Saji et al. 2000). The percentage of ER α positive cells increases after OVX, presumably due to decreased level of circulatory estrogens (Berry et al. 2003, Song et al. 2012). Approximately 60% of epithelial cells coexpress ER α and ER β during lactation, but cells coexpressing ER α and ER β are rarely detected during pregnancy (Saji et al. 2000).

Function of ER α in Mammary Gland Development

ER α is the first discovered estrogen receptor and for many years it was the only known ER. Its expression level is much higher than that of ER β , known as the primary estrogen receptor expressed in the mammary gland. The function of ER α in mammary gland development has been elucidated using the ER α knockout mice (Bocchinfuso et al. 1997). The mammary gland is the same before puberty in the ER α knockout mouse and wild type mouse. At the beginning of female puberty, terminal end buds start to form and ducts start to elongate in the mammary gland of wild type mouse. However, there is no

terminal end buds detected in the ER α knockout mouse (Mallepell et al. 2006). Only prepubertal rudimentary ductal structures can be found in the mammary gland of the ER α knock-out mouse, indicating that ER α is essential for postnatal mammary gland growth but not required for embryonic mammary growth (Bocchinfuso et al. 1997). In order to investigate whether the developmental defect is intrinsic to mammary epithelium or the mammary stroma, the tissue recombination experiments were carried out. ER α -/- mammary epithelial cells from ER α -/- mice failed to develop normal structure when transplanted into the wild type clear fat pad, while the wild type mammary epithelium is able to develop normally in the clear pad of ER α knockout recipient (Mallepell et al. 2006). These results suggest that the primary target of estrogen is the epithelial cells and the direct response of the mammary stroma to estrogen is not required for mammary gland development.

ER α regulate transcription of many genes involved in promoting cell proliferation and inhibiting cell apoptosis. Cyclin D, a major regulator controlling entry into the proliferative stage of the cell cycle, is one of the target genes regulated by ER α (Liu et al. 2002). Impaired mammary gland was found in cyclin D1 knockout mice, indicating that the mammary epithelial cells is unable to respond appropriately to morphogenetic hormone signals (Sicinski et al. 1997). High levels of cyclin D can be found in breast cancer tumors and are positively associated with ER α (Simpson et al. 1997). ER α is able to increase transcription of cyclin D hence leading to up-regulation of cyclin D protein (Prall et al.

1997, Steeg et al. 1998). Estrogens induce cyclin D expression through a ERE-independent pathway since the cyclin D promoter region does not contain a ERE sequence (Herber et al. 1994). Instead, the estrogen-ER α complex binds to sequences containing the AP1 site or cAMP response element (CRE) to promote cyclin D transcription (Liu et al. 2002).

c-Myc is another estrogen-target gene involved in regulation of mammary cell growth. The regulatory region of c-myc gene contains ERE, where the estrogen-ER α complex binds to regulate c-myc transcription (Dubik et al. 1992). Similar to ER α , overexpression of c-myc can be found in 70% of breast cancer tumor samples (Deming et al. 2000). In 15.5% of breast cancer tumor samples, c-myc expression level is three times higher than that in normal breast tissue (Bièche et al. 1999). Estrogen significantly increases c-myc protein levels which result in enhanced cell cycle progression (Mawson et al. 2005).

The proto-oncogene, c-fos, is widely expressed in the mammary gland. c-Fos is both a direct and indirect transcriptional regulator of cyclin genes and functions as a critical link between estrogen stimulation and cell cycle progression (Brown et al. 1998). Estrogens induce c-fos expression and c-fos transactivation by estrogens modulates expression of multiple genes through interactions with AP1 site in corresponding promoters to promote cell proliferation (Cohen et al. 1989).

Breast cancer type 1 susceptibility protein (BRCA1) is responsible for DNA repairing and serves as a tumor suppressor (Deng et al. 2000). BRCA1 mutations can be

found in approximately 5% of all breast cancer cases and 45% of familial breast cancer cases (Yoshida et al. 2004). BRCA1 inhibits ER α activity in two ways. One way is that BRCA1 binds to the AF2 domain of ER α to inhibit ER α 's transcriptional activity (Fan et al. 1999). The other way is that BRCA1 downregulates ER α coactivator p300 gene expression which also leads to reduced ER α activity (Fan et al. 2002).

Besides cell proliferation, apoptosis is another important aspect during mammary gland development. In mammary gland due to the drop in estrogen levels, apoptosis reaches the peak at the end of luteal phase (Ferguson et al. 1981). Estrogen is able to regulate a large set of genes that are related to apoptosis. Apoptosis regulator Bcl-2 family contains proapoptosis and antiapoptosis members. The Bax, BAD, Bak and Bok are proteins that promote cell death, whereas Bcl-2, Bcl-xL and Bcl-w inhibit cell death (Yang et al. 1995). Bcl-2 is induced by estrogen through the classical ER α signaling pathway (Martin et al. 2013). Consistent with ER α expression, expression of Bcl-2 reaches the peak at the end of follicular phase and gradually decreases during the luteal phase (Sabourin et al. 1994). The lower ratio of Bcl-2/Bax is able to increase caspase-3 expression and promotes apoptosis (Martin et al. 2013). Estrogen treatment significantly increases Bcl-2 level but only slightly increases Bax level, consistent with the negative effect of estrogen on apoptosis (Helguero et al. 2005).

Although ER α is essential for mammary gland development, the detailed mechanism is still not fully understood. In normal mammary gland, ER α cannot be detected

in proliferating mammary epithelial cells indicating that estrogen stimulates growth indirectly (Clarke et al. 1997, Russo et al. 1999, Cheng et al. 2005). The paracrine model is the most accepted model explaining such observation. In this model, ER α positive cells do not proliferate but produce and secrete growth factors when they are stimulated by estrogen. These growth factors stimulate the neighboring ER α negative cells to proliferate (Clarke et al. 1997, Mallepell et al. 2006).

The EGF family member, amphiregulin, is the most important growth factor secreted by ER α positive cells and mediates estrogen action (Ciarloni et al. 2007). Local administration of EGF or amphiregulin in OVX mice induces mammary cell proliferation and ductal growth, indicating that EGF or amphiregulin acts downstream of estrogen signaling (Snedeker et al. 1991, Kenney et al. 1996). The morphology of mammary gland in amphiregulin knockout mice is the same as that in OVX mice suggesting that amphiregulin is required in epithelial cell proliferation, terminal end buds formation and ductal elongation (Ciarloni et al. 2007). Consistent with the paracrine model, estrogen induces amphiregulin protein secretion and inhibits the expression of EGFR in ER α positive cells. In ER α negative cells, secreted amphiregulin activates EGFR signaling and stimulates cell proliferation (Kariagina et al. 2010).

However, in some breast cancer tumor cells, ER α has been observed to co-localize with cell proliferation markers. The percentage of this type of cells varies from 0% to 5% of total cells among different patients (Shoker et al. 1999). Therefore some researchers

proposed that ER α stimulates cell proliferation through both autocrine and paracrine models. It has been shown that the half-life of ER α is very short, only one or two hours. Once ER α is activated by estrogen, it is quickly degraded subsequently (Cheng et al. 2004). This characteristic of ER α may account for the special separation of ER α staining and cell proliferation marker in normal breast tissue at detection. In the breast cancer cells however, ER α is not degraded as quickly and can be detected in all phases of cell cycle (Tan et al. 2009). Therefore these researchers believe that a switch from a paracrine to an autocrine mechanism might be an important part of the tumorigenic process (Cheng et al. 2004, Tan et al. 2009).

Function of ER β in Mammary Gland Development

Compared with ER α , the other estrogen receptor, ER β , is much less studied and its function remains elusive. In the ER β knockout mice, the histology of ovary and uterus is only mildly affected and the overall structure of mammary gland during puberty is relatively normal (Krege et al. 1998). The side branching is delayed in the ER β knockout mice, which may be due to the loss of ER β or decreased progesterone secretion (Antal et al. 2008). During pregnancy, mammary glands of the ER β knockout mice have normal structures and can lactate normally as wild-type mammary glands do (Förster et al. 2002). However, mammary glands in the ER β knockout mice have less developed lobuloalveolar structures, suggesting that ER β is involved in alveologensis and differentiation (Förster et

al. 2002, Walker et al. 2004). Observations from ER β knockout mice indicate that ER β is not essential for mammary gland development and ER α is the major type of estrogen receptor which is able to mediate the action of estrogen on its own.

However, ER β expression has been detected in mammary epithelial cells and stromal cells and many studies have reported that ER β negatively regulates proliferation of both normal and breast tumor cells (Lazennec et al. 2001, Liu et al. 2002, Visvader et al. 2003, Paruthiyil et al. 2004, Helguero et al. 2005, Song et al. 2012). The expression of ER β in MCF7 and T47D xenografts has been shown to inhibit tumor growth (Paruthiyil et al. 2004, Hartman et al. 2009). Moreover, ER β expression is found decreased or lost in many primary breast tumors and the ER β level is significantly associated with the survival rate (Zhao et al. 2003, Girault et al. 2004, Gruvberger-Saal et al. 2007, Honma et al. 2008).

It has been shown that ER β may inhibit cell proliferation at multiple levels, although detailed molecular mechanism of the ER β inhibitory effects is still under investigation. ER β inhibits the expression of positive cell cycle progression molecules including c-myc, cyclin A, cyclin D and cyclin E, and promotes the expression of negative ones including p21 and p27, leading to a G1/2 cell cycle arrest. (Paruthiyil et al. 2004, Strom et al. 2004). ER β inhibits TGF α expression, suggesting that ER β may affect the production and/or secretion of growth factor to regulate cell proliferation (Lazennec et al. 2001, Hartman et al. 2009). ER β may also regulate cell proliferation through interacting with ER α . ER α homodimers increase target gene transcription while the heterodimers of

ER α /ER β do not have such effect, indicating a negative role of ER β on ER α actions. ER α induced cyclin D and amphiregulin expression is suppressed by ER β (Song et al. 2012). ERK1/2 activated by ER α is also repressed ER β (Cotrim et al. 2012).

Although the inhibitory effect of ER β has been demonstrated by many studies, in one *in vivo* study using OVX mice, activation of ER β was shown to stimulate cell proliferation (Cheng et al. 2004). Another *in vitro* study suggests that ER β has stimulatory effect on cell growth in an ER negative cell line (Hou et al. 2004). Therefore the role of ER β in cell proliferation are still controversial from studies using different experimental models.

ER β has been shown to play a role in mammary cell apoptosis as well. Many studies suggested that ER β is able to increase mammary cell death in normal breast cells and breast cancer cells (Helguero et al. 2005, Hodges-Gallagher et al. 2008). Activation of ER α significantly decreases expression of Bcl-x, a positive regulator of apoptosis, in contrast to the fact that activation of ER β keeps Bcl-x level high (Helguero et al. 2005).

Function of Estrogen in Breast Cancer

Estrogens are essential for various normal physiological processes in the body. However, evidence has shown that estrogens are positively related to breast cancer and extended exposure to estrogen in females is associated with increased risk of breast cancer (Butler et al. 1979). The risk of breast cancer is significantly higher in women with higher estrogen levels in blood plasma (Butler et al. 1979). Bilateral oophorectomy before age 35

reduces the risk of breast cancer by 40-75% (Feinleib 1968, McPerson et al. 2000). Women with early menarche (before age 12) and/or late menopause (after age 55) that have more menstrual cycles also have a higher risk of breast cancer (Ritte et al. 2012). Late age of first pregnancy and nulliparity increase the risk of breast cancer while the first pregnancy before age 20 reduces breast cancer risk by 50% (Clemons et al. 2001, Bernstein 2002, Yue et al. 2012). Early pregnancy and high estrogen levels during pregnancy may reduce the risk of breast cancer by altering the sensitivity of mammary epithelial cells to stimulatory effect of estrogen on cell proliferation after pregnancy. In addition, fully development of breasts reduces the number of stem or progenitor cells leading to reduced risk of tumorigenesis (Britt et al. 2007, Siwko et al. 2008). HRT has been reported to increase the risk of breast cancer, especially for women who have received estrogen and progestin regimen (Fernandez et al. 2003, Heiss et al. 2008, Hernán et al. 2008). It is still debating whether the oral contraceptives would increase the risk of breast cancer (Kumle et al. 2002, Hunter et al. 2010, Marchbanks et al. 2012, Nelson et al. 2012).

Block of estrogen synthesis with inhibitors of aromatase, the key enzyme in estrogen biosynthesis, has been used to prevent and treat breast cancer in postmenopausal women with ER positive tumor cells. Although the estrogen concentration is very low in these patients, aromatase in their breast tissue is still able to synthesize estrogen which stimulates breast cell proliferation. The development of aromatase inhibitor began in the 1970s (Thompson Jr et al. 1979, Marsh et al. 1985, Brueggemeier et al. 1990). Nowadays

the third generation of aromatase inhibitor (exemestane) significantly reduces the risk of invasive breast cancers by 65% in postmenopausal women (Goss et al. 2011). The aromatase inhibitors can be used alone or combined with tamoxifen for early breast cancer therapy, and anastrozole and letrozole have been proved to be more effective than tamoxifen as the first line therapy in postmenopausal women with advanced breast cancer (Bonnetterre et al. 2000, Nabholz et al. 2000, Bonnetterre et al. 2001).

ER α and Breast Cancer

In normal breast tissue, only approximately 10% of cells are ER α positive (Khan et al. 1998). This percentage increases in proliferative benign disease that is often related to ductal carcinoma in situ (DCIS) (Shoker et al. 1999). Overexpression of ER α leads to overproliferation and tumorigenesis in mammary gland (Frech et al. 2005). About 70% breast cancer tumors are considered as ER α positive tumors containing 40% to 70% ER α positive cells (Regan et al. 2006). Nowadays, as a diagnostic marker, ER α expression status is regularly measured in breast cancer patients. Deregulation of ER α may change the cell cycle progression and directly induce ER α positive cancer cells to proliferate. The crosstalk between ER α and other signaling pathways plays an important role in invasion and metastasis of breast cancer cells. ER α positive tumors are often metastatic to bone and lung (Alanko et al. 1985, Maki et al. 2000, Kennecke et al. 2010). ER α regulates the

deacetylation of tubulins, which promotes breast cancer cell migration, is the first key step in metastatic process (Alberts et al. 2002, Azuma et al. 2009).

ER β and Breast Cancer

In contrast to the positive correlation of ER α expression level and breast cancer, ER β expression level decreases in breast tumors (Zhao et al. 2003, Girault et al. 2004, Gruvberger-Saal et al. 2007, Honma et al. 2008). Hypermethylation of DNA in the promoter region of ER β may partial account for the down-regulation of ER β in breast cancer cells (Zhao et al. 2003, Rody et al. 2005). High levels of ER β expression in breast tumor were found to associate with a better response to tamoxifen and a longer survival time (Honma et al. 2008). This observation may be due to the inhibition of cell proliferation by ER β . It has been shown that ER β expression level is inversely correlated with cell proliferation marker Ki67 (Roger et al. 2001). In addition, clinical studies also found that expression of ER β in ER α positive breast tumors reduces cell motility and invasion (Järvinen et al. 2000).

The function of ER β as a tumor suppressor in mammary epithelial cells has been studied in a number of studies. Paruthiyil and his colleagues found that ER β inhibits breast cancer cell proliferation by inhibiting cyclin A, cyclin D and c-myc gene transcription and increasing the expression of cell cycle inhibitors, p21 and p27. Overexpression of ER β prevents tumor formation in the mouse xenograft model (Paruthiyil et al. 2004). Moreover,

ER β is able to counteract the effects of ER α in mammary gland. The expression level of p21 is much higher in ER α and ER β co-expressing cells than that in ER α only cells (Chang et al. 2006). Relative expression levels of ER α and ER β in mammary gland modulate the cell proliferation rate and apoptosis (Ström et al. 2004, Chang et al. 2006). Furthermore, ER β inhibits breast cancer cell migration and invasion in a ligand independent manner (Lazennec et al. 2001). Platet and his colleagues found that cell invasion was decreased 2 fold by the expression of ER β (Platet et al. 2000). ER β also inhibits angiogenesis by downregulating the expression and secretion of vascular endothelial growth factor (VEGF) and platelet-derived growth factor β (PDGF β) (Hartman et al. 2006).

Selective ER Modulators and Breast Cancer

Selective estrogen receptor modulators (SERMs) are a class of compounds that bind to ER α and ER β and exert estrogenic or anti-estrogenic activities in different tissues. Some SERMs are used to treat breast cancer, including tamoxifen, raloxifene, toremifene and lasofoxifene.

Tamoxifen has been widely used in the clinic to treat early and advanced ER α positive breast cancer for over 35 years. Tamoxifen is also used to prevent breast cancer in women who are at high risk of developing the disease. In addition it is the most common drug to treat male breast cancer (Gethins 2012). Similar to estrogen, tamoxifen binds to ER α and induces dimerization. However, unlike estrogen, tamoxifen inhibits activation of

the AF2 domain on ER α and blocks transcription of ER α target genes. The effect of tamoxifen in different tissues depends on the existence of different coactivators and corepressors in different cells (Jordan et al. 2007). For instance, tamoxifen performs agonistic activity in endometrial cells where the coactivator SRC level is relative high. However, tamoxifen exhibits antagonistic activity in mammary cells where the SRC level is lower (Shang et al. 2002). Thus, tamoxifen used to treat breast cancer increases the risk of endometrial cancer (Bergman et al. 2000, Swerdlow et al. 2005).

Like tamoxifen, another SERM, raloxifene, has a mixed pharmacological effects on breast cancer. It acts as estrogen agonist or antagonist in different tissues (Brzozowski et al. 1997, Wijayaratne et al. 1999). In the bone, raloxifene acts as estrogen agonist to prevent and treat osteoporosis, whereas in the mammary gland and uterus raloxifene acts as estrogen antagonist to inhibit cell proliferation (Haskell 2003). Therefore, compared with tamoxifen, raloxifene treatment has a 50% reduction in the risk of endometrial cancer (DeMichele et al. 2008).

The SERM, toremifene, is used to treat metastatic breast cancer in postmenopausal women with ER α positive tumors or tumors with unknown ER status. Toremifene has structure and properties similar to tamoxifen (Hayes et al. 1995). Hence the tamoxifen treatment and toremifene treatment have similar efficacy and adverse effects (Marttunen et al. 1998).

Lasofoxifene is mainly used to prevent and treat osteoporosis and vaginal atrophy. In the postmenopausal women, 0.5mg of lasofoxifene per day significantly reduces the risk of total breast cancer by 79% and ER α positive breast cancer by 83%. Lasofoxifene treatment increases endometrial thickness, but does not increase the risk of endometrial cancer (LaCroix et al. 2010).

Medical Application

Contraceptive Application

Estrogens, especially 17 β -estradiol, are essential for female fertility. The estrogen-based contraceptive methods include oral pills, transdermal patches, vaginal rings and injectable contraceptives. Currently combined oral contraceptive pills (COCP) are the most popular method of birth control with more than 100 million women worldwide and about 12 million women in the United States using COCP as her birth control method (Mosher et al. 2004). In last 50 years after the first COCP approved in 1960, COCP went through a remarkable evolution which reflected our increased knowledge about hormonal safety issues. The doses of the hormones in the pills have dramatically decreased and new hormone compounds have been introduced (Hatcher 2007, Speroff et al. 2010).

The estrogen and progestin combination in COCP reduces circulating levels of gonadotropins (FSH and LH) and prevents ovulation through an effect on both pituitary

and hypothalamus, hence providing safe, effective and reversible contraceptive (Vandenberg et al. 1974, Meyers et al. 2001, Beral 2003).

Estrogen in the pill also serves two other purposes. First, it stabilizes the endometrium so that irregular shedding and incidence of breakthrough bleeding can be minimized. Second, estrogen is required to potentiate the action of progesterone components by stimulating the expression of progesterone receptors (Kraus et al. 1993, Ing et al. 1997).

Besides being a contraceptive, COCP also solves many menstrual related problems. The menstrual cycle related health benefits include decreased dysmenorrhea (painful periods), decreased menstrual blood loss, reduction in premenstrual syndrome (PMS) symptoms, reduction of premenstrual dysphoric disorder (PMDD), reduced risk of postovulatory ovarian cysts and improvement in menstrual migraines (Hatcher 2007). In addition, COCP has been used to treat other medical conditions, such as polycystic ovary syndrome (PCOS), endometriosis, amenorrhea and adenomyosis (Hatcher 2007). Sometimes COCP is used to treat acne or hirsutism. Three different oral contraceptives have been FDA approved to treat moderate acne if the patient is at least 14 or 15 years old, has already begun menstruating, and needs contraception (Food 2011).

However, hormone-based contraceptives have side effects. There is evidence supporting that the COCP increases the risk of venous thromboembolism (Jick et al. 2000).

Contradictory research results exist in studies on the effect of hormone-based contraceptives on the risk of breast cancer, liver cancer and cervical cancer (Hatcher 2007).

Hormone Replacement Therapy

Menopause is a natural event which normally occurs in women in their late 40s or early 50s. Due to the decrease in estrogen production, a number of physiological changes take place such as hot flushes, night sweating, mood and sleep disturbances, fatigue, difficulties with memory and urogenital dysfunction. About 80% women have one or multiple symptoms that may last for several years (Hickey et al. 2012). HRT is an effective therapy to ameliorate these symptoms suggested by the Women's Health Initiative study, Heart and Estrogen/progestin Replacement study (Hulley et al. 1998, Keating et al. 1999, Genazzani 2001, Rossouw et al. 2002). There are two main types of HRT: estrogen hormone therapy and progesterone/progestin-estrogen hormone therapy. Estrogen can be given oral, transdermal or intravaginal daily. Progesterone can be given oral, transdermal or delivered via an intra-uterine device daily or sequentially.

Besides the effective control of menopausal symptoms, HRT has several side effects. About 14% women who receive transdermal HRT have local skin irritation (Ibarra de Palacios et al. 2002). Other adverse effects of HRT include irregular bleeding, vaginal discharge, flushing, headache, nausea, vomiting and depression. WHI data have suggested that HRT may significantly increase the incidence of stroke (Rossouw et al. 2002). Current

users of estrogen regimen have an increased risk of breast cancer and uterus cancer, and this risk increases with duration of use (Hofseth et al. 1999, Rossouw et al. 2002). The adverse effect of estrogen on uterine endometrium can be opposed by progestins. However, estrogen-plus-progestin HRT regimen impose substantially higher risk of breast cancer than estrogen alone regimen (Grady et al. 1995, Persson et al. 1999, Ross et al. 2000).

Due to the adverse effects of HRT, new regimens need to be developed. The key method to improve HRT is the development of estrogen mimics that can act as estrogen in some tissues, but not in others. SERMs are such compounds that mimic estrogen in a tissue-specific manner. Tamoxifen which is widely used for the treatment of breast cancer can act as estrogen in uterus, bone and cardiovascular system, but not in breast (Love et al. 1992, Grey et al. 1995, Macgregor et al. 1998). Another SERM, raloxifene has the beneficial effect of estrogen in bone and cardiovascular system while it does not have estrogenic effects in uterus and breast (Delmas et al. 1997, Barrett-Connor et al. 1998, Goldstein 1998).

Summary

Estrogen is an essential steroid hormone regulating growth and differentiation in various systems and tissues. The biological functions of estrogen are mediated by two types of estrogen receptors, ER α and ER β , which may have different functions in different tissues. This thesis examines the role of ER α and ER β in mammary gland and other tissues and

explores the potential use of ER β in breast cancer treatment and hormone replacement therapy.

References

- Abney, T. O. and R. B. Myers (1991). "17 β - Estradiol Inhibition of Leydig Cell Regeneration in the Ethane Dimethylsulfonate - Treated Mature Rat." Journal of Andrology **12**(5): 295-304.
- Adami, H. O., I. Persson, R. Hoover, C. Schairer and L. Bergkvist (1989). "Risk of cancer in women receiving hormone replacement therapy." International Journal of Cancer **44**(5): 833-839.
- Alanko, A., E. Heinonen, T. Scheinin, E. M. Tolppanen and R. Vihko (1985). "Significance of estrogen and progesterone receptors, disease - free interval, and site of first metastasis on survival of breast cancer patients." Cancer **56**(7): 1696-1700.
- Alberts, B., A. Johnson, J. Lewis, M. Raff and K. Roberts (2002). Molecular biology of the cell 4th edition, National Center for Biotechnology Informations Bookshelf.
- Anderson, D. C. (1974). "Sex - hormone - binding globulin." Clinical Endocrinology **3**(1): 69-96.
- Anderson, G. L., M. C. Limacher, A. R. Assaf, T. Bassford, S. A. Beresford, H. R. Black, D. E. Bonds, R. L. Brunner, R. G. Brzyski and B. Caan (2004). "Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial." Journal of the American Medical Association **291**(14).
- Anderson, R. (1978). "Embryonic and fetal development of the mammary apparatus." Lactation **4**: 3-40.
- Anderson, R. and I. Wahab (1990). "Changes in parenchyma and stroma of goat udders during pregnancy, lactation and involution." Small Ruminant Research **3**(6): 605-615.
- Ankrapp, D. P., J. M. Bennett and S. Z. Haslam (1998). "Role of epidermal growth factor in the acquisition of ovarian steroid hormone responsiveness in the normal mouse mammary gland." Journal of Cellular Physiology **174**(2): 251-260.
- Antal, M. C., A. Krust, P. Chambon and M. Mark (2008). "Sterility and absence of histopathological defects in nonreproductive organs of a mouse ER β -null mutant." Proceedings of the National Academy of Sciences USA **105**(7): 2433-2438.
- Anwar, A., P. McTernan, L. Anderson, J. Askaa, C. Moody, A. Barnett, M. Eggo and S. Kumar (2001). "Site - specific regulation of oestrogen receptor α and β by oestradiol in human adipose tissue." Diabetes, Obesity and Metabolism **3**(5): 338-349.
- Arai, N., A. Ström, J. J. Rafter and J.-Å. Gustafsson (2000). "Estrogen receptor β mRNA in colon cancer cells: growth effects of estrogen and genistein." Biochemical and Biophysical Research Communications **270**(2): 425-431.

- Arimatsu, Y. and H. Hatanaka (1986). "Estrogen treatment enhances survival of cultured fetal rat amygdala neurons in a defined medium." Developmental Brain Research **26**(1): 151-159.
- Arnold, S. F., J. D. Obourn, H. Jaffe and A. C. Notides (1995). "Phosphorylation of the human estrogen receptor by mitogen-activated protein kinase and casein kinase II: consequence on DNA binding." The Journal of Steroid Biochemistry and Molecular Biology **55**(2): 163-172.
- Aronica, S. and B. Katzenellenbogen (1993). "Stimulation of estrogen receptor-mediated transcription and alteration in the phosphorylation state of the rat uterine estrogen receptor by estrogen, cyclic adenosine monophosphate, and insulin-like growth factor-I." Molecular Endocrinology **7**(6): 743-752.
- Atanassova, N., C. McKinnell, J. Fisher and R. M. Sharpe (2005). "Neonatal treatment of rats with diethylstilboestrol (DES) induces stromal-epithelial abnormalities of the vas deferens and cauda epididymis in adulthood following delayed basal cell development." Reproduction **129**(5): 589-601.
- Azuma, K., T. Urano, K. Horie-Inoue, S.-i. Hayashi, R. Sakai, Y. Ouchi and S. Inoue (2009). "Association of estrogen receptor α and histone deacetylase 6 causes rapid deacetylation of tubulin in breast cancer cells." Cancer Research **69**(7): 2935-2940.
- Ball, P. and R. Knuppen (1980). "Catecholestrogens (2- and 4-hydroxyoestrogens): chemistry, biogenesis, metabolism, occurrence and physiological significance." Acta Endocrinol Suppl (Copenh) **232**: 1-127.
- Ballard, P. L. (1989). "Hormonal regulation of pulmonary surfactant." Endocrine Reviews **10**(2): 165-181.
- Barkhem, T., B. Carlsson, Y. Nilsson, E. Enmark, J.-Å. Gustafsson and S. Nilsson (1998). "Differential response of estrogen receptor α and estrogen receptor β to partial estrogen agonists/antagonists." Molecular Pharmacology **54**(1): 105-112.
- Barrett-Connor, E., N. K. Wenger, D. Grady, L. Mosca, P. Collins, M. Kornitzer, D. A. Cox, E. Moscarelli and P. W. Anderson (1998). "Hormone and nonhormone therapy for the maintenance of postmenopausal health: the need for randomized controlled trials of estrogen and raloxifene." Journal of Women's Health **7**(7): 839-847.
- Beattie, C. W., N. W. Hansen and P. A. Thomas (1985). "Steroid receptors in human lung cancer." Cancer Research **45**(9): 4206-4214.
- Beral, V. (2003). "Breast cancer and hormone-replacement therapy in the Million Women Study." Lancet **362**(9382): 419-427.
- Bergman, L., M. L. Beelen, M. P. Gallee, H. Hollema, J. Benraadt and F. E. van Leeuwen (2000). "Risk and prognosis of endometrial cancer after tamoxifen for breast cancer." The Lancet **356**(9233): 881-887.
- Bernstein, L. (2002). "Epidemiology of endocrine-related risk factors for breast cancer." Journal of Mammary Gland biology and Neoplasia **7**(1): 3-15.

Berry, S., P. Jobst, S. Ellis, R. Howard, A. Capuco and R. Akers (2003). "Mammary Epithelial Proliferation and Estrogen Receptor α Expression in Prepubertal Heifers: Effects of Ovariectomy and Growth Hormone." Journal of Dairy Science **86**(6): 2098-2105.

Bhat, R. A., D. C. Harnish, P. E. Stevis, C. R. Lyttle and B. S. Komm (1998). "A novel human estrogen receptor β : identification and functional analysis of additional N-terminal amino acids." The Journal of Steroid Biochemistry and Molecular Biology **67**(3): 233-240.

Bièche, I., I. Laurendeau, S. Tozlu, M. Olivi, D. Vidaud, R. Lidereau and M. Vidaud (1999). "Quantitation of MYC gene expression in sporadic breast tumors with a real-time reverse transcription-PCR assay." Cancer Research **59**(12): 2759-2765.

Birge, S. J. (2000). "HRT and cognition: what the evidence shows." OBG Manage **12**(10): 40-59.

Bocchinfuso, W. P. and K. S. Korach (1997). "Mammary gland development and tumorigenesis in estrogen receptor knockout mice." Journal of Mammary Gland Biology and Neoplasia **2**(4): 323-334.

Bocchinfuso, W. P. and K. S. Korach (1997). "Mammary gland development and tumorigenesis in estrogen receptor knockout mice." J Mammary Gland Biol Neoplasia **2**(4): 323-334.

Bonnetterre, J., A. Buzdar, J. M. A. Nabholz, J. F. Robertson, B. Thürlimann, M. von Euler, T. Sahmoud, A. Webster and M. Steinberg (2001). "Anastrozole is superior to tamoxifen as first - line therapy in hormone receptor positive advanced breast carcinoma." Cancer **92**(9): 2247-2258.

Bonnetterre, J., B. Thürlimann, J. Robertson, M. Krzakowski, L. Mauriac, P. Koralewski, I. Vergote, A. Webster, M. Steinberg and M. Von Euler (2000). "Anastrozole versus tamoxifen as first-line therapy for advanced breast cancer in 668 postmenopausal women: results of the Tamoxifen or Arimidex Randomized Group Efficacy and Tolerability study." Journal of Clinical Oncology **18**(22): 3748-3757.

Brisken, C., S. Park, T. Vass, J. P. Lydon, B. W. O'Malley and R. A. Weinberg (1998). "A paracrine role for the epithelial progesterone receptor in mammary gland development." Proceedings of the National Academy of Sciences USA **95**(9): 5076-5081.

Britt, K., A. Ashworth and M. Smalley (2007). "Pregnancy and the risk of breast cancer." Endocrine-related Cancer **14**(4): 907-933.

Britt, K. and J. Findlay (2002). "Estrogen actions in the ovary revisited." Journal of Endocrinology **175**(2): 269-276.

Britton, D., I. R. Hutcheson, J. Knowlden, D. Barrow, M. Giles, R. McClelland, J. Gee and R. Nicholson (2006). "Bidirectional cross talk between ER α and EGFR signalling pathways regulates tamoxifen-resistant growth." Breast Cancer Research and Treatment **96**(2): 131-146.

Brooks, S. C. and L. Horn (1971). "Hepatic sulfation of estrogen metabolites." Biochim Biophys Acta **231**(1): 233-241.

Brown, J. R., E. Nigh, R. J. Lee, H. Ye, M. A. Thompson, F. Saudou, R. G. Pestell and M. E. Greenberg (1998). "Fos family members induce cell cycle entry by activating cyclin D1." Molecular and Cellular Biology **18**(9): 5609-5619.

Brown, R. W., L. Campagna, J. K. Dunn and P. T. Cagle (1997). "Immunohistochemical identification of tumor markers in metastatic adenocarcinoma: a diagnostic adjunct in the determination of primary site." American Journal of Clinical Pathology **107**(1): 12-19.

Brueggemeier, R. W., P.-K. Li, H.-H. Chen, P. P. Moh and N. E. Katlic (1990). "Biochemical and pharmacological development of steroidal inhibitors of aromatase." The Journal of Steroid Biochemistry and Molecular Biology **37**(3): 379-385.

Brzozowski, A. M., A. C. Pike, Z. Dauter, R. E. Hubbard, T. Bonn, O. Engström, L. Öhman, G. L. Greene, J.-Å. Gustafsson and M. Carlquist (1997). "Molecular basis of agonism and antagonism in the oestrogen receptor." Nature **389**(6652): 753-758.

Brzozowski, A. M., A. C. W. Pike, Z. Dauter, R. E. Hubbard, T. Bonn, O. Engstrom, L. Ohman, G. L. Greene, J. A. Gustafsson and M. Carlquist (1997). "Molecular basis of agonism and antagonism in the oestrogen receptor." Nature **389**(6652): 753-757.

Buchanan, D. L., T. Kurita, J. A. Taylor, D. B. Lubahn, G. R. Cunha and P. S. Cooke (1998). "Role of stromal and epithelial estrogen receptors in vaginal epithelial proliferation, stratification, and cornification." Endocrinology **139**(10): 4345-4352.

Buhi, W., I. Alvarez and A. Kouba (2008). "Secreted proteins of the oviduct." Cells Tissues Organs **166**(2): 165-179.

Bunone, G., P. Briand, R. Miksicek and D. Picard (1996). "Activation of the unliganded estrogen receptor by EGF involves the MAP kinase pathway and direct phosphorylation." The EMBO journal **15**(9): 2174.

Butler, W. B., W. L. Kirkland and T. L. Jorgensen (1979). "Induction of plasminogen activator by estrogen in a human breast cancer cell line (MCF-7)." Biochemical and Biophysical Research Communications **90**(4): 1328-1334.

Calle, E. E., H. L. Miracle-McMahill, M. J. Thun and C. W. Heath (1995). "Estrogen replacement therapy and risk of fatal colon cancer in a prospective cohort of postmenopausal women." Journal of the National Cancer Institute **87**(7): 517-523.

Campbell-Thompson, M., I. J. Lynch and B. Bhardwaj (2001). "Expression of estrogen receptor (ER) subtypes and ER β isoforms in colon cancer." Cancer Research **61**(2): 632-640.

Chambliss, K. L., I. S. Yuhanna, R. G. Anderson, M. E. Mendelsohn and P. W. Shaul (2002). "ER β has nongenomic action in caveolae." Molecular Endocrinology **16**(5): 938-946.

Chan, C. M., A. E. Lykkesfeldt, M. G. Parker and M. Dowsett (1999). "Expression of nuclear receptor interacting proteins TIF-1, SUG-1, receptor interacting protein 140, and corepressor SMRT in tamoxifen-resistant breast cancer." Clinical Cancer Research **5**(11): 3460-3467.

- Chan, H., W. Zhou, W. Fu, W. Ko and P. Wong (1995). "Different regulatory pathways involved in ATP - stimulated chloride secretion in rat epididymal epithelium." Journal of Cellular Physiology **164**(2): 271-276.
- Chang, E. C., J. Frasor, B. Komm and B. S. Katzenellenbogen (2006). "Impact of estrogen receptor β on gene networks regulated by estrogen receptor α in breast cancer cells." Endocrinology **147**(10): 4831-4842.
- Chen, D., H. Ma, H. Hong, S. S. Koh, S.-M. Huang, B. T. Schurter, D. W. Aswad and M. R. Stallcup (1999). "Regulation of transcription by a protein methyltransferase." Science **284**(5423): 2174-2177.
- Chen, H., T. R. Tritton, N. Kenny, M. Absher and J. F. Chiu (1996). "Tamoxifen induces TGF - β 1 activity and apoptosis of human MCF - 7 breast cancer cells in vitro." Journal of Cellular Biochemistry **61**(1): 9-17.
- Cheng, G., Y. Li, Y. Omoto, Y. Wang, T. Berg, M. Nord, P. Vihko, M. Warner, Y.-S. Piao and J.-Å. Gustafsson (2005). "Differential regulation of estrogen receptor (ER) α and ER β in primate mammary gland." Journal of Clinical Endocrinology & Metabolism **90**(1): 435-444.
- Cheng, G., Y. Li, Y. Omoto, Y. Wang, T. Berg, M. Nord, P. Vihko, M. Warner, Y. S. Piao and J. A. Gustafsson (2005). "Differential regulation of estrogen receptor (ER)alpha and ERbeta in primate mammary gland." The Journal of Clinical Endocrinology & Metabolism **90**(1): 435-444.
- Cheng, G., Z. Weihua, S. Mäkinen, S. Mäkelä S. Saji, M. Warner, J.-Å. Gustafsson and O. Hovatta (2002). "A role for the androgen receptor in follicular atresia of estrogen receptor beta knockout mouse ovary." Biology of Reproduction **66**(1): 77-84.
- Cheng, G., Z. Weihua, M. Warner and J. A. Gustafsson (2004). "Estrogen receptors ER alpha and ER beta in proliferation in the rodent mammary gland." Proceedings of the National Academy of Sciences USA **101**(11): 3739-3746.
- Cho, H. W., R. Nie, K. Carnes, Q. Zhou, N. Sharief and R. A. Hess (2003). "The antiestrogen ICI 182,780 induces early effects on the adult male mouse reproductive tract and long-term decreased fertility without testicular atrophy." Reproductive Biology and Endocrinology **1**(1): 57.
- Ciarloni, L., S. Mallepell and C. Brisken (2007). "Amphiregulin is an essential mediator of estrogen receptor alpha function in mammary gland development." Proceedings of the National Academy of Sciences USA **104**(13): 5455-5460.
- Clarke, R. B., E. Anderson, A. Howell and C. S. Potten (2003). "Regulation of human breast epithelial stem cells." Cell Proliferation **36**(s1): 45-58.
- Clarke, R. B., A. Howell and E. Anderson (1997). "Estrogen sensitivity of normal human breast tissue in vivo and implanted into athymic nude mice: analysis of the relationship between estrogen-induced proliferation and progesterone receptor expression." Breast Cancer Research and Treatment **45**(2): 121-133.

Clarke, R. B., A. Howell, C. S. Potten and E. Anderson (1997). "Dissociation between steroid receptor expression and cell proliferation in the human breast." Cancer Research **57**(22): 4987-4991.

Cleland, W. H., C. R. Mendelson and E. R. Simpson (1985). "Effects of aging and obesity on aromatase activity of human adipose cells." Journal of Clinical Endocrinology & Metabolism **60**(1): 174-177.

Clemons, M. and P. Goss (2001). "Estrogen and the risk of breast cancer." New England Journal of Medicine **344**(4): 276-285.

Cobellis, G., R. Pierantoni, S. Minucci, R. Pernas-Alonso, R. Meccariello and S. Fasano (1999). "c-fos activity in *Rana esculenta* testis: seasonal and estradiol-induced changes." Endocrinology **140**(7): 3238-3244.

Cobellis, L., F. M. Reis, L. Driul, G. Vultaggio, I. Ferretti, E. Villa and F. Petraglia (2002). "Estrogen receptor α mRNA variant lacking exon 5 is co-expressed with the wild-type in endometrial adenocarcinoma." European Journal of Obstetrics & Gynecology and Reproductive Biology **102**(1): 92-95.

Cohen, D. and T. Curran (1989). "The structure and function of the fos proto-oncogene." Critical Reviews in Oncogenesis **1**(1): 65.

Collins, P. and C. Webb (1999). "Estrogen hits the surface." Nature Medicine **5**(10): 1130-1131.

Conrad, K. P., G. M. Joffe, H. Kruszyna, R. Kruszyna, L. Rochelle, R. Smith, J. Chavez and M. Mosher (1993). "Identification of increased nitric oxide biosynthesis during pregnancy in rats." The FASEB Journal **7**(6): 566-571.

Cooke, P., D. Buchanan, P. Young, T. Setiawan, J. Brody, K. Korach, J. Taylor, D. Lubahn and G. Cunha (1997). "Stromal estrogen receptors mediate mitogenic effects of estradiol on uterine epithelium." Proceedings of the National Academy of Sciences USA **94**(12): 6535-6540.

Cooke, P. S., F. Uchima, D. K. Fujii, H. A. Bern and G. R. Cunha (1986). "Restoration of normal morphology and estrogen responsiveness in cultured vaginal and uterine epithelia transplanted with stroma." Proceedings of the National Academy of Sciences USA **83**(7): 2109-2113.

Cotrim, C., V. Fabris, M. Doria, K. Lindberg, J.-Å. Gustafsson, F. Amado, C. Lanari and L. Helguero (2012). "Estrogen receptor beta growth-inhibitory effects are repressed through activation of MAPK and PI3K signalling in mammary epithelial and breast cancer cells." Oncogene **32**(19): 2390-2402.

Couse, J., S. C. Hewitt, D. Bunch, M. Sar, V. Walker, B. Davis and K. Korach (1999). "Postnatal sex reversal of the ovaries in mice lacking estrogen receptors and." Science Signaling **286**(5448): 2328.

- Cowley, S. M. and M. G. Parker (1999). "A comparison of transcriptional activation by ER α and ER β ." The Journal of Steroid Biochemistry and Molecular Biology **69**(1): 165-175.
- Curtis, S. W., T. Washburn, C. Sewall, R. DiAugustine, J. Lindzey, J. F. Couse and K. S. Korach (1996). "Physiological coupling of growth factor and steroid receptor signaling pathways: estrogen receptor knockout mice lack estrogen-like response to epidermal growth factor." Proceedings of the National Academy of Sciences USA **93**(22): 12626-12630.
- Danielian, P., R. White, J. Lees and M. Parker (1992). "Identification of a conserved region required for hormone dependent transcriptional activation by steroid hormone receptors." The EMBO journal **11**(3): 1025.
- Darimont, B. D., R. L. Wagner, J. W. Apriletti, M. R. Stallcup, P. J. Kushner, J. D. Baxter, R. J. Fletterick and K. R. Yamamoto (1998). "Structure and specificity of nuclear receptor-coactivator interactions." Genes & development **12**(21): 3343-3356.
- Davis, R. A., F. Kern, R. Showalter, E. Sutherland, M. Sinensky and F. R. Simon (1978). "Alterations of hepatic Na⁺, K⁺-ATPase and bile flow by estrogen: effects on liver surface membrane lipid structure and function." Proceedings of the National Academy of Sciences USA **75**(9): 4130-4134.
- Delage-Mourroux, R., P. G. Martini, I. Choi, D. M. Kraichely, J. Hoeksema and B. S. Katzenellenbogen (2000). "Analysis of estrogen receptor interaction with a repressor of estrogen receptor activity (REA) and the regulation of estrogen receptor transcriptional activity by REA." Journal of Biological Chemistry **275**(46): 35848-35856.
- Delmas, P. D., N. H. Bjarnason, B. H. Mitlak, A.-C. Ravoux, A. S. Shah, W. J. Huster, M. Draper and C. Christiansen (1997). "Effects of raloxifene on bone mineral density, serum cholesterol concentrations, and uterine endometrium in postmenopausal women." New England Journal of Medicine **337**(23): 1641-1647.
- DeMichele, A., A. B. Troxel, J. A. Berlin, A. L. Weber, G. R. Bunin, E. Turzo, R. Schinnar, D. Burgh, M. Berlin and S. C. Rubin (2008). "Impact of raloxifene or tamoxifen use on endometrial cancer risk: a population-based case-control study." Journal of Clinical Oncology **26**(25): 4151-4159.
- Deming, S., S. Nass, R. Dickson and B. Trock (2000). "C-myc amplification in breast cancer: a meta-analysis of its occurrence and prognostic relevance." British Journal of Cancer **83**(12): 1688.
- Deng, C.-X. and S. G. Brodie (2000). "Roles of BRCA1 and its interacting proteins." Bioessays **22**: 728-737.
- Dey, S. and H. Lim (1996). "Implantation." Reproductive Endocrinology, Surgery and Technology **1**: 421-435.

Di Domenico, M., G. Castoria, A. Bilancio, A. Migliaccio and F. Auricchio (1996). "Estradiol activation of human colon carcinoma-derived Caco-2 cell growth." Cancer Research **56**(19): 4516-4521.

Doershuk, C., B. Fisher and L. Matthews (1974). "Specific airway resistance from the perinatal period into adulthood. Alterations in childhood pulmonary disease." The American Review of Respiratory Disease **109**(4): 452-457.

Donovan, J. and J. Slingerland (2000). "Transforming growth factor-beta and breast cancer: Cell cycle arrest by transforming growth factor- β and its disruption in cancer." Breast Cancer Research **2**(2): 116.

Dubik, D., T. C. Dembinski and R. P. Shiu (1987). "Stimulation of c-myc oncogene expression associated with estrogen-induced proliferation of human breast cancer cells." Cancer Research **47**(24 Part 1): 6517-6521.

Dubik, D. and R. Shiu (1992). "Mechanism of estrogen activation of c-myc oncogene expression." Oncogene **7**(8): 1587-1594.

Dunbar, M. E., P. R. Dann, G. W. Robinson, L. Hennighausen, J.-P. Zhang and J. J. Wysolmerski (1999). "Parathyroid hormone-related protein signaling is necessary for sexual dimorphism during embryonic mammary development." Development **126**(16): 3485-3493.

Dupont, S., A. Krust, A. Gansmuller, A. Dierich, P. Chambon and M. Mark (2000). "Effect of single and compound knockouts of estrogen receptors alpha (ERalpha) and beta (ERbeta) on mouse reproductive phenotypes." Development **127**(19): 4277-4291.

Eghbali-Fatourehchi, G., S. Khosla, A. Sanyal, W. J. Boyle, D. L. Lacey and B. L. Riggs (2003). "Role of RANK ligand in mediating increased bone resorption in early postmenopausal women." Journal of Clinical Investigation **111**(8): 1221-1230.

El-Tanani, M. K. and C. D. Green (1997). "Two separate mechanisms for ligand-independent activation of the estrogen receptor." Molecular Endocrinology **11**(7): 928-937.

Endoh, H., K. Maruyama, Y. Masuhiro, Y. Kobayashi, M. Goto, H. Tai, J. Yanagisawa, D. Metzger, S. Hashimoto and S. Kato (1999). "Purification and identification of p68 RNA helicase acting as a transcriptional coactivator specific for the activation function 1 of human estrogen receptor α ." Molecular and Cellular Biology **19**(8): 5363-5372.

Ewan, K. B., H. A. Oketch-Rabah, S. A. Ravani, G. Shyamala, H. L. Moses and M. H. Barcellos-Hoff (2005). "Proliferation of estrogen receptor- α -positive mammary epithelial cells is restrained by transforming growth factor- β 1 in adult mice." The American Journal of Pathology **167**(2): 409-417.

Ewan, K. B., G. Shyamala, S. A. Ravani, Y. Tang, R. Akhurst, L. Wakefield and M. H. Barcellos-Hoff (2002). "Latent transforming growth factor- β activation in mammary gland: regulation by ovarian hormones affects ductal and alveolar proliferation." The American Journal of Pathology **160**(6): 2081-2093.

- Förster, C., S. Mäkela, A. Wäri, S. Kietz, D. Becker, K. Hultenby, M. Warner and J.-Å. Gustafsson (2002). "Involvement of estrogen receptor β in terminal differentiation of mammary gland epithelium." Proceedings of the National Academy of Sciences USA **99**(24): 15578-15583.
- Fan, S., Y. X. Ma, C. Wang, R.-Q. Yuan, Q. Meng, J.-A. Wang, M. Erdos, I. D. Goldberg, P. Webb and P. J. Kushner (2002). "p300 Modulates the BRCA1 inhibition of estrogen receptor activity." Cancer Research **62**(1): 141-151.
- Fan, S., J.-A. Wang, R. Yuan, Y. Ma, Q. Meng, M. Erdos, R. Pestell, F. Yuan, K. Auborn and I. Goldberg (1999). "BRCA1 inhibition of estrogen receptor signaling in transfected cells." Science **284**(5418): 1354-1356.
- Feinleib, M. (1968). "Breast cancer and artificial menopause: a cohort study." Journal of the National Cancer Institute **41**(2): 315-329.
- Ferguson, D. and T. Anderson (1981). "Morphological evaluation of cell turnover in relation to the menstrual cycle in the "resting" human breast." British Journal of Cancer **44**(2): 177.
- Ferin, M., R. Jewelewicz and M. P. Warren (1993). The menstrual cycle: Physiology, reproductive disorders, and infertility, Oxford University Press.
- Fernandez, E., S. Gallus, C. Bosetti, S. Franceschi, E. Negri and C. La Vecchia (2003). "Hormone replacement therapy and cancer risk: A systematic analysis from a network of case - control studies." International Journal of Cancer **105**(3): 408-412.
- Filardo, E. J., J. A. Quinn, K. I. Bland and A. R. Frackelton (2000). "Estrogen-induced activation of Erk-1 and Erk-2 requires the G protein-coupled receptor homolog, GPR30, and occurs via trans-activation of the epidermal growth factor receptor through release of HB-EGF." Molecular Endocrinology **14**(10): 1649-1660.
- Filardo, E. J., J. A. Quinn, A. R. Frackelton and K. I. Bland (2002). "Estrogen action via the G protein-coupled receptor, GPR30: stimulation of adenylyl cyclase and cAMP-mediated attenuation of the epidermal growth factor receptor-to-MAPK signaling axis." Molecular Endocrinology **16**(1): 70-84.
- Findlay, J. K., K. Britt, J. B. Kerr, L. O'Donnell, M. E. Jones, A. E. Drummond and E. R. Simpson (2001). "The road to ovulation: the role of oestrogens." Reproduction, Fertility and Development **13**(7-8): 543-547.
- Fiorelli, G., L. Picariello, V. Martineti, F. Tonelli and M. L. Brandi (1999). "Functional estrogen receptor β in colon cancer cells." Biochemical and Biophysical Research Communications **261**(2): 521-527.
- Fischer-Dzoga, K., R. W. Wissler and D. Vesselinovitch (1983). "The effect of estradiol on the proliferation of rabbit aortic medial tissue culture cells induced by hyperlipemic serum." Experimental and Molecular Pathology **39**(3): 355-363.

Fisher, C. R., K. H. Graves, A. F. Parlow and E. R. Simpson (1998). "Characterization of mice deficient in aromatase (ArKO) because of targeted disruption of the cyp19 gene." Proceedings of the National Academy of Sciences USA **95**(12): 6965-6970.

Foley, E. F., A. A. Jazaeri, M. A. Shupnik, O. Jazaeri and L. W. Rice (2000). "Selective loss of estrogen receptor β in malignant human colon." Cancer Research **60**(2): 245-248.

Foley, J., P. Dann, J. Hong, J. Cosgrove, B. Dreyer, D. Rimm, M. Dunbar, W. Philbrick and J. Wysolmerski (2001). "Parathyroid hormone-related protein maintains mammary epithelial fate and triggers nipple skin differentiation during embryonic breast development." Development **128**(4): 513-525.

Food, U. (2011). Drug Administration. Orange book: approved drug products with therapeutic equivalence evaluations.

Franceschi, S. and C. La Vecchia (1998). "Colorectal cancer and hormone replacement therapy: an unexpected finding." European Journal of Cancer Prevention **7**(6): 427-438.

Frech, M. S., E. D. Halama, M. T. Tilli, B. Singh, E. J. Gunther, L. A. Chodosh, J. A. Flaws and P. A. Furth (2005). "Deregulated estrogen receptor α expression in mammary epithelial cells of transgenic mice results in the development of ductal carcinoma in situ." Cancer Research **65**(3): 681-685.

Freedman, L. P. (1999). "Increasing the complexity of coactivation in nuclear receptor signaling." Cell **97**(1): 5-8.

Frenette, G., P. Leclerc, O. D'amours and R. Sullivan (2009). "Estrogen sulfotransferase is highly expressed along the bovine epididymis and is secreted into the intraluminal environment." Journal of Andrology **30**(5): 580-589.

Galien, R. and T. Garcia (1997). "Estrogen receptor impairs interleukin-6 expression by preventing protein binding on the NF- κ B site." Nucleic Acids Research **25**(12): 2424-2429.

Ganjam, v. k. and r. p. Amann (1976). "Steroids in fluids and sperm entering and leaving the bovine epididymis, epididymal tissue, and accessory sex gland secretions." Endocrinology **99**(6): 1618-1630.

Gehin, M., M. Mark, C. Dennefeld, A. Dierich, H. Gronemeyer and P. Chambon (2002). "The function of TIF2/GRIP1 in mouse reproduction is distinct from those of SRC-1 and p/CIP." Molecular and Cellular Biology **22**(16): 5923-5937.

Genazzani, A. R. (2001). Hormone replacement therapy and cardiovascular disease. New York, Parthenon Pub. Group.

Georgescu, S. P., J. H. Li, Q. Lu, R. H. Karas, M. Brown and M. E. Mendelsohn (2005). "Modulator recognition factor 1, an AT-rich interaction domain family member, is a novel corepressor for estrogen receptor α ." Molecular Endocrinology **19**(10): 2491-2501.

Gethins, M. (2012). "Breast cancer in men." Journal of the National Cancer Institute **104**(6): 436-438.

Ghosh, D., J. Griswold, M. Ertman and W. Pangborn (2009). "Structural basis for androgen specificity and oestrogen synthesis in human aromatase." Nature **457**(7226): 219-223.

- Girault, I., C. Andrieu, S. Tozlu, F. Spyrtos, I. Bieche and R. Lidereau (2004). "Altered expression pattern of alternatively spliced estrogen receptor beta transcripts in breast carcinoma." Cancer letters **215**(1): 101-112.
- Glasier, A. and A. S. McNeilly (1990). "Physiology of lactation." Baillière's Clinical Endocrinology and Metabolism **4**(2): 379-395.
- Goldstein, S. R. (1998). "Selective estrogen receptor modulators: a new category of therapeutic agents for extending the health of postmenopausal women." American Journal of Obstetrics and Gynecology **179**(6): 1479-1484.
- Gorodeski, G. (1996). "The cervical cycle." Reproductive Endocrinology, Surgery, and Technology **1**: 302-324.
- Gorodeski, G. I. (1998). "Estrogen increases the permeability of the cultured human cervical epithelium by modulating cell deformability." American Journal of Physiology-Cell Physiology **275**(3): C888-C899.
- Goss, P. E., J. N. Ingle, J. E. Alés-Martínez, A. M. Cheung, R. T. Chlebowski, J. Wactawski-Wende, A. McTiernan, J. Robbins, K. C. Johnson and L. W. Martin (2011). "Exemestane for breast-cancer prevention in postmenopausal women." New England Journal of Medicine **364**(25): 2381-2391.
- Grady, D., T. Gebretsadik, K. Kerlikowske, V. Ernster and D. Petitti (1995). "Hormone replacement therapy and endometrial cancer risk: a meta-analysis." Obstetrics & Gynecology **85**(2): 304-313.
- Green, P. S., J. Bishop and J. W. Simpkins (1997). "17 α -Estradiol exerts neuroprotective effects on SK-N-SH cells." The Journal of Neuroscience **17**(2): 511-515.
- Green, S., P. Walter, V. Kumar, A. Krust, J.-M. Bornert, P. Argos and P. Chambon (1986). "Human oestrogen receptor cDNA: sequence, expression and homology to v-erb-A."
- Grey, A., J. Stapleton, M. Evans and I. Reid (1995). "The effect of the anti-estrogen tamoxifen on cardiovascular risk factors in normal postmenopausal women." Journal of Clinical Endocrinology & Metabolism **80**(11): 3191-3195.
- Gruber, C. J., W. Tschugguel, C. Schneeberger and J. C. Huber (2002). "Production and actions of estrogens." New England Journal of Medicine **346**(5): 340-352.
- Gruvberger-Saal, S. K., P. O. Bendahl, L. H. Saal, M. Laakso, C. Hegardt, P. Eden, C. Peterson, P. Malmstrom, J. Isola, A. Borg and M. Ferno (2007). "Estrogen receptor beta expression is associated with tamoxifen response in ERalpha-negative breast carcinoma." Clinical Cancer Research **13**(7): 1987-1994.
- Gudelsky, G. A., D. D. Nansel and J. C. Porter (1981). "Role of estrogen in the dopaminergic control of prolactin secretion." Endocrinology **108**(2): 440-444.
- Gustafsson, J.-A. (1999). "Estrogen receptor beta--a new dimension in estrogen mechanism of action." Journal of Endocrinology **163**(3): 379-383.
- Hadley, M. and J. E. Levine (2006). Endocrinology, 6/e, Pearson Education India.

Hall, J. M., J. F. Couse and K. S. Korach (2001). "The multifaceted mechanisms of estradiol and estrogen receptor signaling." Journal of Biological Chemistry **276**(40): 36869-36872.

Hanstein, B., R. Eckner, J. DiRenzo, S. Halachmi, H. Liu, B. Searcy, R. Kurokawa and M. Brown (1996). "p300 is a component of an estrogen receptor coactivator complex." Proceedings of the National Academy of Sciences USA **93**(21): 11540-11545.

Hartman, J., K. Lindberg, A. Morani, J. Inzunza, A. Ström and J.-Å. Gustafsson (2006). "Estrogen receptor β inhibits angiogenesis and growth of T47D breast cancer xenografts." Cancer Research **66**(23): 11207-11213.

Hartman, J., A. Strom and J. A. Gustafsson (2009). "Estrogen receptor beta in breast cancer--diagnostic and therapeutic implications." Steroids **74**(8): 635-641.

Haskell, S. G. (2003). "Selective estrogen receptor modulators." Southern medical journal **96**(5): 469.

Hatcher, R. A. (2007). Contraceptive technology, Physicians Desk Reference Incorporated.

Hayes, D. F., J. Van Zyl, A. Hacking, L. Goedhals, W. Bezwoda, J. A. Mailliard, S. E. Jones, C. L. Vogel, R. F. Berris and I. Shemano (1995). "Randomized comparison of tamoxifen and two separate doses of toremifene in postmenopausal patients with metastatic breast cancer." Journal of clinical oncology **13**(10): 2556.

Heiss, G., R. Wallace, G. L. Anderson, A. Aragaki, S. A. Beresford, R. Brzyski, R. T. Chlebowski, M. Gass, A. LaCroix and J. E. Manson (2008). "Health risks and benefits 3 years after stopping randomized treatment with estrogen and progestin." Journal of the American Medical Association **299**(9): 1036-1045.

Heldin, C.-H., M. Landström and A. Moustakas (2009). "Mechanism of TGF- β signaling to growth arrest, apoptosis, and epithelial-mesenchymal transition." Current Opinion in Cell Biology **21**(2): 166-176.

Heldring, N., A. Pike, S. Andersson, J. Matthews, G. Cheng, J. Hartman, M. Tujague, A. Ström, E. Treuter and M. Warner (2007). "Estrogen Receptors: how do they signal and what are their targets." Physiological Reviews **87**(3): 905-931.

Helguero, L. A., M. H. Faulds, J. A. Gustafsson and L. A. Haldosen (2005). "Estrogen receptors alfa (ERalpha) and beta (ERbeta) differentially regulate proliferation and apoptosis of the normal murine mammary epithelial cell line HC11." Oncogene **24**(44): 6605-6616.

Hemsell, D. L., J. Grodin, P. Brenner, P. Siiteri and P. MacDonald (1974). "Plasma precursors of estrogen. II. Correlation of the extent of conversion of plasma androstenedione to estrone with age." Journal of Clinical Endocrinology & Metabolism **38**(3): 476-479.

Henderson, V. W. (2000). Estrogen for Alzheimer's disease in women Randomized, double-blind, placebo-controlled trial.

Hennighausen, L. and G. W. Robinson (2001). "Signaling pathways in mammary gland development." Developmental Cell **1**(4): 467-475.

Hens, J. R., P. Dann, J.-P. Zhang, S. Harris, G. W. Robinson and J. Wysolmerski (2007). "BMP4 and PTHrP interact to stimulate ductal outgrowth during embryonic mammary development and to inhibit hair follicle induction." Development **134**(6): 1221-1230.

Henttu, P., E. Kalkhoven and M. G. Parker (1997). "AF-2 activity and recruitment of steroid receptor coactivator 1 to the estrogen receptor depend on a lysine residue conserved in nuclear receptors." Molecular and Cellular Biology **17**(4): 1832-1839.

Herber, B., M. Truss, M. Beato and R. Müller (1994). "Inducible regulatory elements in the human cyclin D1 promoter." Oncogene **9**(4): 1295-1304.

Hernán, M. A., A. Alonso, R. Logan, F. Grodstein, K. B. Michels, W. C. Willett, J. E. Manson and J. M. Robins (2008). "Observational studies analyzed like randomized experiments: an application to postmenopausal hormone therapy and coronary heart disease." Epidemiology **19**(6): 766-779.

Hernandez, J. S., R. W. Watson, T. C. Wood and R. M. Weinshilboum (1992). "Sulfation of estrone and 17 beta-estradiol in human liver. Catalysis by thermostable phenol sulfotransferase and by dehydroepiandrosterone sulfotransferase." Drug Metabolism and Disposition **20**(3): 413-422.

Hershberger, P. A., A. C. Vasquez, B. Kanterewicz, S. Land, J. M. Siegfried and M. Nichols (2005). "Regulation of endogenous gene expression in human non-small cell lung cancer cells by estrogen receptor ligands." Cancer Research **65**(4): 1598-1605.

Hess, R. and K. Carnes (2004). "The role of estrogen in testis and the male reproductive tract: a review and species comparison." Animal Reproduction **1**(1): 5-30.

Hess, R. A. (2003). "Estrogen in the adult male reproductive tract: a review." Reproductive Biology and Endocrinology **1**(1): 52.

Hess, R. A., D. Bunick, K.-H. Lee, J. Bahr, J. A. Taylor, K. S. Korach and D. B. Lubahn (1997). "A role for oestrogens in the male reproductive system." Nature **390**(6659): 509-512.

Hess, R. A., D. H. Gist, D. Bunick, D. B. Lubahn, A. Farrell, J. Bahr, P. S. Cooke and G. L. Greene (1997). "Estrogen receptor (α and β) expression in the excurrent ducts of the adult male rat reproductive tract." Journal of Andrology **18**(6): 602-611.

Hewitt, S. C., E. H. Goulding, E. Eddy and K. S. Korach (2002). "Studies using the estrogen receptor α knockout uterus demonstrate that implantation but not decidualization-associated signaling is estrogen dependent." Biology of Reproduction **67**(4): 1268-1277.

Hewitt, S. C. and K. S. Korach (2002). "Estrogen receptors: structure, mechanisms and function." Reviews in Endocrine and Metabolic Disorders **3**(3): 193-200.

Hickey, M., J. Elliott and S. L. Davison (2012). "Hormone replacement therapy." British Medical Journal **344**.

Hodges-Gallagher, L., C. D. Valentine, S. El Bader and P. J. Kushner (2008). "Estrogen receptor beta increases the efficacy of antiestrogens by effects on apoptosis and cell cycling in breast cancer cells." Breast Cancer Research and Treatment **109**(2): 241-250.

Hoff, M. B. and W. W. Chang (1979). "The effect of estrogen on epithelial cell proliferation and differentiation in the crypts of the descending colon of the mouse: A radioautographic study." American Journal of Anatomy **155**(4): 507-516.

Hoff, M. B., W. W. Chang and K. M. Mak (1980). "Effect of estrogen on cell proliferation in colonic mucosa of the mouse." Virchows Archiv B **35**(1): 263-273.

Hofseth, L. J., A. M. Raafat, J. R. Osuch, D. R. Pathak, C. A. Slomski and S. Z. Haslam (1999). "Hormone replacement therapy with estrogen or estrogen plus medroxyprogesterone acetate is associated with increased epithelial proliferation in the normal postmenopausal breast." Journal of Clinical Endocrinology & Metabolism **84**(12): 4559-4565.

Honma, N., R. Horii, T. Iwase, S. Saji, M. Younes, K. Takubo, M. Matsuura, Y. Ito, F. Akiyama and G. Sakamoto (2008). "Clinical importance of estrogen receptor-beta evaluation in breast cancer patients treated with adjuvant tamoxifen therapy." Journal of Clinical Oncology **26**(22): 3727-3734.

Honma, N., R. Horii, T. Iwase, S. Saji, M. Younes, K. Takubo, M. Matsuura, Y. Ito, F. Akiyama and G. Sakamoto (2008). "Clinical importance of estrogen receptor- β evaluation in breast cancer patients treated with adjuvant tamoxifen therapy." Journal of Clinical Oncology **26**(22): 3727-3734.

Hou, Y. F., S. T. Yuan, H. C. Li, J. Wu, J. S. Lu, G. Liu, L. J. Lu, Z. Z. Shen, J. Ding and Z. M. Shao (2004). "ERbeta exerts multiple stimulative effects on human breast carcinoma cells." Oncogene **23**(34): 5799-5806.

Hulley, S., D. Grady, T. Bush, C. Furberg, D. Herrington, B. Riggs and E. Vittinghoff (1998). "Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women." Journal of the American Medical Association **280**(7): 605-613.

Hunter, D. J., G. A. Colditz, S. E. Hankinson, S. Malspeis, D. Spiegelman, W. Chen, M. J. Stampfer and W. C. Willett (2010). "Oral contraceptive use and breast cancer: a prospective study of young women." Cancer Epidemiology Biomarkers & Prevention **19**(10): 2496-2502.

Ibarra de Palacios, P., G. Schmidt, T. Sergejew, E. Quebe-Fehling, L. Lockhart and L. Krinsky (2002). "Comparative study to evaluate skin irritation and adhesion of Estradot® and Climara® in healthy postmenopausal women." Climacteric **5**(4): 383-389.

Ignar-Trowbridge, D., M. Pimentel, M. Parker, J. McLachlan and K. Korach (1996). "Peptide growth factor cross-talk with the estrogen receptor requires the A/B domain and occurs independently of protein kinase C or estradiol." Endocrinology **137**(5): 1735-1744.

Ignar-Trowbridge, D. M., K. G. Nelson, M. C. Bidwell, S. W. Curtis, T. F. Washburn, J. A. McLachlan and K. S. Korach (1992). "Coupling of dual signaling pathways: epidermal growth factor action involves the estrogen receptor." Proceedings of the National Academy of Sciences USA **89**(10): 4658-4662.

Ing, N. H. and M. B. Tornesi (1997). "Estradiol up-regulates estrogen receptor and progesterone receptor gene expression in specific ovine uterine cells." Biology of Reproduction **56**(5): 1205-1215.

Issa, J.-P. J., Y. L. Ottaviano, P. Celano, S. R. Hamilton, N. E. Davidson and S. B. Baylin (1994). "Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon." Nature Genetics **7**(4): 536-540.

Järvinen, T. A., M. Peltö-Huikko, K. Holli and J. Isola (2000). "Estrogen receptor β is coexpressed with ER α and PR and associated with nodal status, grade, and proliferation rate in breast cancer." The American Journal of Pathology **156**(1): 29-35.

Jameson, J. L. and L. J. De Groot (2010). Endocrinology: adult and pediatric, Saunders.

Jansen, R. P. (1984). "Endocrine response in the fallopian tube." Endocrine Reviews **5**(4): 525-551.

Jensen, E. (1962). "On the mechanism of estrogen action." Perspectives in Biology and Medicine **6**: 47.

Jensen, E. V., G. Cheng, C. Palmieri, S. Saji, S. Mäkelä and S. Van Noorden, T. Wahlström, M. Warner, R. C. Coombes and J.-Å. Gustafsson (2001). "Estrogen receptors and proliferation markers in primary and recurrent breast cancer." Proceedings of the National Academy of Sciences USA **98**(26): 15197-15202.

Jick, H., J. A. Kaye, C. Vasilakis-Scaramozza and S. S. Jick (2000). "Risk of venous thromboembolism among users of third generation oral contraceptives compared with users of oral contraceptives with levonorgestrel before and after 1995: cohort and case-control analysis." British Medical Journal **321**(7270): 1190-1195.

Jilka, R. (1998). "Cytokines, bone remodeling, and estrogen deficiency: a 1998 update." Bone **23**(2): 75.

Joel, P. B., J. Smith, T. W. Sturgill, T. L. Fisher, J. Blenis and D. A. Lannigan (1998). "pp90rsk1 regulates estrogen receptor-mediated transcription through phosphorylation of Ser-167." Molecular and Cellular Biology **18**(4): 1978-1984.

Jones, R. E. and K. H. Lopez (2006). Human reproductive biology.

Jordan, V. C. and B. W. O'Malley (2007). "Selective estrogen-receptor modulators and antihormonal resistance in breast cancer." Journal of Clinical Oncology **25**(36): 5815-5824.

Kacsoh, B. (2000). Endocrine physiology, McGraw-Hill.

Kahlert, S., S. Nuedling, M. van Eickels, H. Vetter, R. Meyer and C. Grohé (2000). "Estrogen receptor α rapidly activates the IGF-1 receptor pathway." Journal of Biological Chemistry **275**(24): 18447-18453.

Karas, R. H., B. L. Patterson and M. E. Mendelsohn (1994). "Human vascular smooth muscle cells contain functional estrogen receptor." Circulation **89**(5): 1943-1950.

Kariagina, A., J. Xie, J. R. Leippardt and S. Z. Haslam (2010). "Amphiregulin mediates estrogen, progesterone, and EGFR signaling in the normal rat mammary gland and in hormone-dependent rat mammary cancers." Hormones and Cancer **1**(5): 229-244.

Karlsson, S. (2006). "Histopathology and histomorphometry of the urogenital tract in 15-month old male and female rats treated neonatally with SERMs and estrogens." Experimental and Toxicologic Pathology **58**(1): 1-12.

Kato, S. (2001). "Estrogen receptor-mediated cross-talk with growth factor signaling pathways." Breast Cancer **8**(1): 3-9.

Kato, S., H. Endoh, Y. Masuhiro, T. Kitamoto, S. Uchiyama, H. Sasaki, S. Masushige, Y. Gotoh, E. Nishida and H. Kawashima (1995). "Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase." Science **270**(5241): 1491-1494.

Keating, N. L., P. D. Cleary, A. S. Rossi, A. M. Zaslavsky and J. Z. Ayanian (1999). "Use of hormone replacement therapy by postmenopausal women in the United States." Annals of Internal Medicine **130**(7): 545-553.

Kennecke, H., R. Yerushalmi, R. Woods, M. C. U. Cheang, D. Voduc, C. H. Speers, T. O. Nielsen and K. Gelmon (2010). "Metastatic behavior of breast cancer subtypes." Journal of Clinical Oncology **28**(20): 3271-3277.

Kenney, N. J., G. H. Smith, K. Rosenberg, M. L. Cutler and R. B. Dickson (1996). "Induction of ductal morphogenesis and lobular hyperplasia by amphiregulin in the mouse mammary gland." Cell Growth & Differentiation **7**(12): 1769-1781.

Khachaturian, Z. S. (1985). "Diagnosis of Alzheimer's disease." Archives of Neurology **42**(11): 1097.

Khan, S. A., M. A. Rogers, K. K. Khurana, M. M. Meguid and P. J. Numann (1998). "Estrogen receptor expression in benign breast epithelium and breast cancer risk." Journal of the National Cancer Institute **90**(1): 37-42.

Khurana, S., S. Ranmal and N. Ben-Jonathan (2000). "Exposure of newborn male and female rats to environmental estrogens: delayed and sustained hyperprolactinemia and alterations in estrogen receptor expression." Endocrinology **141**(12): 4512-4517.

Kim, N., K. Min, M. Pessina, R. Munarriz, I. Goldstein and A. Traish (2004). "Effects of ovariectomy and steroid hormones on vaginal smooth muscle contractility." International Journal of Impotence Research **16**(1): 43-50.

Kleerekoper, M. (2001). Estrogen and the Skeleton. Hormonal Carcinogenesis III, Springer: 357-362.

Kleuser, B., D. Malek, R. Gust, H. H. Pertz and H. Potteck (2008). "17- β -Estradiol inhibits transforming growth factor- β signaling and function in breast cancer cells via activation of extracellular signal-regulated kinase through the G protein-coupled receptor 30." Molecular Pharmacology **74**(6): 1533-1543.

Kobayashi, Y., T. Kitamoto, Y. Masuhiro, M. Watanabe, T. Kase, D. Metzger, J. Yanagisawa and S. Kato (2000). "p300 mediates functional synergism between AF-1 and AF-2 of estrogen receptor α and β by interacting directly with the N-terminal A/B domains." Journal of Biological Chemistry **275**(21): 15645-15651.

Kouros-Mehr, H., E. M. Slorach, M. D. Sternlicht and Z. Werb (2006). "GATA-3 maintains the differentiation of the luminal cell fate in the mammary gland." Cell **127**(5): 1041-1055.

Kraus, W. and B. Katzenellenbogen (1993). "Regulation of progesterone receptor gene expression and growth in the rat uterus: modulation of estrogen actions by progesterone and sex steroid hormone antagonists." Endocrinology **132**(6): 2371-2379.

Kraus, W. L. and J. T. Kadonaga (1998). "p300 and estrogen receptor cooperatively activate transcription via differential enhancement of initiation and reinitiation." Genes & development **12**(3): 331-342.

Krege, J. H., J. B. Hodgin, J. F. Couse, E. Enmark, M. Warner, J. F. Mahler, M. Sar, K. S. Korach, J.-Å. Gustafsson and O. Smithies (1998). "Generation and reproductive phenotypes of mice lacking estrogen receptor β ." Proceedings of the National Academy of Sciences USA **95**(26): 15677-15682.

Kronenberg, M. S. and J. H. Clark (1985). "Changes in keratin expression during the estrogen-mediated differentiation of rat vaginal epithelium." Endocrinology **117**(4): 1480-1489.

Kuiper (1997). "Carlsson B. Grandien K. Enmark E. Häggblad J. Nilsson S. Gustafsson JA. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta." Endocrinology **138**(3): 863-870.

Kuiper, G., E. Enmark, M. Peltö-Huikko, S. Nilsson and J.-A. Gustafsson (1996). "Cloning of a novel receptor expressed in rat prostate and ovary." Proceedings of the National Academy of Sciences USA **93**(12): 5925-5930.

Kuiper, G. G., J. G. Lemmen, B. Carlsson, J. C. Corton, S. H. Safe, P. T. van der Saag, B. van der Burg and J.-Å. Gustafsson (1998). "Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β ." Endocrinology **139**(10): 4252-4263.

Kumle, M., E. Weiderpass, T. Braaten, I. Persson, H.-O. Adami and E. Lund (2002). "Use of Oral Contraceptives and Breast Cancer Risk The Norwegian-Swedish Women's Lifestyle and Health Cohort Study." Cancer Epidemiology Biomarkers & Prevention **11**(11): 1375-1381.

Kushner, P. J., D. A. Agard, G. L. Greene, T. S. Scanlan, A. K. Shiau, R. M. Uht and P. Webb (2000). "Estrogen receptor pathways to AP-1." The Journal of Steroid Biochemistry and Molecular Biology **74**(5): 311-317.

LaCroix, A. Z., T. Powles, C. K. Osborne, K. Wolter, J. R. Thompson, D. D. Thompson, D. C. Allred, R. Armstrong, S. R. Cummings and R. Eastell (2010). "Breast cancer

incidence in the randomized PEARL trial of lasofoxifene in postmenopausal osteoporotic women." Journal of the National Cancer Institute **102**(22): 1706-1715.

Laherty, C. D., A. N. Billin, R. M. Lavinsky, G. S. Yochum, A. C. Bush, J.-M. Sun, T.-M. Mullen, J. R. Davie, D. W. Rose and C. K. Glass (1998). "SAP30, a component of the mSin3 corepressor complex involved in N-CoR-mediated repression by specific transcription factors." Molecular Cell **2**(1): 33-42.

Lannigan, D. A. (2003). "Estrogen receptor phosphorylation." Steroids **68**(1): 1-9.

Larner, J. M. and R. B. Hochberg (1985). "The clearance and metabolism of estradiol and estradiol-17-esters in the rat." Endocrinology **117**(3): 1209-1214.

Lavinsky, R. M., K. Jepsen, T. Heinzel, J. Torchia, T.-M. Mullen, R. Schiff, A. L. Del-Rio, M. Ricote, S. Ngo and J. Gemsch (1998). "Diverse signaling pathways modulate nuclear receptor recruitment of N-CoR and SMRT complexes." Proceedings of the National Academy of Sciences USA **95**(6): 2920-2925.

Lazari, M. F. M., T. F. G. Lucas, F. Yasuhara, G. R. O. Gomes, E. R. Siu, C. Royer, S. A. F. Fernandes and C. S. Porto (2009). "Estrogen receptors and function in the male reproductive system." Arquivos Brasileiros de Endocrinologia & Metabologia **53**(8): 923-933.

Lazennec, G., D. Bresson, A. Lucas, C. Chauveau and F. Vignon (2001). "ER beta inhibits proliferation and invasion of breast cancer cells." Endocrinology **142**(9): 4120-4130.

Lazennec, G., D. Bresson, A. Lucas, C. Chauveau and F. Vignon (2001). "ER β inhibits proliferation and invasion of breast cancer cells." Endocrinology **142**(9): 4120-4130.

Lecce, G., G. Meduri, M. ANCELIN, C. BERGERON and M. PERROT-APPLANAT (2001). "Presence of estrogen receptor β in the human endometrium through the cycle: expression in glandular, stromal, and vascular cells." Journal of Clinical Endocrinology & Metabolism **86**(3): 1379-1386.

Lee, K.-H., C. Finnigan-Bunick, J. Bahr and D. Bunick (2001). "Estrogen regulation of ion transporter messenger RNA levels in mouse efferent ductules are mediated differentially through estrogen receptor ER α and ER β ." Biology of Reproduction **65**(5): 1534-1541.

Leers, J., E. Treuter and J.-Å. Gustafsson (1998). "Mechanistic principles in NR box-dependent interaction between nuclear hormone receptors and the coactivator TIF2." Molecular and Cellular Biology **18**(10): 6001-6013.

Leese, H. (1988). "The formation and function of oviduct fluid." Journal of Reproduction and Fertility **82**(2): 843-856.

Lemmen, J. G., J. L. Broekhof, G. G. Kuiper, J.-Å. Gustafsson, P. T. van der Saag and B. van der Burg (1999). "Expression of estrogen receptor alpha and beta during mouse embryogenesis." Mechanisms of Development **81**(1): 163-167.

Leon, R. L., J. D. Huber and C. L. Rosen (2011). "Potential age-dependent effects of estrogen on neural injury." The American Journal of Pathology **178**(6): 2450-2460.

Lewis-Wambi, J. S. and V. C. Jordan (2009). "Estrogen regulation of apoptosis: how can one hormone stimulate and inhibit." Breast Cancer Research **11**(3): 206.

Li, S. (1994). "Relationship between cellular DNA synthesis, PCNA expression and sex steroid hormone receptor status in the developing mouse ovary, uterus and oviduct." Histochemistry **102**(5): 405-413.

Liu, H., K. Liu and D. L. Bodenner (2005). "Estrogen receptor inhibits interleukin-6 gene expression by disruption of nuclear factor κ B transactivation." Cytokine **31**(4): 251-257.

Liu, M.-M., C. Albanese, C. M. Anderson, K. Hilty, P. Webb, R. M. Uht, R. H. Price, R. G. Pestell and P. J. Kushner (2002). "Opposing action of estrogen receptors α and β on cyclin D1 gene expression." Journal of Biological Chemistry **277**(27): 24353-24360.

Liu, M. M., C. Albanese, C. M. Anderson, K. Hilty, P. Webb, R. M. Uht, R. H. Price, Jr., R. G. Pestell and P. J. Kushner (2002). "Opposing action of estrogen receptors alpha and beta on cyclin D1 gene expression." The Journal of Biological Chemistry **277**(27): 24353-24360.

Liu, S., G. Dontu, I. D. Mantle, S. Patel, N.-s. Ahn, K. W. Jackson, P. Suri and M. S. Wicha (2006). "Hedgehog signaling and Bmi-1 regulate self-renewal of normal and malignant human mammary stem cells." Cancer Research **66**(12): 6063-6071.

Liu, S. and F. Mauvais-Jarvis (2009). "Rapid, nongenomic estrogen actions protect pancreatic islet survival." Islets **1**(3): 273-275.

Love, R. R., R. B. Mazess, H. S. Barden, S. Epstein, P. A. Newcomb, V. C. Jordan, P. P. Carbone and D. L. DeMets (1992). "Effects of tamoxifen on bone mineral density in postmenopausal women with breast cancer." New England Journal of Medicine **326**(13): 852-856.

Lubahn, D. B., J. S. Moyer, T. S. Golding, J. F. Couse, K. S. Korach and O. Smithies (1993). "Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene." Proceedings of the National Academy of Sciences USA **90**(23): 11162-11166.

Lucas, T. F., E. R. Siu, C. A. Esteves, H. P. Monteiro, C. A. Oliveira, C. S. Porto and M. F. M. Lazari (2008). "17beta-estradiol induces the translocation of the estrogen receptors ESR1 and ESR2 to the cell membrane, MAPK3/1 phosphorylation and proliferation of cultured immature rat Sertoli cells." Biology of Reproduction **78**(1): 101-114.

Ma, Z., S. Santagati, C. Patrone, G. Pollio, E. Vegeto and A. Maggi (1994). "Insulin-like growth factors activate estrogen receptor to control the growth and differentiation of the human neuroblastoma cell line SK-ER3." Molecular Endocrinology **8**(7): 910-918.

MacCalman, C. D., S. Getsios, R. Farookhi and O. W. Blaschuk (1997). "Estrogens potentiate the stimulatory effects of follicle-stimulating hormone on N-cadherin messenger ribonucleic acid levels in cultured mouse Sertoli cells." Endocrinology **138**(1): 41-48.

Macgregor, J. I. and V. C. Jordan (1998). "Basic guide to the mechanisms of antiestrogen action." Pharmacological Reviews **50**(2): 151-196.

Mahato, D., E. H. Goulding, K. S. Korach and E. M. Eddy (2000). "Spermatogenic cells do not require estrogen receptor α for development or function." Endocrinology **141**(3): 1273-1273.

Maki, D. D. and R. I. Grossman (2000). "Patterns of disease spread in metastatic breast carcinoma: influence of estrogen and progesterone receptor status." American Journal of Neuroradiology **21**(6): 1064-1066.

Mallepell, S., A. Krust, P. Chambon and C. Brisken (2006). "Paracrine signaling through the epithelial estrogen receptor alpha is required for proliferation and morphogenesis in the mammary gland." Proceedings of the National Academy of Sciences USA **103**(7): 2196-2201.

Mallepell, S., A. Krust, P. Chambon and C. Brisken (2006). "Paracrine signaling through the epithelial estrogen receptor α is required for proliferation and morphogenesis in the mammary gland." Proceedings of the National Academy of Sciences USA **103**(7): 2196-2201.

Manolagas, S. C. and R. L. Jilka (1995). "Bone marrow, cytokines, and bone remodeling. Emerging insights into the pathophysiology of osteoporosis." The New England Journal of Medicine **332**(5): 305.

Marchbanks, P. A., K. M. Curtis, M. G. Mandel, H. G. Wilson, G. Jeng, S. G. Folger, J. A. McDonald, J. R. Daling, L. Bernstein and K. E. Malone (2012). "Oral contraceptive formulation and risk of breast cancer." Contraception **85**(4): 342-350.

Marieb, E. N. and K. Hoehn (2007). Human anatomy & physiology, Pearson Education.

Marsh, D. A., H. J. Brodie, W. Garrett, C. H. Tsai-Morris and A. M. Brodie (1985). "Aromatase inhibitors. Synthesis and biological activity of androstenedione derivatives." Journal of Medicinal Chemistry **28**(6): 788-795.

Martin, L.-A. and M. Dowsett (2013). "BCL-2: A New Therapeutic Target in Estrogen Receptor-Positive Breast Cancer?" Cancer Cell **24**(1): 7-9.

Martin, L., C. Finn and G. Trinder (1973). "Hypertrophy and hyperplasia in the mouse uterus after oestrogen treatment: an autoradiographic study." Journal of Endocrinology **56**(1): 133-NP.

Martineti, V., L. Picariello, I. Tognarini, S. C. Sala, A. Gozzini, C. Azzari, C. Mavilia, A. Tanini, A. Falchetti and G. Fiorelli (2005). "ER β is a potent inhibitor of cell proliferation in the HCT8 human colon cancer cell line through regulation of cell cycle components." Endocrine-related Cancer **12**(2): 455-469.

Marttunen, M. B., P. Hietanen, A. Tiitinen and O. Ylikorkala (1998). "Comparison of effects of tamoxifen and toremifene on bone biochemistry and bone mineral density in postmenopausal breast cancer patients." Journal of Clinical Endocrinology & Metabolism **83**(4): 1158.

Martucci, C. P. and J. Fishman (1993). "P450 enzymes of estrogen metabolism." Pharmacology & Therapeutics **57**(2): 237-257.

Massaro, D., L. B. Clerch and G. D. Massaro (2007). "Estrogen receptor- α regulates pulmonary alveolar loss and regeneration in female mice: morphometric and gene expression studies." American Journal of Physiology-Lung Cellular and Molecular Physiology **293**(1): L222-L228.

Massaro, G. D., J. P. Mortola and D. Massaro (1996). "Estrogen modulates the dimensions of the lung's gas-exchange surface area and alveoli in female rats." American Journal of Physiology-Lung Cellular and Molecular Physiology **270**(1): L110-L114.

Matsuda, S., Y. Kadowaki, M. Ichino, T. Akiyama, K. Toyoshima and T. Yamamoto (1993). "17 beta-estradiol mimics ligand activity of the c-erbB2 protooncogene product." Proceedings of the National Academy of Sciences USA **90**(22): 10803-10807.

Matsuda, T., T. Yamamoto, A. Muraguchi and F. Saatcioglu (2001). "Cross-talk between transforming growth factor- β and estrogen receptor signaling through Smad3." Journal of Biological Chemistry **276**(46): 42908-42914.

Matsuzaki, S., T. Fukaya, T. Suzuki, T. Murakami, H. Sasano and A. Yajima (1999). "Oestrogen receptor α and β mRNA expression in human endometrium throughout the menstrual cycle." Molecular Human Reproduction **5**(6): 559-564.

Mawson, A., A. Lai, J. S. Carroll, C. M. Sergio, C. J. Mitchell and B. Sarcevic (2005). "Estrogen and insulin/IGF-1 cooperatively stimulate cell cycle progression in MCF-7 breast cancer cells through differential regulation of c-Myc and cyclin D1." Molecular and Cellular Endocrinology **229**(1): 161-173.

Mazumdar, A., R.-A. Wang, S. K. Mishra, L. Adam, R. Bagheri-Yarmand, M. Mandal, R. K. Vadlamudi and R. Kumar (2000). "Transcriptional repression of oestrogen receptor by metastasis-associated protein 1 corepressor." Nature Cell Biology **3**(1): 30-37.

McDonnell, D. P., D. L. Clemm, T. Hermann, M. E. Goldman and J. Pike (1995). "Analysis of estrogen receptor function in vitro reveals three distinct classes of antiestrogens." Molecular Endocrinology **9**(6): 659-669.

McEwen, B. S. and S. E. Alves (1999). "Estrogen actions in the central nervous system." Endocrine Reviews **20**(3): 279-307.

McInerney, E. M., M.-J. Tsai, B. W. O'Malley and B. S. Katzenellenbogen (1996). "Analysis of estrogen receptor transcriptional enhancement by a nuclear hormone receptor coactivator." Proceedings of the National Academy of Sciences USA **93**(19): 10069-10073.

McKenna, N. J., R. B. Lanz and B. W. O'Malley (1999). "Nuclear receptor coregulators: cellular and molecular biology." Endocrine Reviews **20**(3): 321-344.

McKinnell, C., N. Atanassova, K. Williams, J. Fisher, M. Walker, K. Turner, P. Saunders and R. Sharpe (2001). "Suppression of androgen action and the induction of gross abnormalities of the reproductive tract in male rats treated neonatally with diethylstilbestrol." Journal of Andrology **22**(2): 323-338.

Meisel, R. and B. Sachs (1994). "The physiology of male sexual behavior." The Physiology of Reproduction **2**: 3-105.

Meistrich, M., T. Hughes and W. Bruce (1975). "Alteration of epididymal sperm transport and maturation in mice by oestrogen and testosterone."

Meyers, M. J., J. Sun, K. E. Carlson, G. A. Marriner, B. S. Katzenellenbogen and J. A. Katzenellenbogen (2001). "Estrogen receptor-beta potency-selective ligands: structure-activity relationship studies of diarylpropionitriles and their acetylene and polar analogues." Journal of Medicinal Chemistry **44**(24): 4230-4251.

Migliaccio, A., G. Castoria, M. Di Domenico, A. de Falco, A. Bilancio, M. Lombardi, M. V. Barone, D. Ametrano, M. S. Zannini and C. Abbondanza (2000). "Steroid-induced androgen receptor–oestradiol receptor β –Src complex triggers prostate cancer cell proliferation." The EMBO journal **19**(20): 5406-5417.

Migliaccio, A., M. Di Domenico, G. Castoria, A. De Falco, P. Bontempo, E. Nola and F. Auricchio (1996). "Tyrosine kinase/p21ras/MAP-kinase pathway activation by estradiol-receptor complex in MCF-7 cells." The EMBO journal **15**(6): 1292.

Millier, S. G., P. F. Whitelaw and C. D. Smyth (1994). "Follicular oestrogen synthesis: the 'two-cell, two-gonadotrophin' model revisited." Molecular and Cellular Endocrinology **100**(1): 51-54.

Mollerup, S., K. Jørgensen, G. Berge and A. Haugen (2002). "Expression of estrogen receptors α and β in human lung tissue and cell lines." Lung Cancer **37**(2): 153-159.

Mooradian, A. D. (1993). "Antioxidant properties of steroids." The Journal of Steroid Biochemistry and Molecular Biology **45**(6): 509-511.

Morani, A., M. Warner and J. Å. Gustafsson (2008). "Biological functions and clinical implications of oestrogen receptors alfa and beta in epithelial tissues." Journal of Internal Medicine **264**(2): 128-142.

Mosher, W. D., G. M. Martinez, A. Chandra, J. C. Abma and S. J. Willson (2004). Use of contraception and use of family planning services in the United States, 1982-2002, US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics.

Mosselman, S., J. Polman and R. Dijkema (1996). "ER β : identification and characterization of a novel human estrogen receptor." FEBS letters **392**(1): 49-53.

Motrich, R. D., A. A. Ponce and V. E. Rivero (2007). "Effect of tamoxifen treatment on the semen quality and fertility of the male rat." Fertility and Sterility **88**(2): 452-461.

Mowa, C. and T. Iwanaga (2000). "Developmental changes of the oestrogen receptor-alpha and-beta mRNAs in the female reproductive organ of the rat--an analysis by in situ hybridization." Journal of Endocrinology **167**(3): 363-369.

Mulac-Jericevic, B., J. P. Lydon, F. J. DeMayo and O. M. Conneely (2003). "Defective mammary gland morphogenesis in mice lacking the progesterone receptor B isoform." Proceedings of the National Academy of Sciences USA **100**(17): 9744-9749.

Musey, P., K. Wright, J. Preedy and D. Collins (1997). "Formation and metabolism of steroid conjugates: effect of conjugation on excretion and tissue distribution." Steroid Biochemistry **2**: 81-132.

Musgrove, E. A., C. Lee, M. F. Buckley and R. L. Sutherland (1994). "Cyclin D1 induction in breast cancer cells shortens G1 and is sufficient for cells arrested in G1 to complete the cell cycle." Proceedings of the National Academy of Sciences USA **91**(17): 8022-8026.

Nabholtz, J., A. Buzdar, M. Pollak, W. Harwin, G. Burton, A. Mangalik, M. Steinberg, A. Webster and M. Von Euler (2000). "Anastrozole is superior to tamoxifen as first-line therapy for advanced breast cancer in postmenopausal women: results of a North American multicenter randomized trial." Journal of Clinical Oncology **18**(22): 3758-3767.

Nelson, H. D., B. Zakher, A. Cantor, R. Fu, J. Griffin, E. S. O'Meara, D. S. Buist, K. Kerlikowske, N. T. van Ravesteyn and A. Trentham-Dietz (2012). "Risk Factors for Breast Cancer for Women Aged 40 to 49 Years A Systematic Review and Meta-analysis." Annals of Internal Medicine **156**(9): 635-648.

Nelson, L. R. and S. E. Bulun (2001). "Estrogen production and action." Journal of the American Academy of Dermatology **45**(3): S116-S124.

Nephew, K. P., X. Long, E. Osborne, K. A. Burke, A. Ahluwalia and R. M. Bigsby (2000). "Effect of estradiol on estrogen receptor expression in rat uterine cell types." Biology of Reproduction **62**(1): 168-177.

Newcomb, P. A. and B. E. Storer (1995). "Postmenopausal hormone use and risk of large-bowel cancer." Journal of the National Cancer Institute **87**(14): 1067-1071.

Newton, C. J., R. Buric, T. Trapp, S. Brockmeier, U. Pagotto and G. K. Stalla (1994). "The unliganded estrogen receptor (ER) transduces growth factor signals." The Journal of steroid biochemistry and molecular biology **48**(5): 481-486.

Nilsson, O. and S. Reinius (1969). "Light and electron microscopic structure of the oviduct." The Mammalian Oviduct: 57-84.

Nilsson, S., S. Makela, E. Treuter, M. Tujague, J. Thomsen, G. Andersson, E. Enmark, K. Pettersson, M. Warner and J. A. Gustafsson (2001). "Mechanisms of estrogen action." Physiological Reviews **81**(4): 1535-1565.

Norris, J. D., D. Fan, A. Sherk and D. P. McDonnell (2002). "A negative coregulator for the human ER." Molecular Endocrinology **16**(3): 459-468.

Nunno, L. D., L. G. Larsson, J. J. Rinehart and R. S. Beissner (2000). "Estrogen and progesterone receptors in non-small cell lung cancer in 248 consecutive patients who underwent surgical resection." Archives of Pathology & Laboratory Medicine **124**(10): 1467-1470.

O'donnell, L., K. M. Robertson, M. E. Jones and E. R. Simpson (2001). "Estrogen and spermatogenesis." Endocrine Reviews **22**(3): 289-318.

Ogawa, S., S. Inoue, T. Watanabe, H. Hiroi, A. Orimo, T. Hosoi, Y. Ouchi and M. Muramatsu (1998). "The Complete Primary Structure of Human Estrogen Receptor β

(hER β) and Its Heterodimerization with ER α in Vivo and in Vitro." Biochemical and Biophysical Research Communications **243**(1): 122-126.

Ojeda, S. and S. McCann (1974). "Development of dopaminergic and estrogenic control of prolactin release in the female rat." Endocrinology **95**(6): 1499-1505.

Oliveira, C. A., G. A. Mahecha, K. Carnes, G. S. Prins, P. T. Saunders, L. R. França and R. A. Hess (2004). "Differential hormonal regulation of estrogen receptors ER α and ER β and androgen receptor expression in rat efferent ductules." Reproduction **128**(1): 73-86.

Oliveira, C. A., Q. Zhou, K. Carnes, R. Nie, D. E. Kuehl, G. L. Jackson, L. R. Franca, M. Nakai and R. A. Hess (2002). "ER function in the adult male rat: short-and long-term effects of the antiestrogen ICI 182,780 on the testis and efferent ductules, without changes in testosterone." Endocrinology **143**(6): 2399-2409.

Ollayos, C., G. Riordan and J. Rushin (1994). "Estrogen receptor detection in paraffin sections of adenocarcinoma of the colon, pancreas, and lung." Archives of Pathology & Laboratory Medicine **118**(6): 630-632.

Omoto, Y., Y. Kobayashi, K. Nishida, E. Tsuchiya, H. Eguchi, K. Nakagawa, Y. Ishikawa, T. Yamori, H. Iwase and Y. Fujii (2001). "Expression, function, and clinical implications of the estrogen receptor β in human lung cancers." Biochemical and Biophysical Research Communications **285**(2): 340-347.

Onate, S. A., V. Boonyaratanakornkit, T. E. Spencer, S. Y. Tsai, M.-J. Tsai, D. P. Edwards and B. W. O'Malley (1998). "The steroid receptor coactivator-1 contains multiple receptor interacting and activation domains that cooperatively enhance the activation function 1 (AF1) and AF2 domains of steroid receptors." Journal of Biological Chemistry **273**(20): 12101-12108.

Overpeck, J. G., S. H. Colson, J. R. Hohmann, M. S. Applestine and J. F. Reilly (1978). "Concentrations of circulating steroids in normal prepubertal and adult male and female humans, chimpanzees, rhesus monkeys, rats, mice, and hamsters: a literature survey." Journal of Toxicology and Environmental Health, Part A Current Issues **4**(5-6): 785-803.

Palijan, A., I. Fernandes, M. Verway, M. Kourelis, Y. Bastien, L. E. Tavera-Mendoza, A. Sacheli, V. Bourdeau, S. Mader and J. H. White (2009). "Ligand-dependent corepressor LCoR is an attenuator of progesterone-regulated gene expression." Journal of Biological Chemistry **284**(44): 30275-30287.

Palmieri, C., G. Cheng, S. Saji, M. Zelada-Hedman, Z. Weihua, S. Van Noorden, T. Wahlstrom, R. Coombes, M. Warner and J. Gustafsson (2002). "Estrogen receptor beta in breast cancer." Endocrine-related Cancer **9**(1): 1-13.

Parakkal, P. F. (1974). "Cyclical changes in the vaginal epithelium of the rat seen by scanning electron microscopy." Anatomical Record **178**(3): 529-537.

Parakkal, P. F. and A. Gregoire (1972). "Differentiation of vaginal epithelium in the normal and hormone-treated rhesus monkey." Biology of Reproduction **6**(1): 117-130.

Paruthiyil, S., H. Parmar, V. Kerekatte, G. R. Cunha, G. L. Firestone and D. C. Leitman (2004). "Estrogen receptor beta inhibits human breast cancer cell proliferation and tumor formation by causing a G2 cell cycle arrest." Cancer Research **64**(1): 423-428.

Paruthiyil, S., H. Parmar, V. Kerekatte, G. R. Cunha, G. L. Firestone and D. C. Leitman (2004). "Estrogen receptor β inhibits human breast cancer cell proliferation and tumor formation by causing a G2 cell cycle arrest." Cancer Research **64**(1): 423-428.

Pascoe, P. A. (1996). *Endocrinology*: By Mac E. Hadley. Upper Saddle River, NJ, Prentice Hall, 1996, \$80.00 (xiii+ 518 Pages), ISBN 0-13-317926-5, Elsevier Current Trends.

Patrone, C., T. N. Cassel, K. Pettersson, Y.-S. Piao, G. Cheng, P. Ciana, A. Maggi, M. Warner, J.-Å. Gustafsson and M. Nord (2003). "Regulation of postnatal lung development and homeostasis by estrogen receptor β ." Molecular and Cellular Biology **23**(23): 8542-8552.

Pelletier, G. and M. El-Alfy (2000). "Immunocytochemical localization of estrogen receptors α and β in the human reproductive organs." Journal of Clinical Endocrinology & Metabolism **85**(12): 4835-4840.

Pentikäinen, V., K. Erkkilä, L. Suomalainen, M. Parvinen and L. Dunkel (2000). "Estradiol acts as a germ cell survival factor in the human testis in vitro." Journal of Clinical Endocrinology & Metabolism **85**(5): 2057-2067.

Persson, I., E. Weiderpass, L. Bergkvist, R. Bergstrom and C. Schairer (1999). "Risks of breast and endometrial cancer after estrogen and estrogen-progestin replacement." Cancer Causes Control **10**(4): 253-260.

Pettersson, K., K. Grandien, G. G. Kuiper and J.-Å. Gustafsson (1997). "Mouse estrogen receptor β forms estrogen response element-binding heterodimers with estrogen receptor α ." Molecular Endocrinology **11**(10): 1486-1496.

Pettersson, K. and J.-Å. Gustafsson (2001). "Role of estrogen receptor beta in estrogen action." Annual Review of Physiology **63**(1): 165-192.

Pezzi, V., J. Mathis, W. E. Rainey and B. R. Carr (2003). "Profiling transcript levels for steroidogenic enzymes in fetal tissues." The Journal of Steroid Biochemistry and Molecular Biology **87**(2): 181-189.

Pfaff, D., S. Schwartz-Giblin, M. McCarthy, L. Kow, E. Knobil and J. Neill (1994). Physiology of reproduction.

Pietras, R. J., D. C. Márquez, H.-W. Chen, E. Tsai, O. Weinberg and M. Fishbein (2005). "Estrogen and growth factor receptor interactions in human breast and non-small cell lung cancer cells." Steroids **70**(5): 372-381.

Platet, N., S. Cunat, D. Chalbos, H. Rochefort and M. Garcia (2000). "Unliganded and liganded estrogen receptors protect against cancer invasion via different mechanisms." Molecular Endocrinology **14**(7): 999-1009.

- Power, R. F., S. K. Mani, J. Codina, O. M. Conneely and B. W. O'Malley (1991). "Dopaminergic and ligand-independent activation of steroid hormone receptors." Science **254**(5038): 1636-1639.
- Pragnell, M., K. Snay, J. Trimmer, N. MacLusky, F. Naftolin, L. Kaczmarek and M. Boyle (1990). "Estrogen induction of a small, putative K⁺ channel mRNA in rat uterus." Neuron **4**(5): 807-812.
- Prall, O. W., B. Sarcevic, E. A. Musgrove, C. K. Watts and R. L. Sutherland (1997). "Estrogen-induced activation of Cdk4 and Cdk2 during G1-S phase progression is accompanied by increased cyclin D1 expression and decreased cyclin-dependent kinase inhibitor association with cyclin E-Cdk2." Journal of Biological Chemistry **272**(16): 10882-10894.
- Prescott, E., A. M. Bjerg, P. K. Andersen, P. Lange and J. Vestbo (1997). "Gender difference in smoking effects on lung function and risk of hospitalization for COPD: results from a Danish longitudinal population study." European Respiratory Journal **10**(4): 822-827.
- Pyo Kim, H., J. Young Lee, J. Kim Jeong, S. Won Bae, H. Kyu Lee and I. Jo (1999). "Nongenomic stimulation of nitric oxide release by estrogen is mediated by estrogen receptor α localized in caveolae." Biochemical and Biophysical Research Communications **263**(1): 257-262.
- Qing, Z., R. NIE, G. S. PRINS, P. T. SAUNDERS, B. S. KATZENELLENBOGEN and R. A. HESS (2002). "Localization of androgen and estrogen receptors in adult male mouse reproductive tract." Journal of Andrology **23**(6): 870-881.
- Quirk, S., R. Cowan, R. Harman, C.-L. Hu and D. Porter (2004). "Ovarian follicular growth and atresia: the relationship between cell proliferation and survival." Journal of Animal Science **82**(13 suppl): E40-E52.
- Regan, M. M., G. Viale, M. G. Mastropasqua, E. Maiorano, R. Golouh, A. Carbone, B. Brown, M. Suurkõla, G. Langman and L. Mazzucchelli (2006). "Re-evaluating adjuvant breast cancer trials: assessing hormone receptor status by immunohistochemical versus extraction assays." Journal of the National Cancer Institute **98**(21): 1571-1581.
- Ripoll, C., A. B. Ropero, P. Alonso-Magdalena, I. Quesada, E. Fuentes and A. Nadal (2008). "Rapid Regulation of Pancreatic-and-Cell Signalling Systems by Estrogens." Infectious Disorders-Drug Targets **8**(1): 61-64.
- Ritte, R., A. Lukanova, A. Tjønneland, A. Olsen, K. Overvad, S. Mesrine, G. Fagherazzi, L. Dossus, B. Teucher and K. Steindorf (2012). "Height, age at menarche and risk of hormone receptor - positive and - negative breast cancer: A cohort study." International Journal of Cancer.
- Rody, A., U. Holtrich, C. Solbach, K. Kourtis, G. Von Minckwitz, K. Engels, S. Kissler, R. Gätje, T. Karn and M. Kaufmann (2005). "Methylation of estrogen receptor β promoter

correlates with loss of ER- β expression in mammary carcinoma and is an early indication marker in premalignant lesions." Endocrine-related Cancer **12**(4): 903-916.

Roger, P., M. E. Sahla, S. Mäkelä J. Å. Gustafsson, P. Baldet and H. Rochefort (2001). "Decreased expression of estrogen receptor β protein in proliferative preinvasive mammary tumors." Cancer Research **61**(6): 2537-2541.

Rosenfeld, M. G., V. V. Lunyak and C. K. Glass (2006). "Sensors and signals: a coactivator/corepressor/epigenetic code for integrating signal-dependent programs of transcriptional response." Genes & Development **20**(11): 1405-1428.

Ross, R. K., A. Paganini-Hill, P. C. Wan and M. C. Pike (2000). "Effect of hormone replacement therapy on breast cancer risk: estrogen versus estrogen plus progestin." Journal of the National Cancer Institute **92**(4): 328-332.

Rossouw, J. E., G. L. Anderson, R. L. Prentice, A. Z. LaCroix, C. Kooperberg, M. Stefanick, R. D. Jackson, S. A. Beresford, B. V. Howard and K. C. Johnson (2002). "Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial." Journal of the American Medical Association **288**(3): 321-333.

Ruan, W., V. Catanese, R. Wiczorek, M. Feldman and D. Kleinberg (1995). "Estradiol enhances the stimulatory effect of insulin-like growth factor-I (IGF-I) on mammary development and growth hormone-induced IGF-I messenger ribonucleic acid." Endocrinology **136**(3): 1296-1302.

Russo, J., X. Ao, C. Grill and I. Russo (1999). "Pattern of distribution of cells positive for estrogen receptor α and progesterone receptor in relation to proliferating cells in the mammary gland." Breast Cancer Research and Treatment **53**(3): 217-227.

Sabourin, J., A. Martin, J. Baruch, J. Truc, A. Gompel and P. Poitout (1994). "bcl - 2 expression in normal breast tissue during the menstrual cycle." International Journal of Cancer **59**(1): 1-6.

Saji, S., E. V. Jensen, S. Nilsson, T. Rylander, M. Warner and J.-Å. Gustafsson (2000). "Estrogen receptors α and β in the rodent mammary gland." Breast Cancer Research **2**(Suppl 1): S. 11.

Santen, R. J., P. Fan, Z. Zhang, Y. Bao, R. X. Song and W. Yue (2009). "Estrogen signals via an extra-nuclear pathway involving IGF-1R and EGFR in tamoxifen-sensitive and -resistant breast cancer cells." Steroids **74**(7): 586-594.

Sar, M. and F. Welsch (1999). "Differential expression of estrogen receptor-beta and estrogen receptor-alpha in the rat ovary." Endocrinology **140**(2): 963-971.

Saville, B., M. Wormke, F. Wang, T. Nguyen, E. Enmark, G. Kuiper, J.-Å. Gustafsson and S. Safe (2000). "Ligand-, cell-, and estrogen receptor subtype (α/β)-dependent activation at GC-rich (Sp1) promoter elements." Journal of Biological Chemistry **275**(8): 5379-5387.

Schams, D., S. Kohlenberg, W. Amselgruber, B. Berisha, M. Pfaffl and F. Sinowatz (2003). "Expression and localisation of oestrogen and progesterone receptors in the bovine mammary gland during development, function and involution." Journal of Endocrinology **177**(2): 305-317.

Schramek, D., V. Sigl and J. M. Penninger (2011). "RANKL and RANK in sex hormone-induced breast cancer and breast cancer metastasis." Trends in Endocrinology & Metabolism **22**(5): 188-194.

Schwabe, J. W., L. Chapman, J. T. Finch and D. Rhodes (1993). "The crystal structure of the estrogen receptor DNA-binding domain bound to DNA: how receptors discriminate between their response elements." Cell **75**(3): 567-578.

Scully, K. M., A. S. Gleiberman, J. Lindzey, D. B. Lubahn, K. S. Korach and M. G. Rosenfeld (1997). "Role of estrogen receptor- α in the anterior pituitary gland." Molecular Endocrinology **11**(6): 674-681.

Shang, Y. and M. Brown (2002). "Molecular determinants for the tissue specificity of SERMs." Science **295**(5564): 2465-2468.

Shao, R., E. Egecioglu, B. Weijdegård, J. J. Kopchick, J. Fernandez-Rodriguez, N. Andersson and H. Billig (2007). "Dynamic regulation of estrogen receptor- α isoform expression in the mouse fallopian tube: mechanistic insight into estrogen-dependent production and secretion of insulin-like growth factors." American Journal of Physiology-Endocrinology And Metabolism **293**(5): E1430-E1442.

Shao, R., M. Nutu, L. Karlsson-Lindahl, A. Benrick, B. Weijdegård, S. Lager, E. Egecioglu, J. Fernandez-Rodriguez, K. Gemzell-Danielsson and C. Ohlsson (2009). "Downregulation of cilia-localized $\text{Il-6R}\alpha$ by 17β -estradiol in mouse and human fallopian tubes." American Journal of Physiology-Cell Physiology **297**(1): C140-C151.

Shao, R., B. Weijdegård, J. Fernandez-Rodriguez, E. Egecioglu, C. Zhu, N. Andersson, A. Thurin-Kjellberg, C. Bergh and H. Billig (2007). "Ciliated epithelial-specific and regional-specific expression and regulation of the estrogen receptor- β 2 in the fallopian tubes of immature rats: a possible mechanism for estrogen-mediated transport process in vivo." American Journal of Physiology-Endocrinology And Metabolism **293**(1): E147-E158.

Shayu, D., C. Kesava, R. Soundarajan and A. J. Rao (2005). "Effects of ICI 182780 on estrogen receptor expression, fluid absorption and sperm motility in the epididymis of the bonnet monkey." Reproductive Biology and Endocrinology **3**(10).

Sherwood, O., E. Knobil and J. Neill (1994). "The physiology of reproduction." Relaxin, 2nd ed, Raven Press, New York: 861-1009.

Shevde, N. K., A. C. Bendixen, K. M. Dienger and J. Pike (2000). "Estrogens suppress RANK ligand-induced osteoclast differentiation via a stromal cell independent mechanism involving c-Jun repression." Proceedings of the National Academy of Sciences USA **97**(14): 7829-7834.

Shi, Y., M. Downes, W. Xie, H.-Y. Kao, P. Ordentlich, C.-C. Tsai, M. Hon and R. M. Evans (2001). "Sharp, an inducible cofactor that integrates nuclear receptor repression and activation." Genes & Development **15**(9): 1140-1151.

Shiau, A. K., D. Barstad, P. M. Loria, L. Cheng, P. J. Kushner, D. A. Agard and G. L. Greene (1998). "The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen." Cell **95**(7): 927-937.

Shoker, B. S., C. Jarvis, R. B. Clarke, E. Anderson, J. Hewlett, M. Davies, D. R. Sibson and J. P. Sloane (1999). "Estrogen receptor-positive proliferating cells in the normal and precancerous breast." The American Journal of Pathology **155**(6): 1811-1815.

Shyamala, G., Y.-C. Chou, S. Louie, R. Guzman, G. Smith and S. Nandi (2002). "Cellular expression of estrogen and progesterone receptors in mammary glands: regulation by hormones, development and aging." The Journal of Steroid Biochemistry and Molecular Biology **80**(2): 137-148.

Sicinski, P. and R. A. Weinberg (1997). "A specific role for cyclin D1 in mammary gland development." Journal of Mammary Gland Biology and Neoplasia **2**(4): 335-342.

Siiteri, P. K. and P. C. MacDonald (1966). "Placental estrogen biosynthesis during human pregnancy." Journal of Clinical Endocrinology & Metabolism **26**(7): 751-761.

Silberstein, G., K. Van Horn, G. Shyamala and C. Daniel (1994). "Essential role of endogenous estrogen in directly stimulating mammary growth demonstrated by implants containing pure antiestrogens." Endocrinology **134**(1): 84-90.

Silberstein, G. B. (2001). "Postnatal mammary gland morphogenesis." Microscopy Research and Technique **52**(2): 155-162.

Simoncini, T., P. Mannella, L. Fornari, A. Caruso, G. Varone and A. R. Genazzani (2004). "Genomic and non-genomic effects of estrogens on endothelial cells." Steroids **69**(8): 537-542.

Simpson, J. F., D. E. Quan, F. O'Malley, T. Odom-Maryon and P. E. Clarke (1997). "Amplification of CCND1 and expression of its protein product, cyclin D1, in ductal carcinoma in situ of the breast." The American Journal of Pathology **151**(1): 161.

Sinha, Y. and H. A. Tucker (1969). "Mammary development and pituitary prolactin level of heifers from birth through puberty and during the estrous cycle." Journal of Dairy Science **52**(4): 507-512.

Siwko, S. K., J. Dong, M. T. Lewis, H. Liu, S. G. Hilsenbeck and Y. Li (2008). "Evidence That an Early Pregnancy Causes a Persistent Decrease in the Number of Functional Mammary Epithelial Stem Cells—Implications for Pregnancy - Induced Protection Against Breast Cancer." Stem Cells **26**(12): 3205-3209.

Smith, C. L., O. M. Conneely and B. W. O'Malley (1993). "Modulation of the ligand-independent activation of the human estrogen receptor by hormone and antihormone." Proceedings of the National Academy of Sciences USA **90**(13): 6120-6124.

- Snedeker, S. M., C. F. Brown and R. P. DiAugustine (1991). "Expression and functional properties of transforming growth factor alpha and epidermal growth factor during mouse mammary gland ductal morphogenesis." Proceedings of the National Academy of Sciences USA **88**(1): 276-280.
- Song, X. and Z.-Z. Pan (2012). "Estrogen receptor-beta agonist diarylpropionitrile counteracts the estrogenic activity of estrogen receptor-alpha agonist propylpyrazole-triol in the mammary gland of ovariectomized Sprague Dawley rats." The Journal of Steroid Biochemistry and Molecular Biology **130**(1-2): 26-35.
- Speroff, L. and P. D. Darney (2010). Clinical guide for contraception, Lippincott Williams & Wilkins.
- Spyridopoulos, I., A. B. Sullivan, M. Kearney, J. M. Isner and D. W. Losordo (1997). "Estrogen-receptor-mediated inhibition of human endothelial cell apoptosis: estradiol as a survival factor." Circulation **95**(6): 1505-1514.
- Srivastava, S., G. Toraldo, M. N. Weitzmann, S. Cenci, F. P. Ross and R. Pacifici (2001). "Estrogen decreases osteoclast formation by down-regulating receptor activator of NF-kappa B ligand (RANKL)-induced JNK activation." Journal of Biological Chemistry **276**(12): 8836-8840.
- Stabile, L. P., A. L. G. Davis, C. T. Gubish, T. M. Hopkins, J. D. Luketich, N. Christie, S. Finkelstein and J. M. Siegfried (2002). "Human non-small cell lung tumors and cells derived from normal lung express both estrogen receptor α and β and show biological responses to estrogen." Cancer Research **62**(7): 2141-2150.
- Stabile, L. P. and J. M. Siegfried (2004). "Estrogen receptor pathways in lung cancer." Current Oncology Reports **6**: 259-267.
- Stauffer, S. R., C. J. Coletta, R. Tedesco, G. Nishiguchi, K. Carlson, J. Sun, B. S. Katzenellenbogen and J. A. Katzenellenbogen (2000). "Pyrazole ligands: structure-affinity/activity relationships and estrogen receptor-alpha-selective agonists." Journal of Medicinal Chemistry **43**(26): 4934-4947.
- Steeg, P. S. and Q. Zhou (1998). Cyclins and breast cancer. Prognostic variables in node-negative and node-positive breast cancer, Springer: 107-118.
- Ström, A., J. Hartman, J. S. Foster, S. Kietz, J. Wimalasena and J.-Å. Gustafsson (2004). "Estrogen receptor β inhibits 17 β -estradiol-stimulated proliferation of the breast cancer cell line T47D." Proceedings of the National Academy of Sciences USA **101**(6): 1566-1571.
- Strauss, L., J. Kallio, N. Desai, P. Pakarinen, T. Miettinen, H. Gylling, M. Albrecht, S. Mäkelä, A. Mayerhofer and M. Poutanen (2009). "Increased exposure to estrogens disturbs maturation, steroidogenesis, and cholesterol homeostasis via estrogen receptor α in adult mouse Leydig cells." Endocrinology **150**(6): 2865-2872.
- Strom, A., J. Hartman, J. S. Foster, S. Kietz, J. Wimalasena and J. A. Gustafsson (2004). "Estrogen receptor beta inhibits 17beta-estradiol-stimulated proliferation of the breast

cancer cell line T47D." Proceedings of the National Academy of Sciences USA **101**(6): 1566-1571.

Sun, G., W. Porter and S. Safe (1998). "Estrogen-induced retinoic acid receptor $\alpha 1$ gene expression: role of estrogen receptor-Sp1 complex." Molecular Endocrinology **12**(6): 882-890.

Sun, M., J. E. Paciga, R. I. Feldman, Z.-q. Yuan, D. Coppola, Y. Y. Lu, S. A. Shelley, S. V. Nicosia and J. Q. Cheng (2001). "Phosphatidylinositol-3-OH kinase (PI3K)/AKT2, activated in breast cancer, regulates and is induced by estrogen receptor α (ER α) via interaction between ER α and PI3K." Cancer Research **61**(16): 5985-5991.

Sutter, T., K. Carnevale, N. Arber and I. Weinstein (1996). "Expression of cyclins D1 and E in human colon adenocarcinomas." Journal of Medicine **28**(5-6): 285-309.

Swerdlow, A. J. and M. E. Jones (2005). "Tamoxifen treatment for breast cancer and risk of endometrial cancer: a case-control study." Journal of the National Cancer Institute **97**(5): 375-384.

Sylvia, V., B. Boyan, D. Dean and Z. Schwartz (2000). "The membrane effects of 17 β estradiol on chondrocyte phenotypic expression are mediated by activation of protein kinase C through phospholipase C and G-proteins." The Journal of Steroid Biochemistry and Molecular Biology **73**(5): 211-224.

Taioli, E. and E. L. WYNDER (1994). "Endocrine factors and adenocarcinoma of the lung in women." Journal of the National Cancer Institute **86**(11): 869-870.

Tan, H., Y. Zhong and Z. Pan (2009). "Autocrine regulation of cell proliferation by estrogen receptor-alpha in estrogen receptor-alpha-positive breast cancer cell lines." BMC Cancer **9**(1): 31.

Tan, J., B. C. Paria, S. K. Dey and S. K. Das (1999). "Differential uterine expression of estrogen and progesterone receptors correlates with uterine preparation for implantation and decidualization in the mouse." Endocrinology **140**(11): 5310-5321.

Tena-Sempere, M., L. Gonzalez, L. Pinilla, I. Huhtaniemi and E. Aguilar (2001). "Neonatal imprinting and regulation of estrogen receptor alpha and beta mRNA expression by estrogen in the pituitary and hypothalamus of the male rat." Neuroendocrinology **73**(1): 12-25.

Thompson Jr, E. and P. Siiteri (1979). "Subcellular distribution of aromatase in human placenta and ovary." Hormone Research in Paediatrics **11**(4): 179-185.

Tomkinson, A., J. Reeve, R. Shaw and B. Noble (1997). "The death of osteocytes via apoptosis accompanies estrogen withdrawal in human bone." Journal of Clinical Endocrinology & Metabolism **82**(9): 3128-3135.

Tucker, H. A. (1987). "Quantitative estimates of mammary growth during various physiological states: a review." Journal of Dairy Science **70**(9): 1958-1966.

Tulchinsky, D., C. Hobel, E. Yeager and J. Marshall (1972). "Plasma estrone, estradiol, estriol, progesterone, and 17-hydroxyprogesterone in human pregnancy. I. Normal pregnancy." American Journal of Obstetrics and Gynecology **112**(8): 1095.

Umayahara, Y., R. Kawamori, H. Watada, E. Imano, N. Iwama, T. Morishima, Y. Yamasaki, Y. Kajimoto and T. Kamada (1994). "Estrogen regulation of the insulin-like growth factor I gene transcription involves an AP-1 enhancer." Journal of Biological Chemistry **269**(23): 16433-16442.

van Amerongen, R. and R. Nusse (2009). "Towards an integrated view of Wnt signaling in development." Development **136**(19): 3205-3214.

Vandenberg, G., G. DeVane and S. Yen (1974). "Effects of exogenous estrogen and progestin on pituitary responsiveness to synthetic luteinizing hormone-releasing factor." Journal of Clinical Investigation **53**(6): 1750.

Vazquez-Alcantara, M. A., M. Menjivar, G. A. Garcia, J. C. Diaz-Zagoya and J. Garza-Flores (1989). "Long-acting estrogenic responses of estradiol fatty acid esters." Journal of Steroid Biochemistry **33**(6): 1111-1118.

Veltmaat, J. M., F. Relaix, L. T. Le, K. Kratochwil, F. G. Sala, W. van Veelen, R. Rice, B. Spencer-Dene, A. A. Mailleux and D. P. Rice (2006). "Gli3-mediated somitic Fgf10 expression gradients are required for the induction and patterning of mammary epithelium along the embryonic axes." Development **133**(12): 2325-2335.

Venkov, C. D., A. B. Rankin and D. E. Vaughan (1996). "Identification of authentic estrogen receptor in cultured endothelial cells. A potential mechanism for steroid hormone regulation of endothelial function." Circulation **94**(4): 727-733.

Visvader, J. E. and G. J. Lindeman (2003). "Transcriptional regulators in mammary gland development and cancer." Int J Biochem Cell Biol **35**(7): 1034-1051.

Visvader, J. E. and G. J. Lindeman (2003). "Transcriptional regulators in mammary gland development and cancer." The International Journal of Biochemistry & Cell Biology **35**(7): 1034-1051.

Vladusic, E., A. Hornby, F. Guerra-Vladusic, J. Lakins and R. Lupu (2000). "Expression and regulation of estrogen receptor beta in human breast tumors and cell lines." Oncology Reports **7**(1): 157-224.

Vo, N., C. Fjeld and R. H. Goodman (2001). "Acetylation of nuclear hormone receptor-interacting protein RIP140 regulates binding of the transcriptional corepressor CtBP." Molecular and Cellular Biology **21**(18): 6181-6188.

Waliszewski, P., M. Blaszczyk, E. Wolinska - Witort, M. Drews, M. Snochowski and R. E. Hurst (1997). "Molecular study of sex steroid receptor gene expression in human colon and in colorectal carcinomas." Journal of Surgical Oncology **64**(1): 3-11.

Walker, V. R. and K. S. Korach (2004). "Estrogen receptor knockout mice as a model for endocrine research." Institute for Laboratory Animal Research journal **45**(4): 455-461.

Webb, P., P. Nguyen, J. Shinsako, C. Anderson, W. Feng, M. P. Nguyen, D. Chen, S.-M. Huang, S. Subramanian and E. McKinerney (1998). "Estrogen receptor activation function 1 works by binding p160 coactivator proteins." Molecular Endocrinology **12**(10): 1605-1618.

Wen-Chang, C., N. Junko, O. Hajime and M. Sei-Itsu (1980). "Stimulation of prostaglandin cyclooxygenase and prostacyclin synthetase activities by estradiol in rat aortic smooth muscle cells." Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism **620**(3): 472-482.

Weyant, M. J., A. M. Carothers, N. N. Mahmoud, H. L. Bradlow, H. Remotti, R. T. Bilinski and M. M. Bertagnolli (2001). "Regular Articles-Epidemiology and Prevention-Reciprocal Expression of ERa and ERb Is Associated with Estrogen-mediated Modulation of Intestinal Tumorigenesis." Cancer Research **61**(6): 2547-2551.

Wijayarathne, A. L., S. C. Nagel, L. A. Paige, D. J. Christensen, J. D. Norris, D. M. Fowlkes and D. P. McDonnell (1999). "Comparative analyses of mechanistic differences among antiestrogens." Endocrinology **140**(12): 5828.

Willert, K., J. D. Brown, E. Danenberg, A. W. Duncan, I. L. Weissman, T. Reya, J. R. Yates and R. Nusse (2003). "Wnt proteins are lipid-modified and can act as stem cell growth factors." Nature **423**(6938): 448-452.

Witte, D., M. Chirala, A. Younes, Y. Li and M. Younes (2001). "Estrogen receptor β is expressed in human colorectal adenocarcinoma." Human pathology **32**(9): 940-944.

Wong, C.-W., C. McNally, E. Nickbarg, B. S. Komm and B. J. Cheskis (2002). "Estrogen receptor-interacting protein that modulates its nongenomic activity-crosstalk with Src/Erk phosphorylation cascade." Proceedings of the National Academy of Sciences USA **99**(23): 14783-14788.

Wysolmerski, J. J., S. Cormier, W. M. Philbrick, P. Dann, J.-P. Zhang, J. Roume, A.-L. Delezoide and C. Silve (2001). "Absence of functional type 1 parathyroid hormone (PTH)/PTH-related protein receptors in humans is associated with abnormal breast development and tooth impaction." Journal of Clinical Endocrinology & Metabolism **86**(4): 1788-1794.

Wysolmerski, J. J., W. M. Philbrick, M. E. Dunbar, B. Lanske, H. Kronenberg and A. Broadus (1998). "Rescue of the parathyroid hormone-related protein knockout mouse demonstrates that parathyroid hormone-related protein is essential for mammary gland development." Development **125**(7): 1285-1294.

Xiaomeng, X. and M. L. Thomas (1994). "Estrogen receptor-mediated direct stimulation of colon cancer cell growth in vitro." Molecular and Cellular Endocrinology **105**(2): 197-201.

Xu, J., Y. Qiu, F. J. DeMayo, S. Y. Tsai, M.-J. Tsai and B. W. O'Malley (1998). "Partial hormone resistance in mice with disruption of the steroid receptor coactivator-1 (SRC-1) gene." Science **279**(5358): 1922-1925.

- Xu, L., C. K. Glass and M. G. Rosenfeld (1999). "Coactivator and corepressor complexes in nuclear receptor function." Current Opinion in Genetics & Development **9**(2): 140-147.
- Yang, E., J. Zha, J. Jockel, L. H. Boise, C. B. Thompson and S. J. Korsmeyer (1995). "Bad, a heterodimeric partner for Bcl-x and Bcl-2, displaces bax and promotes cell death." Cell **80**(2): 285-291.
- Yoshida, K. and Y. Miki (2004). "Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage." Cancer Science **95**(11): 866-871.
- Yuan, C.-X., M. Ito, J. D. Fondell, Z.-Y. Fu and R. G. Roeder (1998). "The TRAP220 component of a thyroid hormone receptor-associated protein (TRAP) coactivator complex interacts directly with nuclear receptors in a ligand-dependent fashion." Proceedings of the National Academy of Sciences USA **95**(14): 7939-7944.
- Yue, W., J. D. Yager, J.-P. Wang, E. R. Jupe and R. J. Santen (2012). "Estrogen receptor-dependent and independent mechanisms of breast cancer carcinogenesis." Steroids.
- Zang, E. A. and E. L. Wynder (1996). "Differences in lung cancer risk between men and women: examination of the evidence." Journal of the National Cancer Institute **88**(3-4): 183-192.
- Zhang, F., J. L. Ram, P. R. Standley and J. R. Sowers (1994). "17 beta-Estradiol attenuates voltage-dependent Ca²⁺ currents in A7r5 vascular smooth muscle cell line." American Journal of Physiology-Cell Physiology **266**(4): C975-C980.
- Zhang, H., J. S. Thomsen, L. Johansson, J.-Å. Gustafsson and E. Treuter (2000). "DAX-1 functions as an LXXLL-containing corepressor for activated estrogen receptors." Journal of Biological Chemistry **275**(51): 39855-39859.
- Zhang, W., S. Andersson, G. Cheng, E. R. Simpson, M. Warner and J.-Å. Gustafsson (2003). "Update on estrogen signaling." FEBS Letters **546**(1): 17-24.
- Zhang, W., S. Saji, S. Mäkinen, G. Cheng, E. V. Jensen, M. Warner and J.-Å. Gustafsson (2000). "Estrogen receptor (ER) β , a modulator of ER α in the uterus." Proceedings of the National Academy of Sciences USA **97**(11): 5936-5941.
- Zhao, C., E. W. Lam, A. Sunter, E. Enmark, M. T. De Bella, R. C. Coombes, J. A. Gustafsson and K. Dahlman-Wright (2003). "Expression of estrogen receptor beta isoforms in normal breast epithelial cells and breast cancer: regulation by methylation." Oncogene **22**(48): 7600-7606.
- Zhou, S., Y. Zilberman, K. Wassermann, S. D. Bain, Y. Sadovsky and D. Gazit (2001). "Estrogen modulates estrogen receptor α and β expression, osteogenic activity, and apoptosis in mesenchymal stem cells (MSCs) of osteoporotic mice." Journal of Cellular Biochemistry **81**(S36): 144-155.
- Zhu, B. T. and A. H. Conney (1998). "Functional role of estrogen metabolism in target cells: review and perspectives." Carcinogenesis **19**(1): 1-27.

**CHAPTER 2: Estrogen receptor-beta agonist
diarylpropionitrile counteracts the estrogenic activity of
estrogen receptor-alpha agonist propylpyrazole-triol in the
mammary gland of ovariectomized Sprague Dawley rats**

Abstract

Although estrogen can bind both types of estrogen receptors, estrogen receptor-alpha ($ER\alpha$) is dominant in mediating estrogenic activity in the mammary gland and uterus. Excessive estrogenic activity such as estrogen-based postmenopausal hormone replacement therapy increases the risk for breast and endometrial cancers. The adverse effect of estrogen on uterine endometrium can be opposed by progestins; however, estrogen-plus-progestin regimen imposes substantially greater risk for breast cancer than estrogen alone. In this study, we used $ER\alpha$ selective agonist propylpyrazole-triol (PPT) and $ER\beta$ - selective agonist diarylpropionitrile (DPN) to activate $ER\alpha$ and estrogen receptor-beta ($ER\beta$) separately in an ovariectomized rat model and determined whether PPT-activated $ER\alpha$ function in the mammary gland can be suppressed by DPN activated $ER\beta$. Ovariectomized rats were randomly divided into six groups and treated with DMSO (control), DPN, PPT, PPT/DPN, PPT/Progesterone, and PPT/Progesterone/DPN, respectively. In the mammary gland, PPT but not DPN increased cell proliferation and amphiregulin gene expression; importantly, the stimulatory effect of PPT on mammary cell proliferation and amphiregulin gene expression can be suppressed by DPN. In the uterus,

the effect of PPT on uterine weight and endometrial cell proliferation was not inhibited by DPN but can be inhibited by progesterone. These data provide *in vivo* evidence that PPT activated ER α activity in the mammary gland can be opposed by ER β -selective agonist DPN, which may be explored for the development of better hormone replacement therapy regimen with less risk for breast cancer.

Keywords:

Estrogen receptor

Diarylpropionitrile

Propylpyrazole triol

Mammary gland

Breast cancer

Hormone replacement therapy

Introduction

Estrogen has profound effects on a broad range of tissues and organs involved in many physiological processes. Drop in estrogen production after menopause is responsible for many postmenopausal symptoms, thus estrogen or estrogen-plus progestin can be used for hormone replacement therapy (HRT) to ameliorate postmenopausal symptoms. A major adverse effect associated with estrogen-based HRT is the increased risk for breast cancer and uterine endometrial hyperplasia and malignancy (Rossouw et al. 2002, Banks

et al. 2003). The adverse effect of estrogen on uterine endometrium can be opposed by progestins; however, estrogen-plus-progestin HR regimen imposes substantially greater risk for breast cancer than estrogen alone (Persson et al. 1999, Pike et al. 2000, Ross et al. 2000, Schairer et al. 2000, Banks et al. 2003). While the two types of estrogen receptors, ER α and ER β , bind to natural estrogen with similar affinity, ER α is the dominant receptor that mediates the estrogenic responses in most estrogen regulated tissues including the mammary gland and uterus (Kuiper et al. 1997, Couse et al. 1999, Dupont et al. 2000, Deroo et al. 2006, Mallepell et al. 2006, Harris 2007). Deregulation of ER α expression and activity accounts for the majority of breast and endometrial cancers. Approximately 70% of breast tumors and 60% of endometrial tumors are ER α -positive tumors (Ferrandina et al. 2005, Regan et al. 2006). In many breast tumors, the percentage of ER α -positive cells is much higher than that in the normal mammary gland (Clarke et al. 1997, Russo et al. 1999, Shoker et al. 1999, Regan et al. 2006). Furthermore, ER α may mediate cell proliferation differently in breast tumors. In the normal mammary gland, ER α + / Ki67+ cells are very rare and it is believed that ER α acts in a paracrine manner to promote neighboring ER α -negative cell to proliferate (Shoker et al. 1999, Schairer et al. 2000, Banks et al. 2003, Cheng et al. 2005, Mallepell et al. 2006). In ER α -positive breast tumors or cancer cell lines, the percentage of ER α + / Ki67+ cells are much higher than that in the normal mammary gland and that ER α may directly stimulate ER α -positive cancer cells to proliferate (Clarke et al. 1997, Ross et al. 2000, Tan et al. 2009). Deregulated expression of ER α in transgenic

mice leads to mammary tumorigenesis and makes the uterus more susceptible to estrogen induced uterine tumorigenesis (Couse et al. 1997, Tilli et al. 2003, Frech et al. 2005). Unlike ER α , ER β is not required for mammary gland and uterus development (Krege et al. 1998, Dupont et al. 2000, Förster et al. 2002, Mallepell et al. 2006). Epidemiological studies indicate that ER β expression is lost or decreased in many breast and endometrial tumors, indicating that ER β may function as a tumor suppressor (Roger et al. 2001, Shaaban et al. 2003, Girault et al. 2004).

The precise mechanism(s) by which estrogen promotes tumorigenesis in the mammary gland and uterine endometrium is not fully understood. A major effect of estrogen on the mammary gland and uterus is to stimulate cell proliferation (Berry et al. 2003, Visvader et al. 2003, Cheng et al. 2004, Slayden et al. 2004). It has been found that estrogen-based HRT significantly increases breast epithelial cell proliferation in postmenopausal women (Hofseth et al. 1999). Deregulation of cell proliferation by oncogenes and tumor suppressors is one of the hallmarks of cancer cells (Hanahan et al. 2000, Meyers 2005). Consistent with its role in breast and endometrial malignancy, ER α is essential and sufficient to mediate estrogen induced cell proliferation (Shoker et al. 1999, Dupont et al. 2000, Harris et al. 2002, Berry et al. 2003, Slayden et al. 2004). In contrast to the positive role of ER α in cell proliferation, ER β may function as a negative regulator of cell proliferation. Loss of ER α could lead to increased cell proliferation, whereas overexpression of ER β has been found to inhibit cell proliferation and xenograft tumor

formation in several breast and endometrial cell lines (Dupont et al. 2000, Nilsson et al. 2001, Berry et al. 2003, Paruthiyil et al. 2004, Ström et al. 2004, Cheng et al. 2005, Helguero et al. 2005, Hartman et al. 2006, Chen et al. 2007, Covaleda et al. 2008, Treeck et al. 2008). The molecular mechanism of ER β action is not fully understood (Harris 2007, Deroo et al. 2010, Warner et al. 2010). Studies using in vitro cell lines have demonstrated that ER β can antagonize ER α in gene expression, cell cycle progression, and cell proliferation (Clarke et al. 1997, Couse et al. 1997, Paech et al. 1997, Hall et al. 1999, Harris et al. 2002, Liu et al. 2002, Paruthiyil et al. 2004). ER α and ER β may form a subtle balance to regulate estrogen signaling in mammary and endometrial cell proliferation, loss of the balance may lead to tumor initiation and progression (Matthews et al. 2003).

In addition to genetic modification of estrogen receptor expression, ER-selective agonists have been developed to determine the biological functions of ER α and ER β (Ström et al. 2004, Harris 2006, Harris 2007, Paruthiyil et al. 2009, Minutolo et al. 2011). These ER-selective agonists may also be used for pharmacological interventions of estrogenic activity (Girault et al. 2004, Harris 2006, Harris 2007). Despite the significance of estrogenic activity in mammary cell proliferation and tumorigenesis and that ER β may function as a tumor suppressor, in vivo studies of the ER β -selective agonists in the mammary gland are very limited (Harris et al. 2003, Slayden et al. 2004, Crabtree et al. 2006). It remains unknown whether endogenous ER β can be activated to function as a tumor suppressor in the mammary gland in vivo. In this study, we used ER-selective

agonists propylpyrazole triol (PPT) and diarylpropionitrile (DPN) to separately activate ER α and ER β in an ovariectomized (OVX) rat model and determined whether ER α -mediated estrogenic activity in the mammary gland can be inhibited by DPN activated ER β . In receptor competition binding assay for binding affinity relative to estradiol, PPT is an ER α -selective agonist that has a 410 fold higher relative binding affinity to ER α than to ER β ; DPN has a 70 fold higher relative binding affinity to ER β than to ER α (Stauffer et al. 2000, Meyers et al. 2001). We demonstrated that ER α -mediated estrogenic activity in the mammary gland can be opposed by the ER β -selective agonist DPN in vivo, suggesting that ER β -selective agonists such as DPN may be explored for the development of better HRT regimens to reduce or eradicate the risk for breast cancer.

Methods

Animals

All animal experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Vermont. Ovariectomized (OVX) virgin female Sprague Dawley rats (Charles River - Canada) were housed with a 12-h light and dark cycle and ad libitum access to food and water. Rats were ovariectomized at 5–6 weeks old and rested for two weeks before treatment. Five to six rats were randomly assigned to each group and totally there are six groups, the control group, the DPN group,

the PPT group, the PPT/DPN group, the PPT-plus-progesterone (PPT/P4) group, and the PPT/P4/DPN group. PPT, DPN, and progesterone were obtained from Tocris Bioscience and were dissolved in DMSO for stock solution. The drugs were administered by i.p. injection once a day for three consecutive days; the control group rats received the vehicle DMSO only. The dosage of the different drugs used in this study was as follows: PPT at 500 µg/kg BW (body weight), DPN at 1000 µg/kg BW, P4 at 20 mg/kg BW. BrdU (5-bromo-2'-deoxyuridine, from Sigma) solution in PBS was injected (i.p., 20 mg/rat/d) at the same time when the drug(s) was administered. Rats were sacrificed 16 h after the last injection for biopsy sample collection. The timing of treatment and biopsy after the last treatment was chosen based on other studies. In the literature, various lengths of treatment ranging from a couple of hours to several weeks were used for the evaluation of different endpoint parameters (Persson et al. 1999, Hanahan et al. 2000, Harris et al. 2003, Cheng et al. 2004, Crabtree et al. 2006). The primary endpoint of evaluation in this study was cell proliferation rate, the three day treatment period was chosen as it has been shown in several studies that two to three day treatment significantly increased mammary cell proliferation rate (Hofseth et al. 1999, Persson et al. 1999). Another reason that we did not choose shorter than three days is the concern that the percentage of proliferating cells in OVX rats induced by shorter treatment period would be too low to allow the detection of any inhibitory effect. The drugs were administered in the afternoon for all three treatments; for the last treatment, drug injections for different animals (with ear tag numbers) were administered at 15 min

intervals so that each individual animal was killed at 16 h post the last treatment for biopsy sample collection. Time course studies have shown that estrogen treatment for as short as 4 h significantly increased the percentage of cyclin D-staining cells in the mammary gland; in our previous studies using ER α -positive MCF-7 cell line treated with estrogen, we noticed that the percentage of cells with the Ki-67 proliferation marker started to increase around 12 h (Hofseth et al. 1999, Pike et al. 2000). Based on these time course studies, we expected that the effect from the last treatment can be detected 16 h later. For mammary gland biopsy, the fourth pair of mammary glands was harvested from each rat and weighed. The right-side was fixed in neutral formalin for 48 h before being processed for paraffin embedding. The left-side was snap-frozen and stored in liquid nitrogen for RNA isolation. The uterus from each rat was first measured for uterine wet weight (UWW) and then fixed in neutral formalin for 24–48 h before being processed for paraffin embedding.

The dosage selection for this study was based on the dosages used by other studies, the relative binding affinity, and the relative transcriptional activity via ERE (estrogen response element) (Kuiper et al. 1997, Kuiper et al. 1998, Persson et al. 1999, Ross et al. 2000, Stauffer et al. 2000, Meyers et al. 2001, Berry et al. 2003, Frasor et al. 2003, Sánchez-Criado et al. 2004, Crabtree et al. 2006, Covaleda et al. 2008, Engdahl et al. 2010). PPT from 50 $\mu\text{g}/\text{d}/\text{rat}$ to 1000 $\mu\text{g}/\text{d}/\text{rat}$ was shown with very good response in the uterine endometrium (Harris et al. 2002). The body weight of the rats in this study was approximately 200 g, therefore the dose per rat was about 100 $\mu\text{g}/\text{d}/\text{rat}$ for PPT, 200

$\mu\text{g/d/rat}$ for DPN, and 4 mg/d/rat for progesterone. In the transcriptional activity assay using the U2OS cell system, it was shown that the maximal activity stimulated by PPT was comparable to that by estradiol, and that the EC₅₀ for estradiol via ER α was 8 pM and the EC₅₀ for PPT via ER α was 140 pM (Covalada et al. 2008). The ratio of 8 pM estradiol/140 pM PPT can be converted as 20 $\mu\text{g/kg BW}$ estradiol/500 $\mu\text{g/kg BW}$ PPT, a dosage that were expected to be functional in the mammary gland as well (Visvader et al. 2003, Ferrandina et al. 2005, Meyers 2005, Li et al. 2006). The binding affinity of PPT to ER α is approximately 49% of that of estradiol to ER α , or the conversion of 500 $\mu\text{g/kg BW}$ PPT to 176 $\mu\text{g/kg BW}$ estradiol (Stauffer et al. 2000). DPN at 1000 $\mu\text{g/kg BW}$ was within the range used by other studies for its effect on uterus, hot flush, osteoporosis, and cardioprotection (Frasor et al. 2003, Sánchez-Criado et al. 2004, Bowe et al. 2006, Nikolic et al. 2007, Bliedtner et al. 2010, Engdahl et al. 2010). The binding affinity of DPN to ER β is approximately 18% of that of estradiol to ER β , or the conversion of 1000 $\mu\text{g/kg BW}$ DPN to 205 $\mu\text{g/kg BW}$ estradiol (Meyers et al. 2001). Based on these calculations, the theoretically converted PPT and DPN (to estradiol) would have DPN binding to ER β and PPT binding to ER α at a comparable level. Considering that estradiol may have a two to ten-fold higher binding affinity for ER α than for ER β , the ratio of DPN-ER β /PPT-ER α could be lower than the 1:1 ratio (Liu et al. 2002, Harris 2007, Warner et al. 2010). The binding affinity of PPT to ER β is 0.12% of that of estradiol to ER β , or the conversion of 500 $\mu\text{g/kg BW}$ PPT to 0.42 $\mu\text{g/kg BW}$ estradiol, a concentration that would not interfere

much of the DPN binding to ER β (Stauffer et al. 2000). The binding affinity of DPN to ER α is 0.25% of that of estradiol to ER α , or the conversion of 1000 $\mu\text{g}/\text{kg}$ BW DPN to 2.85 $\mu\text{g}/\text{kg}$ BW estradiol, a concentration that would not interfere much of the PPT binding to ER α (Hartman et al. 2006). Progesterone from 15 mg/kg BW to 30 mg/kg BW was shown to have synergistic effect with estrogen or PPT, therefore 20 mg/kg was selected for this study (Visvader et al. 2003, Ferrandina et al. 2005).

Immunofluorescent (IF) and immunohistochemical (IHC) staining

IF and IHC assays were used to assess the BrdU-labeled cells and the expression of ER α , ER β , and cyclin D1 following conventional procedures. The sources for the antibodies were as follows: ER α antibody (MC-20) from Santa Cruz Biotechnology, ER β (14C8) and BrdU antibodies from Abcam, cyclin D1 antibody (DCS-6) from Fisher Scientific/Pierce. For IF staining, Alexa Fluor 488 (Invitrogen) was used for green fluorescence, Rhodamine Red (Jackson ImmunoResearch) was used for red fluorescence, and DAPI contained in Mounting Medium (Vector Lab) was used for nucleus staining to give blue fluorescence. For IHC staining, VECTASTAIN Elite ABC Kit was used following the manufacturer's procedure. DAB (3, 3'-diaminobenzidine) was used as the peroxidase substrate to develop brown color and Hematoxylin QS was used for counterstaining. Antigen retrieval was carried out by microwaving (700 W) slides in 10 mM citrate buffer (pH 6.0), ER α and BrdU for 11 min, ER β for 25 min, and cyclin D1 for

20 min. ER α antibody was used at 1:200 dilution, ER β antibody at 1:40 dilution, BrdU antibody at 1:150 dilution, and cyclin D1 antibody at 1:80 dilution. Paraffin sections without incubation with primary antibody were used as negative control of staining; sections from ovary-intact tissues were used as positive control of staining. Immunostaining slides were examined under Olympus BX50 Fluorescence Microscope connected with the Optronics MagnaFire digital camera (Microscopy Imaging Center, UVM). Images were taken with Optronics MagnaFire software and Adobe Photoshop was used for further processing of the digital images.

The NIH software Image J was used for cell counting. For each mammary gland, at least 250 ductal epithelial cells and 500 lobular/ alveolar bud epithelial cells were counted. For the uterine endometrium of most rats, at least 400 luminal epithelial cells and 200 glandular epithelial cells were counted. For IHC images of cyclin D1 staining, the staining was assigned at four levels, level 0 is unstained, level 1 is weak staining, level 3 is intensive staining, and level 2 is between level 1 and level 3 for moderate staining. Cells with staining levels 2 and 3 were counted as positive staining cells, and cells with staining levels 0 and 1 were counted as negative staining cells.

RNA isolation and quantitative real-time PCR assay

Total RNA was extracted with Trizol reagent (Invitrogen) and further purified with RNeasy Kit (Qiagen). Two micrograms of RNA from each sample was used for reverse

transcription with SuperScript III (Invitrogen). TaqMan probes for amphiregulin (Areg) and β -actin labeled with FAM dye were purchased from Applied Biosystems (ABI). Quantitative real-time PCR was performed for amphiregulin and β -actin (endogenous control) using TaqMan gene expression assay (ABI) on an ABI 7500 real-time PCR System. PCR for RNA from each sample was performed in duplicate and the average Ct from each sample was used for further calculation. The relative gene expression of amphiregulin transcripts were calculated using the $2^{-\Delta\Delta C_t}$ method. (Saville et al. 2000)

Statistical analysis

Statistical significance among different groups of animals was assessed by one-way ANOVA. Post hoc Tukey's HSD test was used for pairwise multiple comparisons to determine which groups differ, with the significance level (alpha) set as 0.05. A p value less than 0.05 was considered significant.

Results

Expression of ER α and ER β in the mammary gland and uterine endometrium of OVX rats

The expression patterns of ER α and ER β in the mammary gland and uterus of OVX rats were determined by immunofluorescent (IF) and immunohistochemical (IHC) staining, respectively (Fig. 1). In the mammary gland of OVX rats, ER α was detected in more than 50% of luminal epithelial cells in the lobular and ductal structures (Fig. 1). In comparison

to that of the ovary-intact rats, the mammary gland of ovariectomized rats showed more cells with ER α expression but the staining intensity was weaker (Fig. 1, and data not shown). ER β was detected in almost all mammary epithelial cells with strong staining, and ER β -staining was also detected in some stromal cells in the mammary gland (Fig. 1). In the uterus of OVX rats, ER α was detected in almost all endometrial luminal epithelial cells, glandular epithelial cells, and stromal cells; the staining intensity in the epithelial cells was much stronger than that in the stromal cells (Fig. 1). ER β expression was detected mainly in the endometrial luminal epithelial cells and glandular epithelial cells (Fig. 1). These data confirmed that both ER α and ER β were expressed in the mammary gland and uterus of OVX rats.

DPN counteracts the proliferative effect of PPT in the mammary gland

To determine the effect of ER α and/or ER β activation on mammary cell proliferation, OVX rats were treated with ER α -selective agonist PPT alone, ER β -selective agonist DPN alone, or PPT and DPN combined. Mammary cell proliferation rate was determined by the percentage of BrdU-labeled cells in different groups of rats. In comparison to the control group, PPT treatment significantly increased mammary epithelial cell proliferation rate in both ductal and lobular structures (Fig. 2). DPN treatment slightly decreased the percentage of BrdU-labeled mammary epithelial cells; statistically, the percentage of BrdU labeled cells in lobules and total cells (ducts and

lobules combined) were significantly different from that of control rats (Fig. 2). In the PPT/DPN group treated with PPT and DPN to activate both ER α and ER β , mammary cell proliferation rate was significantly lower than that of the PPT group but was not significantly different from that of the control group (Fig. 2). Cyclin D1 expression was evaluated to confirm the effect of PPT and/or DPN on mammary gland cell proliferation. In the mammary glands of the control group and the DPN group, the percentages of cells with moderate and intensive staining for cyclin D1 were very low (Fig. 3). Consistent with the BrdU-staining data, PPT significantly increased the percentage of cells with moderate and intensive staining for cyclin D1 (Fig. 3). In the mammary glands of the PPT/DPN group, the percentage of cells with moderate and intensive staining for cyclin D1 was similar to that of the control group and the DPN group, indicating that PPT-induced cyclin D1 expression was suppressed by DPN (Fig. 3). Collectively, these data indicate that activation of ER α by PPT but not activation of ER β by DPN leads to increased mammary cell proliferation, and that the proliferative effect of PPT on mammary cell proliferation can be suppressed by DPN.

Inhibition of PPT induced amphiregulin expression by DPN in the mammary gland

In the mammary gland, ER α mediates cell proliferation by paracrine regulation and the epidermal growth factor (EGF) family member amphiregulin plays critical role in the paracrine action of ER α (Visvader et al. 2003, Mallepell et al. 2006, Ciarlioni et al. 2007).

Consistent with its estrogenic activity to promote cell proliferation as shown above, PPT significantly increased amphiregulin expression (Fig. 4). DPN alone did not affect much of amphiregulin expression; when co-administered with PPT, PPT induced amphiregulin increase was significantly reduced by DPN in the PPT/DPN group (Fig. 4). These data suggest that the inhibitory effect of DPN on PPT-induced mammary cell proliferation may be mediated by suppression of amphiregulin expression.

DPN does not inhibit the estrogenic activity of PPT in the uterus

Uterine wet weight (UWW) and morphology were used to determine the uterotrophic effect of PPT and DPN (Fig. 5A, and data not shown). In the OVX control group, the uterus was very thin in diameter. Consistent with other studies (Couse et al. 1999, Persson et al. 1999, Harris et al. 2002, Frasor et al. 2003, Tilli et al. 2003, Hartman et al. 2006, Wegorzewska et al. 2008), PPT treatment increased uterine diameter and uterine wet weight, whereas uterus morphology and UWW in the DPN group were very similar to that of the control group (Fig. 5A). Co-administration of DPN with PPT did not inhibit or enhance PPT's effect on UWW (Fig. 5A). Immunostaining of BrdU-labeled cells was used to determine endometrial cell proliferation rate in different groups of rats (Fig. 5B and C). In comparison to the control group, PPT treatment group showed significantly higher percentage of BrdU-labeled endometrial luminal and glandular cells (Fig. 5B and C). DPN treatment did not affect the percentage of BrdU-labeled endometrial luminal and

glandular cells (Fig. 5B and C). When PPT and DPN were co-administered in the PPT/DPN group, PPT-increased cell proliferation was not inhibited by DPN (Fig. 5B and C). These data are consistent with that the estrogenic activity in the uterus is mediated by ER α and that ER β -selective agonist DPN had little effect on the estrogenic activity of ER α -selective agonist PPT in the uterus (Persson et al. 1999, Harris et al. 2002, Berry et al. 2003, Frasor et al. 2003, S á nchez-Criado et al. 2004, Wegorzewska et al. 2008, Bliedtner et al. 2010).

The estrogenic activity of PPT in the uterus is opposed by progesterone and the opposing function of progesterone in the uterus is not affected by DPN

The adverse effect of estrogenic activity on uterine endometrium can be opposed by progestins (Persson et al. 1999, Frasor et al. 2003). Consistent with that the estrogen action in the uterus is mediated by ER α , we found that the estrogenic activity of ER α -agonist PPT can be opposed by progesterone (Fig. 5). The UWW and the percentage of BrdU-labeled endometrial epithelial cells in the PPT/P4 group were significantly lower than that of the PPT group (Fig. 5). UWW and the percentage of BrdU-labeled endometrial epithelial cells in the PPT/P4/DPN group were similar to that of the PPT/P4 group (Fig. 5). These data indicate that progesterone has opposing effect on PPT in the uterus tissues and DPN does not affect the opposing function of progesterone.

The inhibitory function of DPN in mammary cell proliferation remains effective when used together with PPT and progesterone

Progestins when used alone have little effect on mammary cell proliferation; however, progestins and estrogen have synergistic effect on mammary cell proliferation and progestins also have synergistic effect with PPT in promoting mammary gland end bud development (Hofseth et al. 1999, Regan et al. 2006, Ciarloni et al. 2007, Zhao et al. 2008). Consistently, we found that the percentage of BrdU labeled mammary proliferating cells was significantly higher in the PPT/P4 group than that of the PPT group (Figs. 2 and 6). To determine whether the inhibitory effect of DPN on mammary cell proliferation is affected by progesterone, DPN was co-administered with PPT/P4. The percentage of BrdU labeled mammary cells in the PPT/P4/DPN group was significantly lower than that of the PPT/P4 group, indicating that the inhibitory function of DPN was still effective when co-administrated with PPT/P4 (Fig. 6).

Discussion

Mammary gland and uterine endometrium are classic estrogen regulated organs/tissues and the estrogen action is mediated mainly by ER α (Couse et al. 1999, Hanahan et al. 2000, Harris et al. 2002, Berry et al. 2003, Herynk et al. 2004, Hartman et al. 2006, Mallepell et al. 2006, Tan et al. 2009). Excessive estrogenic activity, such as estrogen-based postmenopausal hormone replacement therapy, exposure to environmental endocrine disruptive agents with estrogenic activity, early menarche or late menopause, is associated with increased risk for breast cancer and/or endometrial cancer (Russo et al.

1998, Waard et al. 2005, Shanle et al. 2010). Accumulating evidence indicates that ER β may function as a tumor suppressor and therefore can be exploited for cancer prevention and therapy (Harris 2007, Deroo et al. 2010, Warner et al. 2010). However, in vivo animal model studies on the role of ER β and its interaction with ER α in mammary cell proliferation and tumorigenesis are very limited and the expected tumor suppressor function was not observed in the studies using ER β -selective agonists BAG and ERB-041 (Harris et al. 2003, Helguero et al. 2005, Crabtree et al. 2006). It is unknown whether ERB-041 or other ER β -selective agonists have additive/synergistic or antagonistic effect with ER α -selective agonist PPT in mediating mammary cell proliferation. In this study, we used ER-selective agonists PPT and DPN to activate ER α and ER β separately in an OVX rat model. We demonstrated for the first time that the estrogenic activity of PPT in the mammary gland, including cell proliferation and amphiregulin expression, can be opposed by an ER β -selective agonist in an animal model. We further showed that the estrogenic activity of PPT in the uterus can be opposed by progesterone, and that DPN and progesterone do not interfere with each other for their respective opposing functions in the mammary gland and uterus. These findings indicate that the adverse effect of ER α -mediated estrogenic activity in different organs/tissues can be opposed by different drugs via different mechanisms, which may be explored for the development of better hormone replacement therapy regimen with less risk for breast and endometrial cancer.

The differential effect of DPN on the estrogenic activity of PPT in the mammary gland and uterus supports that the effect of DPN activated ER β on target cells is cell type dependent. In addition, the effect of ER β on cellular activities of target cells is ligand type dependent and the results from ER β -selective agonists including DPN may not represent the physiological role of ER β bound to estrogen (Harris 2006, Harris 2007, Paruthiyil et al. 2009, Deroo et al. 2010). The effect of DPN on mammary cell proliferation in this study is quite different from that of the ER β -selective agonist BAG in the study by Cheng et al. using an OVX mouse model (Cheng et al. 2004). It is unlikely that the difference is caused by different rodent species, but it will be valuable to evaluate ER β -selective agonists in more than one species. It is more likely that different ER β -selective agonists including DPN and BAG may exert different effects; in that case, it will be valuable to determine whether other ER β -selected agonists besides DPN have anti-proliferative function in the mammary gland.

The uterotrophic activity of PPT and several ER β -selective agonists has been well studied in different rodent models. In all these models, PPT has been shown with estrogenic activity to increase uterine wet weight or to stimulate endometrial cell proliferation (Frasor et al. 2003, Harris 2007, Bliedtner et al. 2010). For the ER β -selective agonists evaluated for uterotrophic activity, 8 β -VE2 is the only one known with uterotrophic activity (Helguero et al. 2005, Harris 2007). In the OVX rodent models, DPN does not have uterotrophic activity (Wegorzewska et al. 2008, Engdahl et al. 2010). Data on whether the

uterotrophic activity of PPT can be suppressed by DPN is inconsistent in different animal models. In the OVX model, our data are consistent with that DPN does not suppress the uterotrophic activity of PPT (Sánchez-Criado et al. 2004). In ArKO (aromatase knock-out) and ovary intact immature mouse models, DPN does not have uterotrophic activity but may have some inhibitory activity and reduces the estrogenic activity of PPT (Frasor et al. 2003, Bliedtner et al. 2010). From these different models, it is clear that DPN does not have uterotrophic activity and may instead inhibit the ER α -mediated uterotrophic activity to a certain extent. The reason why DPN has a more pronounced suppressive effect on PPT activity in the mammary gland vs. the uterus is not clear. It could be related to the relative expression levels of ER β or the relative ratios of ER α /ER β in these two types of tissues. It is worth noting that the relative expression patterns of ER α and ER β are different in these two types of tissues, which might also account for the differential effect of DPN in these two types of tissues. As shown in Fig. 1, ER β expression is more widespread than ER α in the mammary gland; in the uterine endometrium, ER α expression is more widespread than ER β . The mammary gland and uterine endometrium respond differently to Tamoxifen, indicating that the cellular contexts, such as coactivators or corespressors, might be different in these two types of tissues that may also be related to the differential effect of DPN (Jordan et al. 2007).

In the PPT/DPN group of rats in this study, the estrogenic activity of PPT in the mammary gland is almost completely abrogated by DPN, as determined by mammary cell

proliferation rate and expression of cyclin D1 and amphiregulin. The mammary cell proliferation rate in the PPT/P4/DPN group is significantly lower than that in the PPT/P4 group, indicating that ER α -mediated activity that functions synergistic with progesterone is significantly inhibited by DPN. However, the mammary cell proliferation rate in the PPT/P4/DPN group is significantly higher than that in the control and PPT/DPN groups, and even a little higher than the PPT group. These data indicate that when co-administered with PPT/P4, PPT activated ER α activity is not completely blocked by DPN. Further studies are needed to determine whether the inhibitory function of DPN is dose-dependent and whether the inhibitory function of DPN can be further increased at other doses.

ER α -mediated activity is believed to be responsible for the increased risk for breast cancer and endometrial cancer in women under HRT. As shown in this study using the OVX postmenopausal rat model, ER α -mediated estrogenic activity activated by PPT in the mammary gland and uterus can be differentially opposed by DPN and progesterone, respectively. It will be interesting to determine whether a regimen containing DPN such as PPT/DPN/progestin is a better option than the estrogen-plus progestin regimen. When considering inclusion of DPN or ER β -selective agonist in the hormone replacement therapy regimen, at least two types of effects need to be considered. The first concern is whether ER β -selective agonist has adverse effects in different organs/tissues. The other concern is that whether some of the beneficial effects of estrogenic activity are abolished or some of the adverse effects of estrogenic activity are enhanced. Based on the reported

animal model studies involving many organ systems, it seems that PPT and DPN might be good candidates with regards to the beneficial vs. adverse effects. Here we will briefly discuss the effects on the mammary gland, uterine endometrium, hot flashes, and bone metabolism. As aforementioned, DPN can inhibit the adverse effect of PPT in the mammary gland and DPN has no adverse effect on uterine endometrium. With regards to hot flashes, both PPT and DPN have been shown to prevent hot flashes in rodent models (Harris et al. 2002, Liu et al. 2002). For bone metabolism, bone sparing activity is found in PPT but not found in several ER β -selective agonists including ERB-041; the effect of DPN on bone metabolism is unknown (Berry et al. 2003, Harris 2007). Further studies will be needed to determine the effect of DPN on bone metabolism and its effect on the bone sparing activity of PPT. In summary, these functional studies of DPN in animal models suggest that DPN merits consideration for the development of better hormone replacement therapy regimen.

Conclusion

Estrogen-based postmenopausal hormone replacement therapy increases the risk for breast and uterine endometrial cancers; estrogen action in the mammary gland and endometrium is mediated mainly by ER α (Couse et al. 1997, Couse et al. 1999, Persson et al. 1999, Banks et al. 2003, Cheng et al. 2004, Paruthiyil et al. 2004, Deroo et al. 2006, Mallepell et al. 2006, Harris 2007). In this study using an ovariectomized postmenopausal

rat model and the ER α -selective agonist PPT and ER β -selective agonist DPN, we provide in vivo evidence that ER α -mediated estrogenic activity in the mammary gland can be opposed by ER β -selective agonist DPN. Suppressing PPT-induced amphiregulin expression by DPN may account for its opposing function in mammary gland cell proliferation. The estrogenic activity of ER α -agonist PPT in the uterus can be opposed by progesterone. These findings indicate that the adverse effect of ER α -mediated estrogenic activity in different organs can be opposed by different mechanisms, which may be explored for the development of better hormone replacement therapy regimen with less risk for breast and endometrial cancer.

Acknowledgements

This project was supported by USDA Hatch and Vermont Cancer Center (VCC)/Lake Champlain Cancer Research Organization (LCCRO) funds. Microimaging was performed in the UVM Microscopy Imaging Center, a facility center supported by VCC, LCCRO, and UVM College of Medicine.

References

- Banks, E., V. Beral, D. Bull, G. Reeves, J. Austoker and R. English (2003). "Breast cancer and hormone-replacement therapy in the Million Women Study." Lancet **362**(9382): 419-427.
- Berry, S., P. Jobst, S. Ellis, R. Howard, A. Capuco and R. Akers (2003). "Mammary Epithelial Proliferation and Estrogen Receptor α Expression in Prepubertal Heifers: Effects of Ovariectomy and Growth Hormone." Journal of Dairy Science **86**(6): 2098-2105.
- Berry, S. D., P. M. Jobst, S. E. Ellis, R. D. Howard, A. V. Capuco and R. M. Akers (2003). "Mammary epithelial proliferation and estrogen receptor alpha expression in prepubertal heifers: effects of ovariectomy and growth hormone." Journal Dairy Science **86**(6): 2098-2105.
- Bliedtner, A., O. Zierau, S. Albrecht, S. Liebhaber and G. Vollmer (2010). "Effects of genistein and estrogen receptor subtype-specific agonists in ArKO mice following different administration routes." Molecular and Cellular Endocrinology **314**(1): 41-52.
- Bowe, J., X. F. Li, J. Kinsey-Jones, A. Heyerick, S. Brain, S. Milligan and K. O'Byrne (2006). "The hop phytoestrogen, 8-prenylnaringenin, reverses the ovariectomy-induced rise in skin temperature in an animal model of menopausal hot flushes." Journal of Endocrinology **191**(2): 399-405.
- Chen, J.-Q., P. A. Russo, C. Cooke, I. H. Russo and J. Russo (2007). "ER β shifts from mitochondria to nucleus during estrogen-induced neoplastic transformation of human breast epithelial cells and is involved in estrogen-induced synthesis of mitochondrial respiratory chain proteins." Biochimica et Biophysica Acta (BBA)-Molecular Cell Research **1773**(12): 1732-1746.
- Cheng, G., Y. Li, Y. Omoto, Y. Wang, T. Berg, M. Nord, P. Vihko, M. Warner, Y.-S. Piao and J.-Å. Gustafsson (2005). "Differential regulation of estrogen receptor (ER) α and ER β in primate mammary gland." Journal of Clinical Endocrinology & Metabolism **90**(1): 435-444.
- Cheng, G., Y. Li, Y. Omoto, Y. Wang, T. Berg, M. Nord, P. Vihko, M. Warner, Y. S. Piao and J. A. Gustafsson (2005). "Differential regulation of estrogen receptor (ER)alpha and ERbeta in primate mammary gland." The Journal of Clinical Endocrinology & Metabolism **90**(1): 435-444.
- Cheng, G., Z. Weihua, M. Warner and J.-Å. Gustafsson (2004). "Estrogen receptors ER α and ER β in proliferation in the rodent mammary gland." Proceedings of the National Academy of Sciences USA **101**(11): 3739-3746.

- Ciarloni, L., S. Mallepell and C. Brisken (2007). "Amphiregulin is an essential mediator of estrogen receptor α function in mammary gland development." Proceedings of the National Academy of Sciences USA **104**(13): 5455-5460.
- Clarke, R. B., A. Howell, C. S. Potten and E. Anderson (1997). "Dissociation between steroid receptor expression and cell proliferation in the human breast." Cancer Research **57**(22): 4987-4991.
- Couse, J. F., V. L. Davis, R. B. Hanson, W. N. Jefferson, J. A. McLachlan, B. C. Bullock, R. R. Newbold and K. S. Korach (1997). "Accelerated onset of uterine tumors in transgenic mice with aberrant expression of the estrogen receptor after neonatal exposure to diethylstilbestrol." Molecular Carcinogenesis **19**(4): 236-242.
- Couse, J. F. and K. S. Korach (1999). "Estrogen receptor null mice: what have we learned and where will they lead us?" Endocrine Reviews **20**(3): 358.
- Covalada, A. M. S., H. van den Berg, J. Vervoort, P. van der Saag, A. Ström, J.-Å. Gustafsson, I. Rietjens and A. J. Murk (2008). "Influence of cellular ER α /ER β ratio on the ER α -agonist induced proliferation of human T47D breast cancer cells." Toxicological Sciences **105**(2): 303-311.
- Crabtree, J. S., X. Zhang, B. J. Peano, Z. Zhang, R. C. Winneker and H. A. Harris (2006). "Development of a mouse model of mammary gland versus uterus tissue selectivity using estrogen-and progesterone-regulated gene markers." The Journal of Steroid Biochemistry and Molecular Biology **101**(1): 11-21.
- Deroo, B. J. and A. V. Buensuceso (2010). "Minireview: Estrogen receptor- β : mechanistic insights from recent studies." Molecular Endocrinology **24**(9): 1703-1714.
- Deroo, B. J. and K. S. Korach (2006). "Estrogen receptors and human disease." Journal of Clinical Investigation **116**(3): 561-570.
- Dupont, S., A. Krust, A. Gansmuller, A. Dierich, P. Chambon and M. Mark (2000). "Effect of single and compound knockouts of estrogen receptors alpha (ERalpha) and beta (ERbeta) on mouse reproductive phenotypes." Development **127**(19): 4277-4291.
- Engdahl, C., C. Jochems, S. H. Windahl, A. E. Bärjesson, C. Ohlsson, H. Carlsten and M. K. Lagerquist (2010). "Amelioration of collagen - induced arthritis and immune - associated bone loss through signaling via estrogen receptor α , and not estrogen receptor β or G protein-coupled receptor 30." Arthritis & Rheumatism **62**(2): 524-533.
- Förster, C., S. Mäkelä, A. Warri, S. Kietz, D. Becker, K. Hultenby, M. Warner and J.-Å. Gustafsson (2002). "Involvement of estrogen receptor β in terminal differentiation of mammary gland epithelium." Proceedings of the National Academy of Sciences USA **99**(24): 15578-15583.
- Ferrandina, G., F. O. Ranelletti, V. Gallotta, E. Martinelli, G. F. Zannoni, M. Gessi and G. Scambia (2005). "Expression of cyclooxygenase-2 (COX-2), receptors for estrogen (ER), and progesterone (PR), p53, ki67, and neu protein in endometrial cancer." Gynecologic Oncology **98**(3): 383-389.

Frasor, J., D. H. Barnett, J. M. Danes, R. Hess, A. F. Parlow and B. S. Katzenellenbogen (2003). "Response-specific and ligand dose-dependent modulation of estrogen receptor (ER) α activity by ER β in the uterus." Endocrinology **144**(7): 3159-3166.

Frech, M. S., E. D. Halama, M. T. Tilli, B. Singh, E. J. Gunther, L. A. Chodosh, J. A. Flaws and P. A. Furth (2005). "Deregulated estrogen receptor α expression in mammary epithelial cells of transgenic mice results in the development of ductal carcinoma in situ." Cancer Research **65**(3): 681-685.

Girault, I., C. Andrieu, S. Tozlu, F. Spyrtos, I. Bièche and R. Lidereau (2004). "Altered expression pattern of alternatively spliced estrogen receptor β transcripts in breast carcinoma." Cancer Letters **215**(1): 101-112.

Hall, J. M. and D. P. McDonnell (1999). "The estrogen receptor β -isoform (ER β) of the human estrogen receptor modulates ER α transcriptional activity and is a key regulator of the cellular response to estrogens and antiestrogens." Endocrinology **140**(12): 5566-5578.

Hanahan, D. and R. A. Weinberg (2000). "The hallmarks of cancer." Cell **100**(1): 57-70.

Harris, H. A. (2006). "The unexpected science of estrogen receptor- β selective agonists: a new class of anti-inflammatory agents?" Nuclear Receptor Signaling **4**.

Harris, H. A. (2007). "Estrogen receptor-beta: recent lessons from in vivo studies." Molecular Endocrinology **21**(1): 1-13.

Harris, H. A., L. M. Albert, Y. Leathurby, M. S. Malamas, R. E. Mewshaw, C. P. Miller, Y. P. Kharode, J. Marzolf, B. S. Komm and R. C. Winneker (2003). "Evaluation of an estrogen receptor- β agonist in animal models of human disease." Endocrinology **144**(10): 4241-4249.

Harris, H. A., J. A. Katzenellenbogen and B. S. Katzenellenbogen (2002). "Characterization of the biological roles of the estrogen receptors, ER α and ER β , in estrogen target tissues in vivo through the use of an ER α -selective ligand." Endocrinology **143**(11): 4172-4177.

Hartman, J., K. Lindberg, A. Morani, J. Inzunza, A. Ström and J.-Å. Gustafsson (2006). "Estrogen receptor β inhibits angiogenesis and growth of T47D breast cancer xenografts." Cancer Research **66**(23): 11207-11213.

Helguero, L. A., M. H. Faulds, J.-Å. Gustafsson and L.-A. Haldosén (2005). "Estrogen receptors α (ER α) and β (ER β) differentially regulate proliferation and apoptosis of the normal murine mammary epithelial cell line HC11." Oncogene **24**(44): 6605-6616.

Herynk, M. H. and S. A. Fuqua (2004). "Estrogen receptor mutations in human disease." Endocrine Reviews **25**(6): 869-898.

Hofseth, L. J., A. M. Raafat, J. R. Osuch, D. R. Pathak, C. A. Slomski and S. Z. Haslam (1999). "Hormone replacement therapy with estrogen or estrogen plus medroxyprogesterone acetate is associated with increased epithelial proliferation in the normal postmenopausal breast." Journal of Clinical Endocrinology & Metabolism **84**(12): 4559-4565.

- Jordan, V. C. and A. M. Brodie (2007). "Development and evolution of therapies targeted to the estrogen receptor for the treatment and prevention of breast cancer." Steroids **72**(1): 7-25.
- Krege, J. H., J. B. Hodgin, J. F. Couse, E. Enmark, M. Warner, J. F. Mahler, M. Sar, K. S. Korach, J.-Å. Gustafsson and O. Smithies (1998). "Generation and reproductive phenotypes of mice lacking estrogen receptor β ." Proceedings of the National Academy of Sciences USA **95**(26): 15677-15682.
- Kuiper, G. G., B. Carlsson, K. Grandien, E. Enmark, J. Häggblad, S. Nilsson and J.-Å. Gustafsson (1997). "Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β ." Endocrinology **138**(3): 863-870.
- Kuiper, G. G., J. G. Lemmen, B. Carlsson, J. C. Corton, S. H. Safe, P. T. van der Saag, B. van der Burg and J. A. Gustafsson (1998). "Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta." Endocrinology **139**(10): 4252-4263.
- Li, J. and R. W. McMurray (2006). "Effects of estrogen receptor subtype-selective agonists on immune functions in ovariectomized mice." International Immunopharmacology **6**(9): 1413-1423.
- Liu, M.-M., C. Albanese, C. M. Anderson, K. Hilty, P. Webb, R. M. Uht, R. H. Price, R. G. Pestell and P. J. Kushner (2002). "Opposing action of estrogen receptors α and β on cyclin D1 gene expression." Journal of Biological Chemistry **277**(27): 24353-24360.
- Mallepell, S., A. Krust, P. Chambon and C. Brisken (2006). "Paracrine signaling through the epithelial estrogen receptor α is required for proliferation and morphogenesis in the mammary gland." Proceedings of the National Academy of Sciences USA **103**(7): 2196-2201.
- Matthews, J. and J. Gustafsson (2003). "Estrogen signaling: a subtle balance between ER α and ER β ." Molecular Interventions **3**(5): 281.
- Meyers, M. J., J. Sun, K. E. Carlson, G. A. Marriner, B. S. Katzenellenbogen and J. A. Katzenellenbogen (2001). "Estrogen receptor- β potency-selective ligands: structure-activity relationship studies of diarylpropionitriles and their acetylene and polar analogues." Journal of Medicinal Chemistry **44**(24): 4230-4251.
- Meyers, R. R. A. (2005). Encyclopedia of molecular cell biology and molecular medicine, Wiley Online Library.
- Minutolo, F., M. Macchia, B. S. Katzenellenbogen and J. A. Katzenellenbogen (2011). "Estrogen receptor β ligands: Recent advances and biomedical applications." Medicinal Research Reviews **31**(3): 364-442.
- Nikolic, I., D. Liu, J. A. Bell, J. Collins, C. Steenbergen and E. Murphy (2007). "Treatment with an estrogen receptor-beta-selective agonist is cardioprotective." Journal of Molecular and Cellular Cardiology **42**(4): 769-780.

Nilsson, S., S. Makela, E. Treuter, M. Tujague, J. Thomsen, G. Andersson, E. Enmark, K. Pettersson, M. Warner and J. A. Gustafsson (2001). "Mechanisms of estrogen action." Physiological Reviews **81**(4): 1535-1565.

Paech, K., P. Webb, G. G. Kuiper, S. Nilsson, J.-Å. Gustafsson, P. J. Kushner and T. S. Scanlan (1997). "Differential ligand activation of estrogen receptors ER α and ER β at AP1 sites." Science **277**(5331): 1508-1510.

Paruthiyil, S., A. Cvaro, X. Zhao, Z. Wu, Y. Sui, R. E. Staub, S. Baggett, C. B. Herber, C. Griffin and M. Tagliaferri (2009). "Drug and cell type-specific regulation of genes with different classes of estrogen receptor β -selective agonists." PloS one **4**(7): e6271.

Paruthiyil, S., H. Parmar, V. Kerekatte, G. R. Cunha, G. L. Firestone and D. C. Leitman (2004). "Estrogen receptor β inhibits human breast cancer cell proliferation and tumor formation by causing a G2 cell cycle arrest." Cancer Research **64**(1): 423-428.

Persson, I., E. Weiderpass, L. Bergkvist, R. Bergström and C. Schairer (1999). "Risks of breast and endometrial cancer after estrogen and estrogen-progestin replacement." Cancer Causes & Control **10**(4): 253-260.

Pike, M. C. and R. K. Ross (2000). "Progestins and menopause: epidemiological studies of risks of endometrial and breast cancer." Steroids **65**(10): 659-664.

Regan, M. M., G. Viale, M. G. Mastropasqua, E. Maiorano, R. Golouh, A. Carbone, B. Brown, M. Suurkõla, G. Langman and L. Mazzucchelli (2006). "Re-evaluating adjuvant breast cancer trials: assessing hormone receptor status by immunohistochemical versus extraction assays." Journal of the National Cancer Institute **98**(21): 1571-1581.

Roger, P., M. E. Sahla, S. Mäkelä J. Å. Gustafsson, P. Baldet and H. Rochefort (2001). "Decreased expression of estrogen receptor β protein in proliferative preinvasive mammary tumors." Cancer Research **61**(6): 2537-2541.

Ross, R. K., A. Paganini-Hill, P. C. Wan and M. C. Pike (2000). "Effect of hormone replacement therapy on breast cancer risk: estrogen versus estrogen plus progestin." Journal of the National Cancer Institute **92**(4): 328-332.

Rossouw, J. E., G. L. Anderson, R. L. Prentice, A. Z. LaCroix, C. Kooperberg, M. Stefanick, R. D. Jackson, S. A. Beresford, B. V. Howard and K. C. Johnson (2002). "Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial." Journal of the American Medical Association **288**(3): 321-333.

Russo, I. H. and J. Russo (1998). "Role of hormones in mammary cancer initiation and progression." Journal of Mammary Gland Biology and Neoplasia **3**(1): 49-61.

Russo, J., X. Ao, C. Grill and I. Russo (1999). "Pattern of distribution of cells positive for estrogen receptor α and progesterone receptor in relation to proliferating cells in the mammary gland." Breast Cancer Research and Treatment **53**(3): 217-227.

Sánchez-Criado, J. E., C. Bellido, M. Tena-Sempere, R. Aguilar and A. Blanco (2004). "Biological role of pituitary estrogen receptors ER α and ER β on progesterone receptor expression and action and on gonadotropin and prolactin secretion in the rat." Neuroendocrinology **79**(5): 247-258.

Saville, B., M. Wormke, F. Wang, T. Nguyen, E. Enmark, G. Kuiper, J. A. Gustafsson and S. Safe (2000). "Ligand-, cell-, and estrogen receptor subtype (alpha/beta)-dependent activation at GC-rich (Sp1) promoter elements." Journal of Biological Chemistry **275**(8): 5379-5387.

Schairer, C., J. Lubin, R. Troisi, S. Sturgeon, L. Brinton and R. Hoover (2000). "Menopausal estrogen and estrogen-progestin replacement therapy and breast cancer risk." Journal of the American Medical Association **283**(4): 485-491.

Shaaban, A. M., P. A. O'Neill, M. P. Davies, R. Sibson, C. R. West, P. H. Smith and C. S. Foster (2003). "Declining Estrogen Receptor-[beta] Expression Defines Malignant Progression of Human Breast Neoplasia." The American Journal of Surgical Pathology **27**(12): 1502-1512.

Shanle, E. K. and W. Xu (2010). "Endocrine disrupting chemicals targeting estrogen receptor signaling: identification and mechanisms of action." Chemical Research in Toxicology **24**(1): 6-19.

Shoker, B. S., C. Jarvis, R. B. Clarke, E. Anderson, J. Hewlett, M. Davies, D. R. Sibson and J. P. Sloane (1999). "Estrogen receptor-positive proliferating cells in the normal and precancerous breast." The American Journal of Pathology **155**(6): 1811-1815.

Shoker, B. S., C. Jarvis, D. R. Sibson, C. Walker and J. P. Sloane (1999). "Oestrogen receptor expression in the normal and pre - cancerous breast." The Journal of Pathology **188**(3): 237-244.

Slayden, O. D. and R. M. Brenner (2004). "Hormonal regulation and localization of estrogen, progestin and androgen receptors in the endometrium of nonhuman primates: effects of progesterone receptor antagonists." Archives of Histology and Cytology **67**(5): 393-409.

Stauffer, S. R., C. J. Coletta, R. Tedesco, G. Nishiguchi, K. Carlson, J. Sun, B. S. Katzenellenbogen and J. A. Katzenellenbogen (2000). "Pyrazole ligands: structure-affinity/activity relationships and estrogen receptor- α -selective agonists." Journal of Medicinal Chemistry **43**(26): 4934-4947.

Ström, A., J. Hartman, J. S. Foster, S. Kietz, J. Wimalasena and J.-Å. Gustafsson (2004). "Estrogen receptor β inhibits 17 β -estradiol-stimulated proliferation of the breast cancer cell line T47D." Proceedings of the National Academy of Sciences USA **101**(6): 1566-1571.

Tan, H., Y. Zhong and Z. Pan (2009). "Autocrine regulation of cell proliferation by estrogen receptor-alpha in estrogen receptor-alpha-positive breast cancer cell lines." BMC Cancer **9**(1): 31.

- Tilli, M. T., M. S. Frech, M. E. Steed, K. S. Hruska, M. D. Johnson, J. A. Flaws and P. A. Furth (2003). "Introduction of estrogen receptor- α into the tTA/TAg conditional mouse model precipitates the development of estrogen-responsive mammary adenocarcinoma." The American Journal of Pathology **163**(5): 1713-1719.
- Trecek, O., I. Juhasz-Boess, C. Lattrich, F. Horn, R. Goerse and O. Ortmann (2008). "Effects of exon-deleted estrogen receptor β transcript variants on growth, apoptosis and gene expression of human breast cancer cell lines." Breast Cancer Research and Treatment **110**(3): 507-520.
- Visvader, J. E. and G. J. Lindeman (2003). "Transcriptional regulators in mammary gland development and cancer." Int J Biochem Cell Biol **35**(7): 1034-1051.
- Waard, F. d. and J. Thijssen (2005). "Hormonal aspects in the causation of human breast cancer: epidemiological hypotheses reviewed, with special reference to nutritional status and first pregnancy." The Journal of Steroid Biochemistry and Molecular Biology **97**(5): 451-458.
- Warner, M. and J.-Å. Gustafsson (2010). "The role of estrogen receptor β (ER β) in malignant diseases—A new potential target for antiproliferative drugs in prevention and treatment of cancer." Biochemical and Biophysical Research Communications **396**(1): 63-66.
- Wegorzewska, I. N., K. Walters, M. J. Weiser, D. F. Cruthirds, E. Ewell, D. O. Larco, R. J. Handa and T. J. Wu (2008). "Postovariectomy weight gain in female rats is reversed by estrogen receptor α agonist, propylpyrazoletriol." American Journal of Obstetrics and Gynecology **199**(1): 67. e61-67. e65.
- Zhao, C., K. Dahlman-Wright and J. A. Gustafsson (2008). "Estrogen receptor beta: an overview and update." Nuclear Receptor Signaling **6**: e003.

Figures

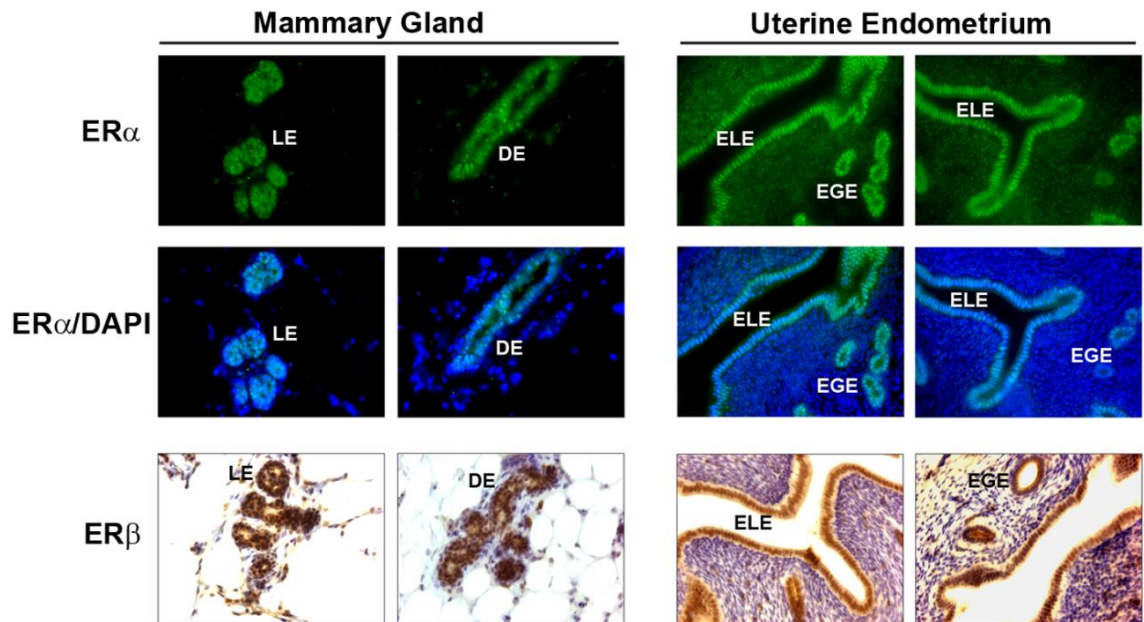


Figure 1. Immunostaining of ER α and ER β in the mammary gland and uterine endometrium of ovariectomized rats.

Five to six week old rats were ovariectomized (OVX), and mammary gland and uterus tissues were harvested two weeks after OVX. The expression pattern of ER α was determined by IF staining (green fluorescence, top panels). Cell nuclei were counterstained with DAPI (blue fluorescence). Images of ER α staining and DAPI staining were overlaid to show the localization of ER α -staining cells (middle panels). The expression pattern of ER β was determined by IHC staining with DAB as substrate to give brown color (bottom panels). In the mammary gland, ER α staining was detected in more than 50% of luminal epithelial cells; ER β was expressed in almost all epithelial cells and some stromal cells. In

the endometrium, ER α was detected in almost all epithelial cells and stromal cells; ER β expression was detected mainly in the luminal and glandular epithelial cells. DE, ducts; ELE, endometrial luminal epithelial cells; EGE, endometrial glandular epithelial cells; LE, lobules. Magnification, 400 \times .

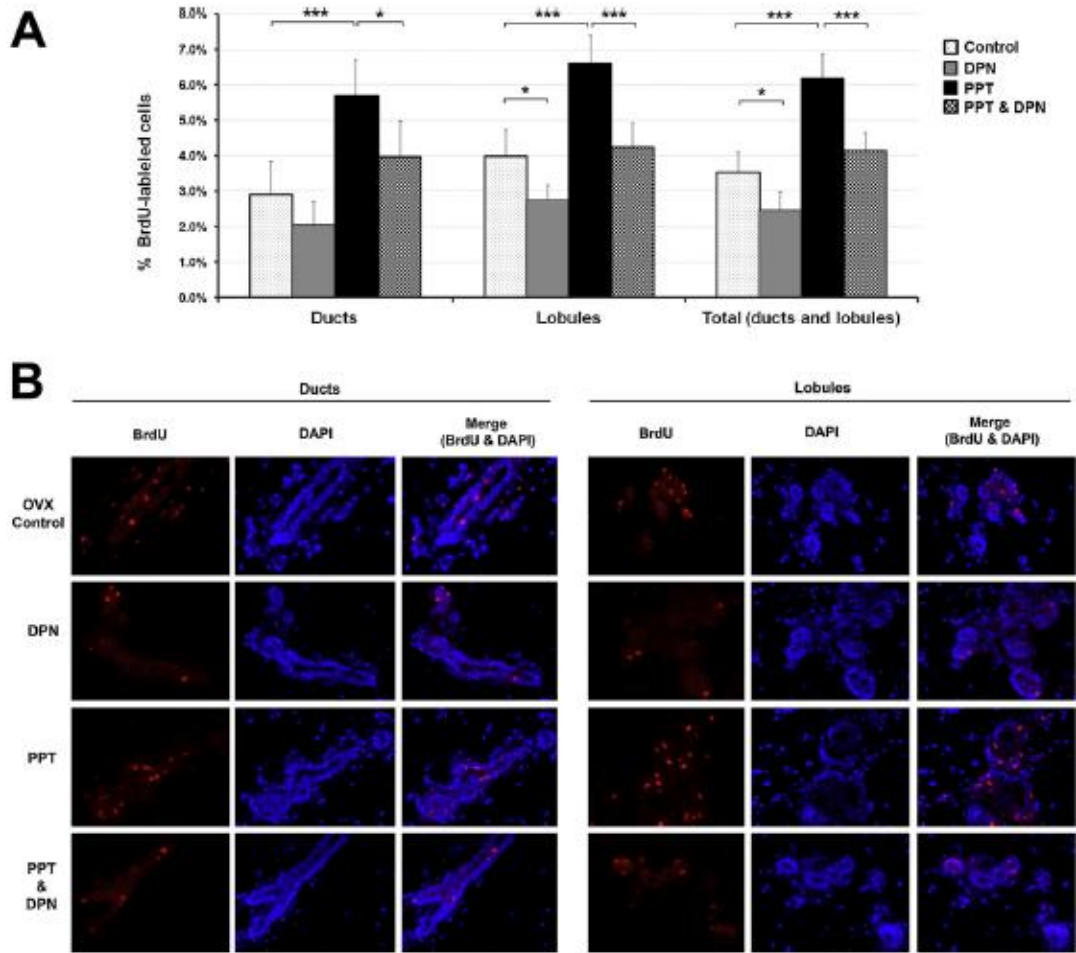


Figure 2. DPN counteracts the proliferative effect of PPT in the mammary gland of OVX rats.

OVX rats were rested for two weeks before treatment for 3 days with DMSO (control), DPN, PPT, PPT and DPN (PPT/DPN), respectively. BrdU was injected concurrently with each drug administration to label proliferating cells. (A) Percentage of BrdU-labeled proliferation cells in the mammary gland of different groups of rats. Mammary epithelial cells in ductal and lobular structures were counted separately, and the total is the combination of ductal and lobular cells. DPN slightly decreased mammary cell

proliferation. PPT significantly increased mammary epithelial cell proliferation. When co-administered with PPT, DPN significantly decreased mammary cell proliferation caused by PPT. Data are shown as means \pm SD. The p values are as follows: 0.45 for DPN vs. control in the ducts, 0.03 for DPN vs. control in the lobules and totals; <0.001 for PPT vs. control in the ducts, lobules, and total; 0.023 for PPT&DPN vs. PPT in the ducts, and <0.001 for PPT&DPN vs. PPT in the lobules and total (*p < 0.05; **p < 0.01; ***p < 0.001). (B) Representative microimages showing BrdU-labeled proliferation cells in the mammary ductal and lobular structures of different groups of rats. BrdU labeled cells were detected by immunofluorescent staining with BrdU stained with red fluorescence; nuclei were counterstained with DAPI to give blue fluorescence. Images of BrdU staining and DAPI staining were merged to show the location of BrdU-labeled cells. Magnification, 400 \times .

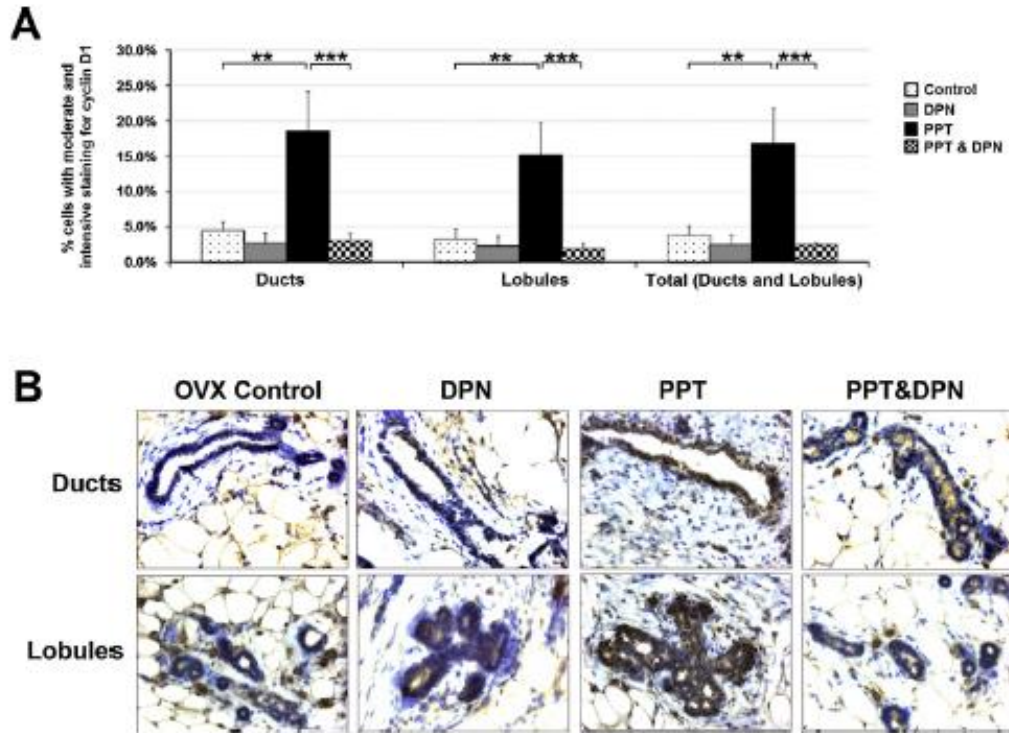


Figure 3. DPN inhibits PPT induced cyclin D1 expression in the mammary gland.

(A) Percentage of cells with moderate and intensive staining for cyclin D1 in the mammary gland of different groups of rats. Mammary epithelial cells in ductal and lobular structures were counted separately, and the total is the combination of ductal and lobular cells.

(B) Cyclin D1 was detected by immunohistochemical staining using DAB as the substrate (brown color) and hematoxylin for counterstaining (blue color). Cells were counted as unstained, weakly stained, moderately stained, and intensively stained for cyclin D1. Cells with moderate or intensive staining were calculated as cyclin D1 staining cells and the percentage of those cells were shown in the graph as means \pm SD. PPT significantly increased the percentage of cyclin D1 staining cells. When co-administered with PPT, DPN

significantly decreased the percentage of cyclin D1 staining cells caused by PPT. The p values are as follows: 1.00 for DPN vs. control in the ducts, lobules, and totals; 0.002 for PPT vs. control in the ducts, lobules, and totals; 0.001 for PPT&DPN vs. PPT in the ducts, lobules, and totals (*p < 0.05; **p < 0.01). (B) Representative cyclin D1 IHC staining images of ductal and lobular structures in the mammary gland of the four groups of rats. Cells with intensive cyclin D1 staining (arrows) were found in the mammary gland treated with PPT and barely observed in the mammary gland of the other three groups (control, DPN, PPT/DPN). Magnification, 400×.

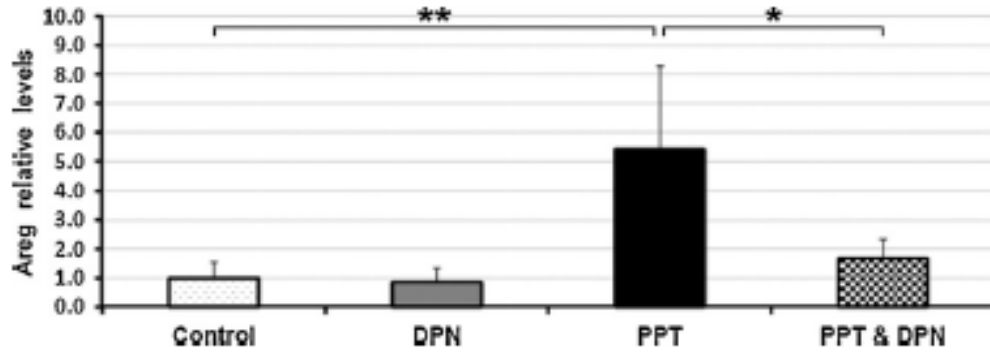


Figure 4. DPN inhibits PPT induced amphiregulin expression in the mammary gland.

RNA levels of amphiregulin were determined by quantitative real-time RT-PCR; β -actin was used as internal control. PPT but not DPN significantly increased amphiregulin expression. Amphiregulin expression in the PPT/DPN group was significantly lower than that in the PPT group. Data are shown as means \pm SD. The p values are as follows: 0.003 for PPT vs. control; 0.013 for PPT&DPN vs. PPT (*p < 0.05; **p < 0.01).

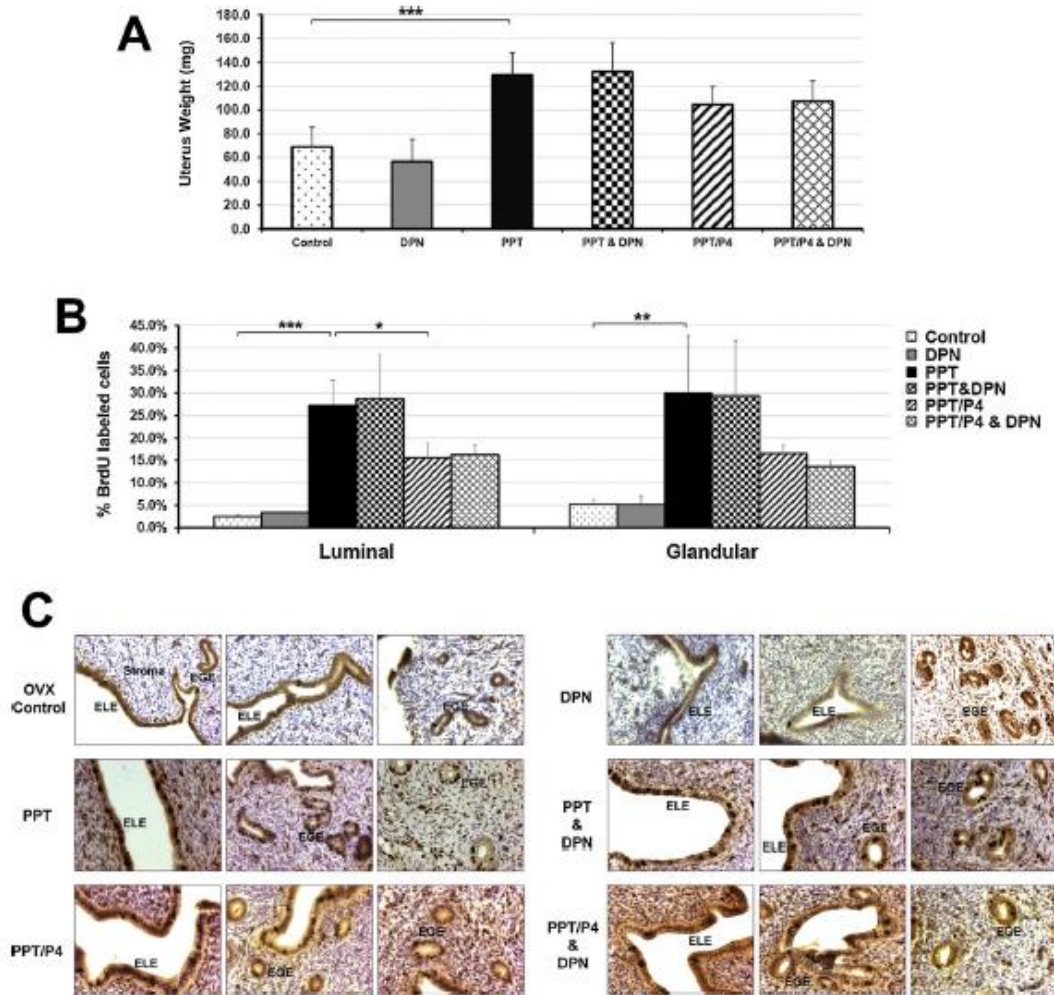


Figure 5. The estrogenic activity of PPT in the uterus is opposed by progesterone but not by DPN.

(A) Uterine wet weight (UWW) in different groups of rats. PPT but not DPN had uterotrophic activity. The uterotrophic activity of PPT was opposed by progesterone but not by DPN. Data are shown as means \pm SD. *** $p < 0.001$ for the PPT group vs. the control group. (B) Percentage of BrdU-labeled endometrial luminal and glandular epithelial cells. PPT but not DPN significantly increased endometrial luminal and glandular

epithelial cell proliferation. The effect of PPT on endometrial epithelial cell proliferation was opposed by progesterone but not by DPN. Data are shown as means \pm SD. The p values are as follows: <0.001 for PPT vs. control in luminal epithelial cells, 0.003 for PPT vs. control in glandular epithelial cells; 0.014 for PPT/P4 vs. PPT in luminal epithelial cells, and 0.078 for PPT/P4 vs. PPT in glandular epithelial cells (*p < 0.05; **p < 0.01; ***p < 0.001). (C) Representative microimages showing BrdU-labeled proliferation cells in endometrial luminal and glandular cells. BrdU-labeled cells were detected by immunohistochemical staining using DAB as substrate (brown color) and hematoxylin for counterstaining. ELE, endometrial luminal epithelial cells; EGE, endometrial glandular epithelial cells. Magnification, 400 \times .

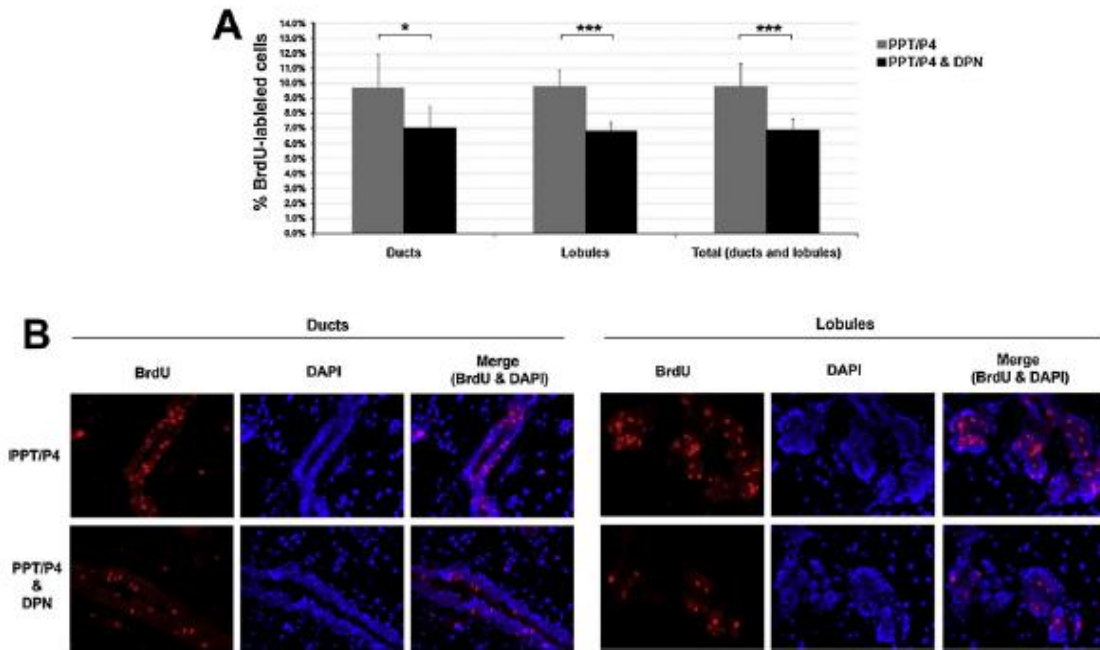


Figure 6. DPN inhibits mammary cell proliferation induced by PPT and progesterone.

(A) Percentage of BrdU-labeled mammary epithelial cells. See Fig. 2 to compare with other groups of rats. PPT and progesterone (P4) were synergistic in promoting mammary epithelial cell proliferation. When co-administered with PPT/P4, DPN significantly decreased mammary cell proliferation caused by PPT/P4. Data are shown as means \pm SD. The p values are as follows: <0.001 for PPT/P4 vs. PPT in the ducts, lobules, and totals; 0.02 for PPT/P4 & DPN vs. PPT/P4 in the ducts, <0.001 for PPT/P4 & DPN vs. PPT/P4 in the lobules and totals (* $p < 0.05$; *** $p < 0.001$). (B) Representative microimages showing BrdU-labeled proliferation cells in the mammary ductal and lobular structures. BrdU-labeled cells were detected by immunofluorescent staining with BrdU stained with red fluorescence; nuclei were counterstained with DAPI to give blue fluorescence. Images of

BrdU staining and DAPI staining were merged to show the location of BrdU-labeled cells.

Magnification, 400×.

CHAPTER 3: Overexpression of estrogen receptor beta inhibits mammary epithelial cell proliferation in Sprague Dawley rats

Abstract

The function of estrogen is mediated by two types of estrogen receptors, estrogen receptor α (ER α) and estrogen receptor β (ER β). The mitogenic action of estrogen in the mammary gland is mediated by mainly by ER α , whereas the function of ER β is less understood. Accumulating evidence indicates that ER β is able to antagonize the activity of ER α in many cellular processes. In the previous studies, we demonstrated that activation of endogenous ER β can counteract the mitogenic action of ER α in the mammary gland. In many breast cancers, ER β expression is decreased and overexpression of ER β in breast cancer cell lines decreased cell proliferation, indicating that high levels of ER β may inhibit cell proliferation. In present study, we used lentiviral infection to overexpress ER β in normal rat mammary gland and determined its effect on mammary cell proliferation. We first evaluated the expression of endogenous ER α and ER β in the mammary gland and the mammary cell proliferation rate during the estrous cycles. In the proestrus phase, ER α was detected in about 20% of mammary epithelial cells; in the diestrus phase, no ER α staining was detected in mammary epithelial cells. In the proestrus phase, ER β was expressed in

more than 50% of mammary epithelial cells and ER β staining was found in some stromal cells as well. In the diestrus phase, ER β was expressed in about 20% of mammary epithelial cells, the staining intensity of ER β was much weaker than that in the proestrus phase. In the mammary glands infected with lentivirus to overexpress ER α or ER β , ER α overexpression significantly increased cell proliferation in diestrus phase, whereas ER β overexpression significantly decreased cell proliferation in both proestrus and diestrus phases. These data provided the first *in vivo* evidence that mammary cell proliferation is decreased by high levels of ER β expression, supporting that ER β is a negative regulator of mammary cell proliferation. Decreased levels of ER β or the ratio of ER β /ER α may contribute breast cancer initiation and progression.

Introduction

Development of the mammary gland occurs in distinct stages, embryonic, neonate, puberty, pregnancy, lactation, and involution (Visvader et al. 2003). The mammary gland only contains simple and rudimentary ducts at birth (Sinha et al. 1969). During the puberty stage, due to a large increase in systemic hormones, the mammary gland cells undergo rapid proliferation, which contributes to extensive ductal branching and elongation (Ruan et al. 1995, Briskin et al. 1998, Silberstein 2001, Mulac-Jericevic et al. 2003). However, the full development and differentiation of mammary gland can be completed only after pregnancy and lactation (Anderson et al. 1990).

Mammary gland cell proliferation, differentiation, and apoptosis undergo dynamic alterations in different phases of the estrous cycle (Schedin et al. 2000). It has been demonstrated by the vast majority of studies that the highest mammary cell proliferation rate is found in the luteal phase of the human menstrual cycle (Ferguson et al. 1981, Vogel et al. 1981, Potten et al. 1988, Söderqvist et al. 1997). In mice and rats, the mammary cell proliferation rate in the diestrus phase is several times higher than that in the proestrus phase (Schedin et al. 2000, Fata et al. 2001). The data on whether alveolar and ductal structures possess different cell proliferation rates are controversial. There is one study showing that the proliferation rate was similar in the alveolar and ductal structures of female breast tissue (Söderqvist et al. 1997). However, Fata and his colleagues found that epithelial cell proliferation rate in the alveolar structure is significantly higher than that in the ductal structure (Fata et al. 2001).

The development of mammary gland is regulated largely by systemic hormones, including estrogen, progesterone, growth hormone, prolactin, oxytocin, glucocorticoids, thyroid hormones, and insulin (Hadley et al. 2006). Estrogen, mainly produced by the ovary, is perhaps the most important hormone in regulating mammary gland development (Berry et al. 2003). Local administration of anti-estrogen into the mammary gland of mouse inhibits mammary cell proliferation and simplifies the pattern of ductal branching (Silberstein et al. 1994). Ovariectomized (the ovaries are removed) animals have reduced mammary gland growth, which can be restored by exogenous estrogen application (Berry

et al. 2003, Cheng et al. 2005). Estrogen also stimulates mammary gland development indirectly by inducing the production of growth factors that play an important role in regulating cell proliferation and differentiation (Mallepell et al. 2006, Ciarloni et al. 2007). For example, amphiregulin, an epidermal growth factor receptor (EGFR) ligand required for ductal elongation and side branching in the mammary gland, is an important paracrine mediator of estrogen activity (Ciarloni et al. 2007).

The biological actions of estrogen are mediated by two types of estrogen receptors, estrogen receptor α (ER α) and estrogen receptor β (ER β) (Zhang et al. 2003, Zhao et al. 2008). Both types of estrogen receptors belong to the nuclear receptor superfamily and are involved in the transcriptional regulation of their target genes (Green et al. 1986, Kuiper et al. 1996, McDonnell et al. 2002). ER α is known as the primary estrogen receptor expressed in mammary gland and its expression level is much higher than that of ER β (Jensen et al. 2001, Pettersson et al. 2001, Shyamala et al. 2002). ER α is expressed only in epithelial cells, it is not expressed in stromal or myoepithelial cells (Saji et al. 2000). In ER α knock-out mice, the mammary glands remain rudimentary after birth and there is no further growth in later stages of development, indicating that ER α is required for mammary gland development (Bocchinfuso et al. 1997). ER α has been shown essential for the mitogenic effect of estrogen on mammary gland cell proliferation both *in vivo* and *in vitro* (Bocchinfuso et al. 1997, Tilli et al. 2003, Mallepell et al. 2006).

Compared with ER α , ER β is much less studied and its function is not fully understood. ER β expression has been detected in epithelial cells, stromal cells and myoepithelial cells (Palmieri et al. 2002, Speirs et al. 2004). ER β knock-out mice have relatively normal mammary gland structures during puberty and are able to lactate normally after pregnancy (Förster et al. 2002, Walker et al. 2004). However, the ER β knockout mice have delayed side branching in the puberty stage and the lobuloalveolar structures are less developed during pregnancy, suggesting that ER β is involved in mammary cell differentiation and alveologenesis (Förster et al. 2002, Walker et al. 2004). Unlike ER α , ER β has been implicated as a negative regulator of cell proliferation in both normal breast tissues and breast tumors (Lazennec et al. 2001, Liu et al. 2002, Visvader et al. 2003, Paruthiyil et al. 2004, Helguero et al. 2005, Song et al. 2012). Introducing ER β into immortalized or breast tumor cell lines inhibits cell proliferation (Lazennec et al. 2001, Liu et al. 2002, Visvader et al. 2003, Paruthiyil et al. 2004, Helguero et al. 2005). ER β overexpression has also been shown to inhibit cell proliferation and tumor growth in MCF7 and T47D xenograft tumors (Paruthiyil et al. 2004, Hartman et al. 2009). Moreover, ER β expression is found to be decreased or lost in many primary breast tumors and ER β levels are inversely correlated with the survival rate (Zhao et al. 2003, Girault et al. 2004, Grubberger-Saal et al. 2007, Honma et al. 2008). In chapter two of this dissertation, we examined the mammary cell proliferation by using ER β selective agonist DPN. We found

that activation of ER β inhibited cell proliferation in OVX rats. There is no report on whether ER β overexpression can inhibit mammary cell proliferation *in vivo*.

In present study, we further investigated whether the effect of ER β on mammary cell proliferation is affected by the expression levels of ER β . We first evaluated the expression of endogenous ER α and ER β in the mammary gland and the mammary cell proliferation rate during the estrous cycles. We demonstrated that mammary epithelial cell proliferation rate is similar in alveolar and ductal structures. Overexpression of ER α significantly increased cell proliferation in the diestrus phase. Overexpression of ER β significantly decreased cell proliferation in both proestrus and diestrus phases. Amphiregulin gene expression is increased by overexpressing ER α but not ER β . These data provided the first *in vivo* evidence that mammary cell proliferation is decreased by high levels of ER β expression, supporting that ER β is a negative regulator of mammary cell proliferation.

Methods

Lentivirus Production

The cDNA of ER α and ER β are the gifts from Dr. Yongkui Jing (Mount Sinai School of Medicine) and Dr. Kenneth S. Korach (National Institute of Environmental Health Science / National Institute of Health). EGFP and human ER α -IRES-EGFP cDNA were subcloned into plenti6/V5-DEST of the ViraPower™ Lentiviral Expression System

(Invitrogen, Carlsbad, CA). Human ER β cDNA was subcloned into pLVX_IRES_ZsGreen1 of the Lenti-X™ Lentiviral Expression Systems (Clontech, Palo Alto, CA). Human ER α and ER β cDNA instead of rat ER α and ER β cDNA were used to make lentivirus. The comparison of amino acid sequence is shown in figure 7. Human ER α and rat ER α proteins have 88% amino acid identity. Human and rat ER β share an overall amino acid identity of 89%. 293FT (Invitrogen, Carlsbad, CA) cells were co-transfected with the lentivirus plasmids and the packaging/helper plasmids for 62-72 hours following the manufacturer's instructions for production of lentiviruses. Generated lentiviruses in the culture medium were collected and filtered through 0.45 μ m PVDF filter (Millipore, Billerica, MA). Lentiviral stocks were concentrated using Lenti-X concentrator (Clontech, Palo Alto, CA).

Animal Treatment and Sample Collection

All animal experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Vermont. Thirteen weeks old virgin female Sprague-Dawley rats were purchased from Charles River Laboratories (Senneville, Canada) and were housed with a 12-hour light and dark cycle with free access to water and food. Thirty two rats were randomly assigned to four groups, the uninfected control group, the EGFP group, the ER α overexpressing group and the ER β overexpression group. Rats were anesthetized first and the replication incompetent lentiviruses expressing

EGFP or estrogen receptors were introduced via intraductal instillation to infect the mammary gland cells. The tit was cut and the central duct of the 4th pair mammary gland was injected with 20 μ l of solution containing lentiviruses, polybrene (8 mg/ml) and fast green (0.5 mg/ml) by using Hamilton syringe. Vaginal smear was used to determine the estrous cycle 10 days (about two menstrual cycles) after the intraductal infusion. Four rats in each group were sacrificed in pro-estrous stage and 4 rats in diestrus stage. Two hours before sacrifice, rats received an intraperitoneal infusion of 5-bromo-2'-deoxyuridine (BrdU) (2 mg/100 g body weight). Half of harvested tissue was fixed in neutral formalin for 48 hours before being processed for paraffin embedding (Pathology lab, Fletcher Allen Health Care, VT) and the other half was snap-frozen and stored in liquid nitrogen for RNA isolation.

Western Blot and Slot Blot

Rat mammary gland tissue samples were homogenized using a Dounce homogenizer, on ice, in lysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2.5 mM sodium pyrophosphate, 1 mM sodium β -glycerophosphate, 5 mM NaF, 1 mM Na₃VO₄, 1 mM phenylmethylsulfonyl fluoride, 10% Nonidet P40, and protease inhibitor mixture (Roche Molecular Biochemicals, Indianapolis, IN)). Fat tissue and cell debris were removed by centrifugation (14,000 \times g for 15 min at 4 $^{\circ}$ C). Cell lysates were sonicated and cellular debris were removed by centrifugation (14,000 \times g for 15min at 4 $^{\circ}$ C). The protein

concentration was determined using the BioRad DC protein assay reagent (BioRad, Hercules, CA) , and 150 µg protein from each treatment were loaded on 10% SDS-PAGE gel and then transferred onto PVDF membranes after electrophoresis (Millipore, Bedford, MA). For immunoblotting, the membranes were blocked with Tris-Buffered Saline and Tween 20 (TBST) containing 5% non-fat milk and 3% Bovine Serum Albumin (BSA) for 1 hour at room temperature. Primary antibody was used at 1:200 dilution and incubated in TBST containing 3% BSA overnight at 4°C. Membranes were washed 4 times, 7 minutes each with TBST before blotting with second antibodies. Horseradish Peroxidase (HRP)-conjugated secondary antibody was diluted at 1:10,000 and incubated with membranes in TBST containing 5% non-fat milk for 1 hour at room temperature. The Western Lightning™ Chemiluminescence Reagent Plus (PerkinElmer, Waltham, MA) was used to detect protein immobilized on the membrane.

Same amount of protein (275 µg) from each treatment was transferred to nitrocellulose membrane using the Minifold II slot-blot system (Schleicher & Schuell, Keene, NH). The membrane was hybridized with anti-ZsGreen antibody (Clontech, Palo Alto, CA) and followed by HRP-conjugated secondary antibody. The Western Lightning™ Chemiluminescence Reagent Plus (PerkinElmer, Waltham, MA) was used to detect protein immobilized on the membrane. Protein lysate from ERβ-ZsGreen lentivirus infected 293FT cells was used as positive control.

Immunofluorescent (IF) and Immunohistochemical (IHC) Staining

293FT cells grown on glass coverslips were briefly washed with ice-cold Dulbecco's PBS and fixed with cold 4% paraformaldehyde containing 0.01% Triton X-100 for 25 min on ice. After washing once briefly with PBS-T (Dulbecco's PBS containing 0.01% Triton X-100), the cells were treated with 0.05% Triton x-100 in PBS for 10 min for permeabilization. The cells were washed once briefly with PBS-T, followed by blocking in PBS-T containing 5% normal serum and 50 mM NH₄Cl for 30 min at room temperature. The cells were then incubated with primary antibodies diluted in PBS-T containing 1% normal serum. Anti-ER α antibody was diluted at 1:200, and anti-ER β at 1:40. After overnight incubation at 4 °C, the cells were washed four times with PBS-T, 7 min each time. Then the cells were incubated for 1 hour at room temperature with fluorescent dye-conjugated secondary antibodies (1:200) in PBST containing 1% normal serum. The cells were washed 4 times with PBS-T, 7 min each time before mounted on slides with VectoShield with DAPI (Vector Lab, Burlingame, CA).

Fixed and embedded mammary tissues were cut into 5- μ m sections and mounted onto gelatin-coated slides. IHC and IF assays were used to assess the BrdU-labeled cells and the expression of ER α and ER β following conventional procedures. The sources for the antibodies were as follows: ER α antibody (MC-20) from Santa Cruz Biotechnology, ER β (14C8) and BrdU antibodies from Abcam, cyclin D1 antibody (DCS-6) from Fisher

Scientific/Pierce. For IF staining, Alexa Fluor 488 (Invitrogen, Billerica, MA) was used for green fluorescence, Rhodamine Red (Jackson ImmunoResearch Laboratories, West Grove, PA) was used for red fluorescence, and DAPI contained in Mounting Medium (Vector Lab, Burlingame, CA) was used for nucleus staining to give blue fluorescence. For IHC staining, VECTASTAIN Elite ABC Kit (Vector Lab, Burlingame, CA) was used following the manufacturer's procedure. DAB (3, 3'-diaminobenzidine; Vector Lab,) was used as the peroxidase substrate to develop brown color and Hematoxylin QS (Vector Lab, Burlingame, CA) was used for counterstaining. Antigen retrieval was carried out by microwaving (700 W) slides in 10 mM citrate buffer (pH 6.0), ER α and BrdU for 11 min and ER β for 25 min. The slides were washed once briefly with PBS-T, followed by blocking in PBS-T containing 5% normal serum for 1 hour at room temperature. ER α antibody was used at 1:200 dilution, ER β antibody at 1:40 dilution and BrdU antibody at 1:150 dilution. Immunostaining slides were examined under Olympus BX50 Fluorescence Microscope (Microscopy Imaging Center, UVM).

Images were taken with QImaging Retiga 2000R digital camera (QImaging, Surrey, BC, Canada) and Adobe Photoshop was used for further processing of the digital images. The brightness, contrast and exposure level were adjusted for cell counting.

The software Image J (NIH, Bethesda, MD) was used for cell counting. For each mammary gland, at least 500 ductal epithelial cells and 600 lobular epithelial cells were counted. For IHC images of BrdU staining, based on the staining intensity, the staining

was assigned at four levels. The level 0 is unstained, level 1 is weak staining, level 2 is moderate and level 3 is intensive staining. The cells with staining levels 0 and 1 were counted as BrdU negative staining cells and the cells with staining levels 2 and 3 were counted as BrdU positive staining cells.

RNA Isolation and Quantitative Real-Time PCR Assay

Total RNA was extracted with Trizol reagent (Invitrogen, Billerica, MA) and further purified with RNeasy Kit (Qiagen, Valencia, CA). The concentrations of isolated RNA were measured with a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE). Two μg of total purified RNA was reverse transcribed using SuperScript III reverse transcriptase (Invitrogen, Billerica, MA). TaqMan Gene Expression Assay Mix (Applied Biosystems, Foster City, CA) were used to quantify the mRNA expression levels of amphiregulin (Areg) and β -actin (endogenous control). Real-time PCR for RNA from each sample was performed in duplicates in a 20 μl reaction system containing 10 μl Universal PCR Master Mix (Applied Biosystems, # 4352042), 1 μl TaqMan Assay Mix, and 9 μl diluted cDNA. The reaction conditions for amplification of DNA were 50 $^{\circ}\text{C}$ for 2 min, 95 $^{\circ}\text{C}$ for 10 min and 40 cycles of 95 $^{\circ}\text{C}$ for 15 s and 60 $^{\circ}\text{C}$ for 1 min. The average Ct from each sample was used for further calculation. The relative gene expression of amphiregulin transcripts were calculated using the $2^{-\Delta\Delta\text{Ct}}$ method (Livak et al. 2001).

Statistical Analysis

All statistical analyses were performed using the JMP software (SAS, Cary, NC). One-way analysis of variance (ANOVA) analysis followed by Tukey's post hoc test were carried out on collected data and p value less than 0.05 was considered statistically significant.

Results

Expression of ER α and ER β in the Mammary Gland during Estrous Cycle

We compared the expression pattern of ER α and ER β in the rat mammary gland in the proestrus phase with that in the diestrus phase. For the ER α staining, 20% of mammary epithelial cells were stained positive in the proestrus phase and there was ER α staining in the diestrus phase; these data are consistent with the data by other studies (Fig. 8). For the ER β staining, ER β staining was found in more than 50% of mammary epithelial cells in the proestrus phase, but only about 20% of mammary epithelial cells showed ER β staining in the diestrus phase (Fig. 8). The staining intensity of ER β was much weaker in the diestrus phase than that in the proestrus phase (Fig. 8). In addition, ER β staining was detected in some stromal cells in both the proestrus and diestrus phases, and no significant difference was observed in the fraction of such ER β positive stromal cells in the two phases. These results first confirmed that ER α is only expressed in the proestrus phase and further

demonstrated that ER β is expressed in both the proestrus and diestrus phases, and ER β expression level was lower in the diestrus phase than that in the proestrus phase.

Overexpression of ER α and ER β in Mammary Gland by Lentiviral Infection

We first infected 293FT cell with ER α – EGFP lentivirus. ER α and EGFP co-expressed in the all the infected cells (Fig. 9). EGFP and ER β – ZsGreen lentiviruses were also tested in 293FT cell. EGFP expressed in the infected cells and ER β and ZsGreen co-expressed in all infected cells (data not shown). Then we infected rat mammary glands and determined the infection efficiency by evaluating the reporter gene expression levels in each group. The expression of reporter gene EGFP was analyzed by western blot (Fig. 10A), and the expression of reporter gene ZsGreen was analyzed with slot blot (Fig. 10B). As expected, expression of EGFP was found in the EGFP group and the ER α group, but not in the uninfected negative control group or the ER β group (Fig. 10A). Protein lysate from ER β -ZsGreen lentivirus infected 293FT cells was used as positive control for the slot blot to analyze the expression of ZsGreen (Fig. 10B). Expression of ZsGreen was detected in ER β -ZsGreen lentivirus infected mammary gland tissues, indicating that ER β -ZsGreen was successfully expressed by the infected mammary gland cells (Fig. 10B). In order to evaluate the infection efficiency, we performed the IHC staining for EGFP in mammary gland tissues from the EGFP group and the ER α group. EGFP staining was observed in over 50% of mammary epithelial cells in both groups, indicating that at least 50% of

epithelial cells were infected by lentiviruses to overexpress ER α and the reporter EGFP protein (Fig. 10C). These results demonstrated that rat mammary gland cells were successfully infected *in situ* by lentiviruses to overexpress ER α or ER β in mammary epithelial cells.

Effect of ER α and ER β Overexpression on Rat Mammary Cell Proliferation

Proliferating cells were labeled with BrdU and the mammary cell proliferation rate was determined by BrdU-staining. Representative images of BrdU-labeled proliferation cells in the mammary lobular and ductal structures are shown in Figure 9A and 10A. As expected, overexpression of EGFP did not affect the cell proliferation rate in both the proestrus and diestrus phases (Fig. 11B and 12B). Overexpression of ER α significantly increased mammary epithelial cell proliferation rate in the lobular structure but not in the ductal structure in the proestrus phase, and dramatically increased the proliferation rate in both the lobular and ductal structures in the diestrus phase, supporting the positive effect of ER α on cell proliferation during the estrous cycle (Fig. 11B and 12B). The proliferation rate was increased about 1.2 times in the proestrus phase and about 1.8 times in the diestrus phase (Fig. 11B and 12B), indicating that after high level of estrogen stimulation in the estrus phase, ER α significantly increased mammary cell proliferation rate in the diestrus phase.

In the ER β group, overexpression of ER β significantly decreased cell proliferation rate in both lobular and ductal structures in the proestrus phase and decreased cell proliferation rate in ductal structure in the diestrus phase, indicating that high levels of ER β can inhibit mammary cell proliferation *in vivo* (Fig. 11B and 12B). Furthermore, the mammary epithelial cell proliferation rate was significantly lower in the ER β group than that in the ER α group in both lobular and ductal structures, indicating the distinct role of these two types of ERs in regulation of cell proliferation during the estrous cycle (Fig. 11B and 12B). Taken together, these data support that ER α overexpression promotes and ER β overexpression inhibits mammary epithelial cell proliferation in both the proestrus and diestrus phases.

Effect of ER β on Amphiregulin Expression

The epidermal growth factor (EGF) family member amphiregulin is an important mediator of estrogen action (Ciarloni et al. 2007). Estrogen induces amphiregulin expression and secretion via ER α , which activates EGFR signaling pathway to stimulate cell proliferation (Visvader et al. 2003, Ciarloni et al. 2007, Kariagina et al. 2010). As shown above, ER β overexpression inhibits mammary cell proliferation, we wondered whether ER β inhibits mammary cell proliferation by suppressing amphiregulin expression. To address this question, we quantified amphiregulin transcript levels in mammary gland tissues overexpressing ER α and ER β was quantified by RT-PCR. In the ER α group the

expression level of amphiregulin increased about 1.5 fold in the proestrus phase (Fig. 13A) and about 3.2 fold in the diestrus phase when compared to the uninfected EGFP group (Fig. 13B). In the ER β group, the expression level of amphiregulin remained about the same as that in the EGFP group in the proestrus phase (Fig. 13A) and slightly but not significantly decreased in the diestrus phase (Fig. 13B). These data support that ER α induces mammary cell proliferation by up-regulating amphiregulin expression. However, the inhibitory effect of ER β on mammary cell proliferation may involve downregulating amphiregulin expression, but there are also other unknown mechanisms that are more responsible for the inhibitory effect.

Discussion

Both ER α and ER β are expressed in the mammary gland and therefore the estrogen action in the mammary gland may be mediated by both types of estrogen receptors (Couse et al. 1999, Hanahan et al. 2000, Harris et al. 2002, Berry et al. 2003, Herynk et al. 2004, Hartman et al. 2006, Mallepell et al. 2006, Tan et al. 2009). Although ER α and ER β share large homology, accumulating evidence from *in vitro* and *in vivo* studies suggests that the two types of estrogen receptors may function differently (Harris 2007, Deroo et al. 2010, Warner et al. 2010). The expression of ER α differs from ER β expression and their expression level is tightly regulated in various estrogen regulated processes (Zhang et al. 2003). ER α expression has been demonstrated as the major regulatory node during normal mammary gland development (Jensen et al. 2001). Previous studies on the expression of

ER α showed ER α staining is detected in 10% to 40% of mammary epithelial cells and ER α expression in mammary epithelial cells is detected only in proestrus phase of the estrous cycle (Clarke et al. 1997, Saji et al. 2000, Cheng et al. 2004, Clarke 2004). ER β expression is found in epithelial cells and also some stromal cells (Palmieri et al. 2002, Schams et al. 2003). However, lack of studies on ER β expression *in vivo* limited exploring the function of ER β in cell proliferation in mammary gland during estrous cycle.

In the present study, we investigated the expression of ER α and ER β in the mammary gland of rats during estrous cycle and provided more *in vivo* expression analysis for further characterizing the function of ER β in mammary gland. Consistent with previous studies, we found that ER α existed in about 20% of mammary epithelial cells in the proestrus phase, but was not detected in mammary epithelial cells in the diestrus phase. Although ER α has been suggested to play a more important role in mammary gland development, interestingly, we observed that much more epithelial cells expressed ER β , with more than 50% in the proestrus phase and 20% in the diestrus phase. Consistent with many prior studies, we did not detect the existence of ER α in stromal cells in the rat mammary gland, and a small fraction of stromal cells was detected with ER β staining in both the proestrus and diestrus phases. A number of studies have shown the existence of ER β in stromal cells, but little is known about whether the fraction of ER β positive stromal cells change during the estrous cycle (Palmieri et al. 2002, Speirs et al. 2002). In the present

studies, we did not observe significant difference of ER β positive stromal cells in the estrous cycle. The function of ER β in the mammary stromal cells is not clear.

Epithelial cell proliferation rate in the mammary gland is highly regulated by steroid hormones which undergo dynamic changes during the estrous cycle. The mammary cell proliferation rates have been studied using different animal models and different proliferation markers. The overall trend is similar where the rate is lower in the proestrus phase and higher in the diestrus phase (Söderqvist et al. 1997, Navarrete et al. 2005). However it is worth noting that the expression of both ER α and ER β decreases from proestrus to diestrus. Considering the stimulatory role of ER α on cell proliferation, it is possible that the increase of proliferation rate in the diestrus phase may be due to the decreased level of ER β . Studies supporting this hypothesis have used breast tumors to show that loss of ER β would result higher proliferation rate in breast tumor (Zhao et al. 2003, Girault et al. 2004, Gruvberger-Saal et al. 2007, Honma et al. 2008).

To further dissect the function of ER β in regulation of mammary cell proliferation during the estrous cycle, we overexpressed ER α and ER β separately in the rat mammary gland using lentivirus carrying target gene and the reporter gene. The expression of the reporter and infection efficiency were quantified first to make sure that most mammary gland cells were infected.

After establishing the ER α and ER β *in vivo* overexpression system, we were able to evaluate the effect of ER α and ER β overexpression on epithelial cell proliferation in rat

mammary gland during the estrous cycle. Our data confirmed that overexpression of ER α significantly increased mammary cell proliferation rate in both the proestrus and diestrus phases, supporting the positive role of ER α in regulation of cell proliferation (Sicinski et al. 1997, Liu et al. 2002, Mallepell et al. 2006). Moreover, overexpression of ER α has a more dramatic stimulatory effect on cell proliferation in the diestrus phase, indicating that ER α regulates cell proliferation in a ligand dependent manner due to high level of estrogen stimulation in estrus phase.

Most studies on the effect of ER β on cell proliferation have been carried out *in vitro* and have suggested that ER β is a negative regulator of cell proliferation (Lazennec et al. 2001, Liu et al. 2002, Visvader et al. 2003, Paruthiyil et al. 2004, Helguero et al. 2005, Song et al. 2012). In our previous studies, we provided the first *in vivo* evidence that activation of endogenous ER β can counteract the mitogenic effect of ER α in the mammary gland (Song and Pan 2012). In the present study, we provided further *in vivo* evidence showing that overexpression of ER β significantly decreased cell proliferation rate in both the proestrus and diestrus phases. These data support that mammary cell proliferation rate is inversely correlated with ER β expression level.

The growth factor amphiregulin is one of the most important estrogen action mediators, which have been shown to be downstream of ER α in regulation of cell proliferation in the mammary gland (Ciarloni et al. 2007). Consistently, we found that amphiregulin transcript levels were significantly higher in ER α overexpressing mammary

gland. However, we did not observe any significant difference in the quantities of amphiregulin transcripts in mammary gland tissues overexpressing ER β , suggesting that unlike ER α , ER β may not affect the production of amphiregulin directly. In chapter two of this dissertation we have shown that cells with both activated ER α and ER β contain lower level of amphiregulin compared to cells with activated ER α alone. Collectively, our *in vivo* animal model studies demonstrated that ER β may counteract the proliferative effect of ER α in mammary gland either by high levels of ER β or by activation of endogenous ER β . There is also the possibility that the ratio of ER α /ER β is critical for mammary gland cell proliferation. In the proestrus phase, without high level of estrogen, the overall proliferation rate is low. In the ER α group, due to the high ratio of ER α /ER β , cell proliferation rate was increased about 1.2 fold. In contrast, cell proliferation rate was decreased 1.4 fold in the ER β group. In the diestrus phase, the proliferation rate was increased 1.8 fold in the ER α group and was decreased 1.6 fold in the ER β group. It has been shown that ER α and ER β can form heterodimers which are able to bind DNA and regulate transcription (Cowley et al. 1997). It is possible that the formation of ER α /ER β heterodimers decreases the number of ER α homodimers which results in lower transcription activity. In most breast tumors, ER α expression is much higher than that in normal breast tissue and ER β expression is found to be decreased or completely lost, which indicates that the ratio of ER α /ER β may be critical in regulating cell proliferation not only in normal mammary cells but also in

breast tumor cells (Zhao et al. 2003, Girault et al. 2004, Regan et al. 2006, Gruvberger-Saal et al. 2007, Honma et al. 2008).

Previous studies have shown that cell death induced by tamoxifen in ER β positive breast tumors is significant higher than that in ER β negative breast tumors (Honma et al. 2008). Thus, the expression level of ER β or the ratio of ER α /ER β might be used as biomarkers in breast cancer diagnosis and examination of the expression level of ER α and ER β may help determine appropriate treatments for different patients. In addition, both ER α and ER β can be served as the pharmaceutical targets to treat breast cancer. Due to the inhibitory effect of ER β on cell proliferation, specific agonists of ER β may be potential drugs for breast cancer.

As discussed above, we further characterized the expression pattern and the effect of ER α and ER β on epithelial cell proliferation in the mammary gland during estrous cycle. We found that both ER α and ER β expression levels are higher in the proestrus phase and decreases in the diestrus phase. By overexpressing ER α and ER β in the mammary gland *in vivo*, we showed that ER α stimulates whereas ER β inhibits epithelial cell proliferation in the mammary gland in both the proestrus and diestrus phases, implicating the distinct mechanism of the two types of estrogen receptors in regulating cell proliferation.

Acknowledgements

This project was supported by USDA Hatch and Vermont Cancer Center (VCC)/Lake Champlain Cancer Research Organization (LCCRO) funds. Microimaging

was performed in the UVM Microscopy Imaging Center, a facility center supported by VCC, LCCRO, and UVM College of Medicine.

References

- Anderson, R. and I. Wahab (1990). "Changes in parenchyma and stroma of goat udders during pregnancy, lactation and involution." Small Ruminant Research **3**(6): 605-615.
- Berry, S. D., P. M. Jobst, S. E. Ellis, R. D. Howard, A. V. Capuco and R. M. Akers (2003). "Mammary epithelial proliferation and estrogen receptor alpha expression in prepubertal heifers: effects of ovariectomy and growth hormone." Journal Dairy Science **86**(6): 2098-2105.
- Bocchinfuso, W. P. and K. S. Korach (1997). "Mammary gland development and tumorigenesis in estrogen receptor knockout mice." Journal Mammary Gland Biol Neoplasia **2**(4): 323-334.
- Bocchinfuso, W. P. and K. S. Korach (1997). "Mammary gland development and tumorigenesis in estrogen receptor knockout mice." Journal of Mammary Gland Biology and Neoplasia **2**(4): 323-334.
- Brisken, C., S. Park, T. Vass, J. P. Lydon, B. W. O'Malley and R. A. Weinberg (1998). "A paracrine role for the epithelial progesterone receptor in mammary gland development." Proceedings of the National Academy of Sciences USA **95**(9): 5076-5081.
- Cheng, G., Y. Li, Y. Omoto, Y. Wang, T. Berg, M. Nord, P. Vihko, M. Warner, Y. S. Piao and J. A. Gustafsson (2005). "Differential regulation of estrogen receptor (ER)alpha and ERbeta in primate mammary gland." The Journal of Clinical Endocrinology & Metabolism **90**(1): 435-444.
- Cheng, G., Z. Weihua, M. Warner and J. A. Gustafsson (2004). "Estrogen receptors ER alpha and ER beta in proliferation in the rodent mammary gland." Proceedings of the National Academy of Sciences USA **101**(11): 3739-3746.
- Ciarloni, L., S. Mallepell and C. Brisken (2007). "Amphiregulin is an essential mediator of estrogen receptor alpha function in mammary gland development." Proceedings of the National Academy of Sciences USA **104**(13): 5455-5460.
- Ciarloni, L., S. Mallepell and C. Brisken (2007). "Amphiregulin is an essential mediator of estrogen receptor α function in mammary gland development." Proceedings of the National Academy of Sciences USA **104**(13): 5455-5460.
- Clarke, R. B. (2004). "Human breast cell proliferation and its relationship to steroid receptor expression." Climacteric **7**(2): 129-137.
- Clarke, R. B., A. Howell, C. S. Potten and E. Anderson (1997). "Dissociation between steroid receptor expression and cell proliferation in the human breast." Cancer Research **57**(22): 4987-4991.
- Couse, J. F. and K. S. Korach (1999). "Estrogen receptor null mice: what have we learned and where will they lead us?" Endocrine Reviews **20**(3): 358.

Cowley, S. M., S. Hoare, S. Mosselman and M. G. Parker (1997). "Estrogen receptors α and β form heterodimers on DNA." Journal of Biological Chemistry **272**(32): 19858-19862.

Deroo, B. J. and A. V. Buensuceso (2010). "Minireview: Estrogen receptor- β : mechanistic insights from recent studies." Molecular Endocrinology **24**(9): 1703-1714.

Förster, C., S. Mäkelä, A. Wäri, S. Kietz, D. Becker, K. Hultenby, M. Warner and J.-Å. Gustafsson (2002). "Involvement of estrogen receptor β in terminal differentiation of mammary gland epithelium." Proceedings of the National Academy of Sciences USA **99**(24): 15578-15583.

Fata, J. E., V. Chaudhary and R. Khokha (2001). "Cellular turnover in the mammary gland is correlated with systemic levels of progesterone and not 17 β -estradiol during the estrous cycle." Biology of Reproduction **65**(3): 680-688.

Ferguson, D. and T. Anderson (1981). "Morphological evaluation of cell turnover in relation to the menstrual cycle in the "resting" human breast." British Journal of Cancer **44**(2): 177.

Girault, I., C. Andrieu, S. Tozlu, F. Spyrtos, I. Bieche and R. Lidereau (2004). "Altered expression pattern of alternatively spliced estrogen receptor beta transcripts in breast carcinoma." Cancer letters **215**(1): 101-112.

Gruvberger-Saal, S. K., P. O. Bendahl, L. H. Saal, M. Laakso, C. Hegardt, P. Eden, C. Peterson, P. Malmstrom, J. Isola, A. Borg and M. Ferno (2007). "Estrogen receptor beta expression is associated with tamoxifen response in ERalpha-negative breast carcinoma." Clinical Cancer Research **13**(7): 1987-1994.

Hadley, M. and J. E. Levine (2006). Endocrinology, 6/e, Pearson Education India.

Hanahan, D. and R. A. Weinberg (2000). "The hallmarks of cancer." Cell **100**(1): 57-70.

Harris, H. A. (2007). "Estrogen receptor-beta: recent lessons from in vivo studies." Molecular Endocrinology **21**(1): 1-13.

Harris, H. A., J. A. Katzenellenbogen and B. S. Katzenellenbogen (2002). "Characterization of the biological roles of the estrogen receptors, ER α and ER β , in estrogen target tissues in vivo through the use of an ER α -selective ligand." Endocrinology **143**(11): 4172-4177.

Hartman, J., K. Lindberg, A. Morani, J. Inzunza, A. Ström and J.-Å. Gustafsson (2006). "Estrogen receptor β inhibits angiogenesis and growth of T47D breast cancer xenografts." Cancer Research **66**(23): 11207-11213.

Hartman, J., A. Strom and J. A. Gustafsson (2009). "Estrogen receptor beta in breast cancer--diagnostic and therapeutic implications." Steroids **74**(8): 635-641.

Helguero, L. A., M. H. Faulds, J. A. Gustafsson and L. A. Haldosen (2005). "Estrogen receptors alfa (ERalpha) and beta (ERbeta) differentially regulate proliferation and apoptosis of the normal murine mammary epithelial cell line HC11." Oncogene **24**(44): 6605-6616.

Herynk, M. H. and S. A. Fuqua (2004). "Estrogen receptor mutations in human disease." Endocrine Reviews **25**(6): 869-898.

Honma, N., R. Horii, T. Iwase, S. Saji, M. Younes, K. Takubo, M. Matsuura, Y. Ito, F. Akiyama and G. Sakamoto (2008). "Clinical importance of estrogen receptor-beta evaluation in breast cancer patients treated with adjuvant tamoxifen therapy." Journal of Clinical Oncology **26**(22): 3727-3734.

Jensen, E. V., G. Cheng, C. Palmieri, S. Saji, S. Mäkelä, S. Van Noorden, T. Wahlström, M. Warner, R. C. Coombes and J.-Å. Gustafsson (2001). "Estrogen receptors and proliferation markers in primary and recurrent breast cancer." Proceedings of the National Academy of Sciences USA **98**(26): 15197-15202.

Kariagina, A., J. Xie, J. R. Leppprandt and S. Z. Haslam (2010). "Amphiregulin mediates estrogen, progesterone, and EGFR signaling in the normal rat mammary gland and in hormone-dependent rat mammary cancers." Hormones and Cancer **1**(5): 229-244.

Kuiper, G., E. Enmark, M. Peltö-Huikko, S. Nilsson and J.-A. Gustafsson (1996). "Cloning of a novel receptor expressed in rat prostate and ovary." Proceedings of the National Academy of Sciences USA **93**(12): 5925-5930.

Lazennec, G., D. Bresson, A. Lucas, C. Chauveau and F. Vignon (2001). "ER beta inhibits proliferation and invasion of breast cancer cells." Endocrinology **142**(9): 4120-4130.

Liu, M.-M., C. Albanese, C. M. Anderson, K. Hilty, P. Webb, R. M. Uht, R. H. Price, R. G. Pestell and P. J. Kushner (2002). "Opposing action of estrogen receptors α and β on cyclin D1 gene expression." Journal of Biological Chemistry **277**(27): 24353-24360.

Liu, M. M., C. Albanese, C. M. Anderson, K. Hilty, P. Webb, R. M. Uht, R. H. Price, Jr., R. G. Pestell and P. J. Kushner (2002). "Opposing action of estrogen receptors alpha and beta on cyclin D1 gene expression." The Journal of Biological Chemistry **277**(27): 24353-24360.

Livak, K. J. and T. D. Schmittgen (2001). "Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method." Methods **25**(4): 402-408.

Mallepell, S., A. Krust, P. Chambon and C. Brisken (2006). "Paracrine signaling through the epithelial estrogen receptor alpha is required for proliferation and morphogenesis in the mammary gland." Proceedings of the National Academy of Sciences USA **103**(7): 2196-2201.

Mallepell, S., A. Krust, P. Chambon and C. Brisken (2006). "Paracrine signaling through the epithelial estrogen receptor α is required for proliferation and morphogenesis in the mammary gland." Proceedings of the National Academy of Sciences USA **103**(7): 2196-2201.

McDonnell, D. P. and J. D. Norris (2002). "Connections and regulation of the human estrogen receptor." Science **296**(5573): 1642-1644.

Mulac-Jericevic, B., J. P. Lydon, F. J. DeMayo and O. M. Conneely (2003). "Defective mammary gland morphogenesis in mice lacking the progesterone receptor B isoform." Proceedings of the National Academy of Sciences USA **100**(17): 9744-9749.

Navarrete, M., C. M. Maier, R. Falzoni, L. Quadros, G. R. Lima, E. C. Baracat and A. Nazario (2005). "Assessment of the proliferative, apoptotic and cellular renovation indices of the human mammary epithelium during the follicular and luteal phases of the menstrual cycle." Breast Cancer Research **7**(3): R306-313.

Palmieri, C., G. Cheng, S. Saji, M. Zelada-Hedman, Z. Weihua, S. Van Noorden, T. Wahlstrom, R. Coombes, M. Warner and J. Gustafsson (2002). "Estrogen receptor beta in breast cancer." Endocrine-related Cancer **9**(1): 1-13.

Paruthiyil, S., H. Parmar, V. Kerekatte, G. R. Cunha, G. L. Firestone and D. C. Leitman (2004). "Estrogen receptor beta inhibits human breast cancer cell proliferation and tumor formation by causing a G2 cell cycle arrest." Cancer Research **64**(1): 423-428.

Pettersson, K. and J.-Å. Gustafsson (2001). "Role of estrogen receptor beta in estrogen action." Annual Review of Physiology **63**(1): 165-192.

Potten, C. S., R. Watson, G. Williams, S. Tickle, S. A. Roberts, M. Harris and A. Howell (1988). "The effect of age and menstrual cycle upon proliferative activity of the normal human breast." British Journal of Cancer **58**(2): 163.

Regan, M. M., G. Viale, M. G. Mastropasqua, E. Maiorano, R. Golouh, A. Carbone, B. Brown, M. Suurkõla, G. Langman and L. Mazzucchelli (2006). "Re-evaluating adjuvant breast cancer trials: assessing hormone receptor status by immunohistochemical versus extraction assays." Journal of the National Cancer Institute **98**(21): 1571-1581.

Ruan, W., V. Catanese, R. Wiczorek, M. Feldman and D. Kleinberg (1995). "Estradiol enhances the stimulatory effect of insulin-like growth factor-I (IGF-I) on mammary development and growth hormone-induced IGF-I messenger ribonucleic acid." Endocrinology **136**(3): 1296-1302.

Söderqvist, G., E. Isaksson, B. von Schoultz, K. Carlström, E. Tani and L. Skoog (1997). "Proliferation of breast epithelial cells in healthy women during the menstrual cycle." American Journal of Obstetrics and Gynecology **176**(1): 123-128.

Saji, S., E. V. Jensen, S. Nilsson, T. Rylander, M. Warner and J.-Å. Gustafsson (2000). "Estrogen receptors α and β in the rodent mammary gland." Breast Cancer Research **2**(Suppl 1): S. 11.

Saji, S., E. V. Jensen, S. Nilsson, T. Rylander, M. Warner and J. A. Gustafsson (2000). "Estrogen receptors alpha and beta in the rodent mammary gland." Proceedings of the National Academy of Sciences USA **97**(1): 337-342.

Schams, D., S. Kohlenberg, W. Amselgruber, B. Berisha, M. Pfaffl and F. Sinowatz (2003). "Expression and localisation of oestrogen and progesterone receptors in the bovine mammary gland during development, function and involution." Journal of Endocrinology **177**(2): 305-317.

Schedin, P., T. Mitrenga and M. Kaeck (2000). "Estrous cycle regulation of mammary epithelial cell proliferation, differentiation, and death in the Sprague-Dawley rat: a model for investigating the role of estrous cycling in mammary carcinogenesis." Journal of Mammary Gland Biology and Neoplasia **5**(2): 211-225.

Shyamala, G., Y.-C. Chou, S. Louie, R. Guzman, G. Smith and S. Nandi (2002). "Cellular expression of estrogen and progesterone receptors in mammary glands: regulation by hormones, development and aging." The Journal of Steroid Biochemistry and Molecular Biology **80**(2): 137-148.

Sicinski, P. and R. A. Weinberg (1997). "A specific role for cyclin D1 in mammary gland development." Journal of Mammary Gland Biology and Neoplasia **2**(4): 335-342.

Silberstein, G. B. (2001). "Postnatal mammary gland morphogenesis." Microscopy Research and Technique **52**(2): 155-162.

Silberstein, G. B., K. Van Horn, G. Shyamala and C. W. Daniel (1994). "Essential role of endogenous estrogen in directly stimulating mammary growth demonstrated by implants containing pure antiestrogens." Endocrinology **134**(1): 84-90.

Sinha, Y. and H. A. Tucker (1969). "Mammary development and pituitary prolactin level of heifers from birth through puberty and during the estrous cycle." Journal of Dairy Science **52**(4): 507-512.

Song, X. and Z.-Z. Pan (2012). "Estrogen receptor-beta agonist diarylpropionitrile counteracts the estrogenic activity of estrogen receptor-alpha agonist propylpyrazole-triol in the mammary gland of ovariectomized Sprague Dawley rats." The Journal of Steroid Biochemistry and Molecular Biology **130**(1-2): 26-35.

Speirs, V., P. J. Carder, S. Lane, D. Dodwell, M. R. Lansdown and A. M. Hanby (2004). "Oestrogen receptor β : what it means for patients with breast cancer." The Lancet Oncology **5**(3): 174-181.

Speirs, V., G. Skliris, S. Burdall and P. Carder (2002). "Distinct expression patterns of ER α and ER β in normal human mammary gland." Journal of Clinical Pathology **55**(5): 371-374.

Tan, H., Y. Zhong and Z. Pan (2009). "Autocrine regulation of cell proliferation by estrogen receptor-alpha in estrogen receptor-alpha-positive breast cancer cell lines." BMC Cancer **9**(1): 31.

Tilli, M. T., M. S. Frech, M. E. Steed, K. S. Hruska, M. D. Johnson, J. A. Flaws and P. A. Furth (2003). "Introduction of estrogen receptor-alpha into the tTA/TAg conditional mouse model precipitates the development of estrogen-responsive mammary adenocarcinoma." American Journal of Pathology **163**(5): 1713-1719.

Visvader, J. E. and G. J. Lindeman (2003). "Transcriptional regulators in mammary gland development and cancer." Int J Biochem Cell Biol **35**(7): 1034-1051.

Visvader, J. E. and G. J. Lindeman (2003). "Transcriptional regulators in mammary gland development and cancer." The International Journal of Biochemistry & Cell Biology **35**(7): 1034-1051.

Vogel, P., N. Georgiade, B. Fetter, F. Vogel and K. McCarty Jr (1981). "The correlation of histologic changes in the human breast with the menstrual cycle." The American Journal of Pathology **104**(1): 23.

Walker, V. R. and K. S. Korach (2004). "Estrogen receptor knockout mice as a model for endocrine research." ILAR journal **45**(4): 455-461.

Warner, M. and J.-Å. Gustafsson (2010). "The role of estrogen receptor β (ER β) in malignant diseases—A new potential target for antiproliferative drugs in prevention and treatment of cancer." Biochemical and Biophysical Research Communications **396**(1): 63-66.

Zhang, W., S. Andersson, G. Cheng, E. R. Simpson, M. Warner and J.-Å. Gustafsson (2003). "Update on estrogen signaling." FEBS Letters **546**(1): 17-24.

Zhao, C., K. Dahlman-Wright and J. A. Gustafsson (2008). "Estrogen receptor beta: an overview and update." Nuclear Receptor Signaling **6**: e003.

Zhao, C., E. W. Lam, A. Sunter, E. Enmark, M. T. De Bella, R. C. Coombes, J. A. Gustafsson and K. Dahlman-Wright (2003). "Expression of estrogen receptor beta isoforms in normal breast epithelial cells and breast cancer: regulation by methylation." Oncogene **22**(48): 7600-7606.

Figures

A

hER α 1 MTMTLHTKASGMALLHQIQGNELEPLNRPQLKIPTEERPLGEVYLDSSKPAVYNYPEGAAY
rER α 1 MTMTLHTKASGMALLHQIQGNELEPLNRPQLKMPMERALGEVYVDNSKPAVENYPEGAAY

hER α 61 EFNAAAAANAQ-----VYGOITGLPYGPGSEAAAFGSNGLGTFPLNSVSPSPMLLLHPPP
rER α 61 EFNAAAAAAGASAPVYGQSSITYGPGSEAAAFGANSGLGAFPOLNSVSPSPMLLLHPPP

hER α 116 QLSPFLOPHGQVPPYYLENEPSGYTVREAGPPAFYRPNSDNRRQGGRRERLASINDKGSMA
rER α 121 HVSPFLHPHGQVPPYYLENEPSAYAVRDTGPPAFYRSNSDNRRQNGRRERLSSSSSEKGNMI

hER α 176 MESAKETRYCAVCNDYASGYHYGVWSCEGCKAFFKRSIQGHNDYMC PATNQCTIDKNRRK
rER α 181 MESAKETRYCAVCNDYASGYHYGVWSCEGCKAFFKRSIQGHNDYMC PATNQCTIDKNRRK

hER α 236 SCQACRLRKCYEVMKGGIRKDRRGGRLKHKRQRDDGEGRGEVGSAGDMRAANLWPSF
rER α 241 SCQACRLRKCYEVMKGGIRKDRRGGRLKHKRQRDDLEGRNEVGTSGDMRAANLWPSF

hER α 296 LMIKRSKKNSLALSILTADQMVSAALLDAEPPILYSEYDPIRPFSEASMMGLLTNLADRELV
rER α 301 LVIKHTKKNSPALSILTADQMVSAALLDAEPPILYSEYDPSRPFSEASMMGLLTNLADRELV

hER α 356 HMINWAKRVPGFVDLTLHDQVHLLLECAWLEILMIGLVWRSMEHPGKLLFAPNLLLDRNQG
rER α 361 HMINWAKRVPGFGDLNLHDQVHLLLECAWLEILMIGLVWRSMEHPGKLLFAPNLLLDRNQG

hER α 416 KCVEGMVEIFDMLLATSSRFMMNLQGEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHI
rER α 421 KCVEGMVEIFDMLLATSSRFMMNLQGEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHI

hER α 476 HRVLDKIITDTLIHLMKAGLTLQQQHRLAQLLLILSHIRHMSNKGMEHLYSMKCKNVVP
rER α 481 HRVLDKINDTLIHLMAKAGLTLQQQHRRLAQLLLILSHIRHMSNKGMEHLYNMGKCKNVVP

hER α 536 LYDLLLEMLDAHRLHAPT SRGCASVEETDQSHLATAGSTSSHSLQKYYITGEAEGFPATV
rER α 541 LYDLLLEMLDAHRLHAPASRMGVPPPEEPSQSQTITTSSTSAHSLQTYIYIPEAEGFPNTI

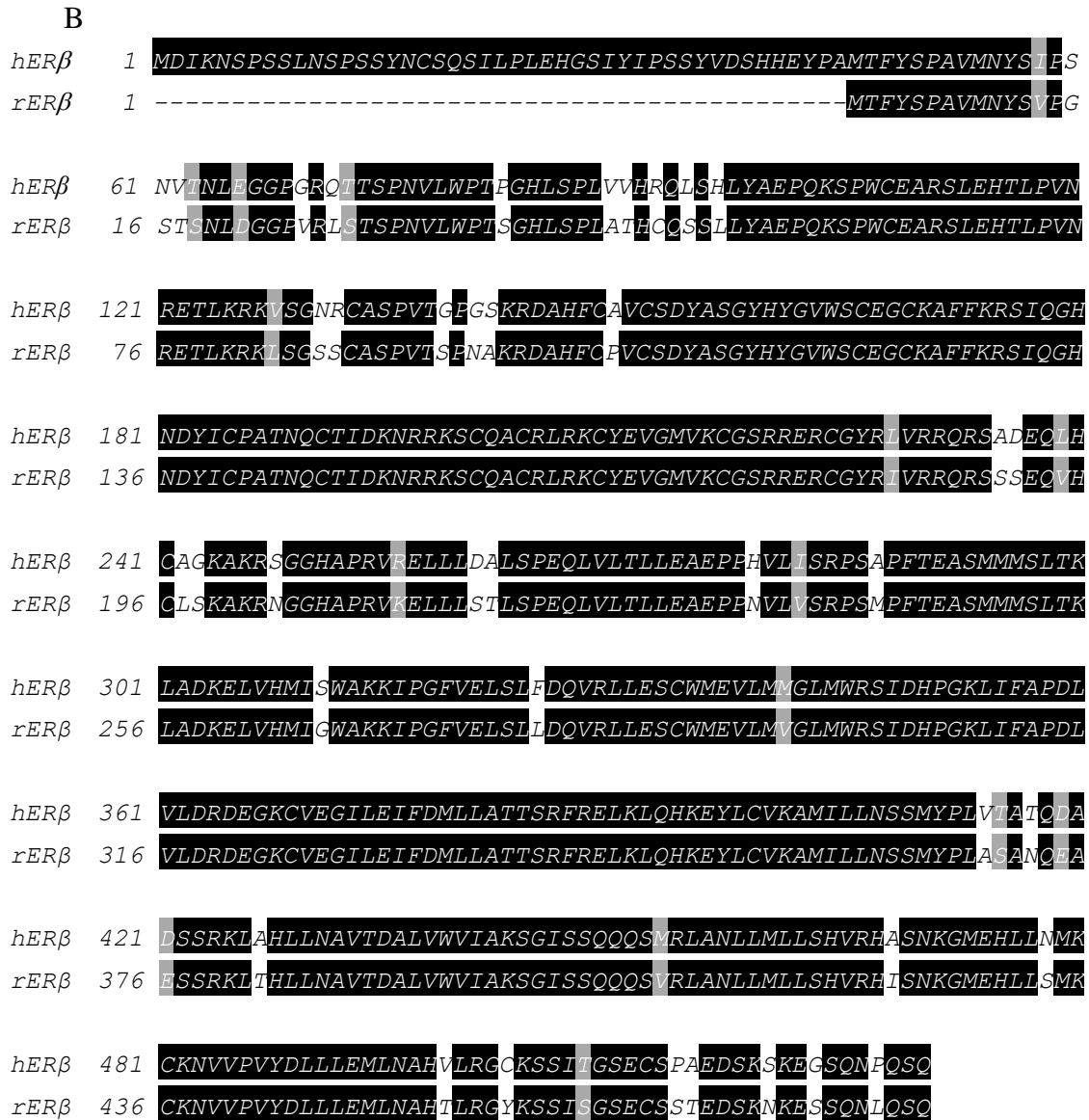


Figure 7. Amino acid sequence comparisons of human and rat ER α and human and rat ER β

The panel A shows the alignment of human ER α amino acid sequence with rat ER α sequences. The panel B shows the alignment of human ER β amino acid sequence with rat

ER β sequences. Identical and similar residues are shaded in black and gray, respectively.

Dashes indicate gaps introduced to maximize the alignment.

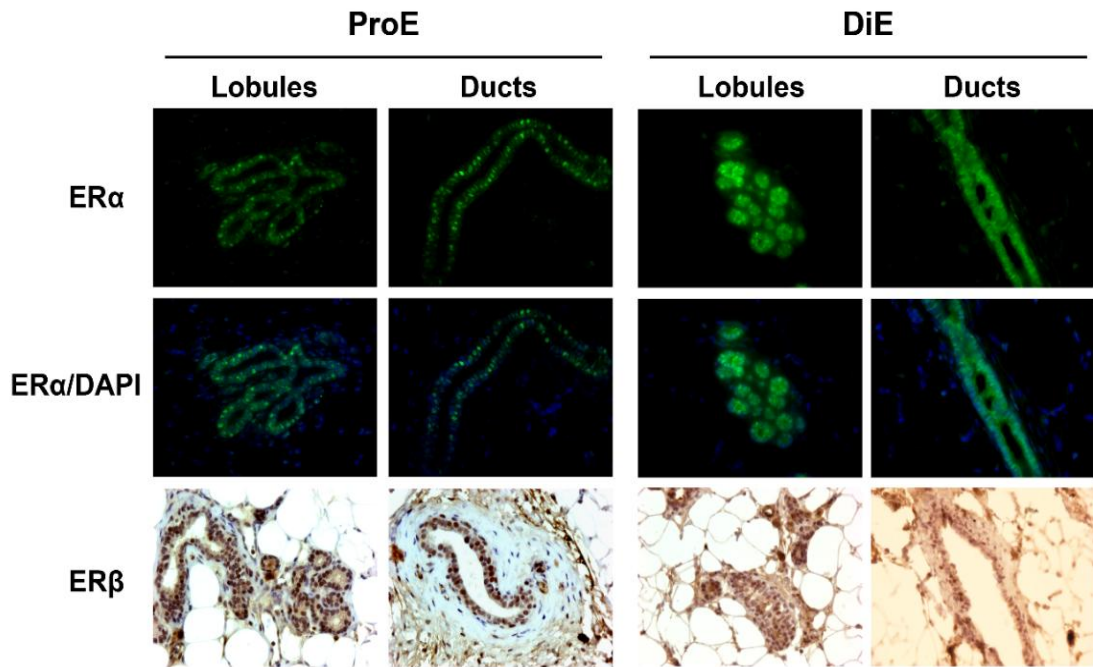


Figure 8. Immunostaining of ER α and ER β in the rat mammary gland.

The expression pattern of ER α was determined by IF staining (green fluorescence, top panels). Cell nuclei were counterstained with DAPI (blue fluorescence). Images of ER α staining and DAPI staining were overlaid to show the localization of ER α -staining cells (middle panels). The expression pattern of ER β was determined by IHC staining with DAB as substrate to give brown color (bottom panels). In the proestrus phase, ER α staining was detected in about 20% of epithelial cells; ER β was expressed in more than 50% of epithelial cells and ER β staining was also detected in some stromal cells. In the diestrus phase, no ER α staining was detected in mammary epithelial cells; ER β was expressed in about 20% of mammary epithelial cells and some stromal cells. Magnification, 400 \times .

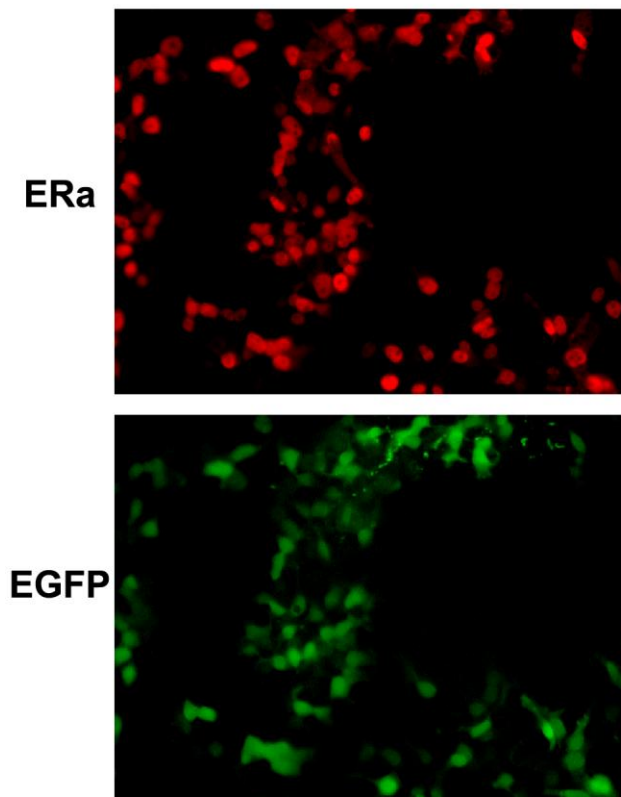


Figure 9. Co-immunostaining of ER α and EGFP in the 293FT cell

The expression of ER α was determined by IF staining (red fluorescence, top panels). The expression of EGFP was evaluated directly under fluorescence microscopy. Both ER α and EGFP were detected in all cells. Magnification, 400 \times .

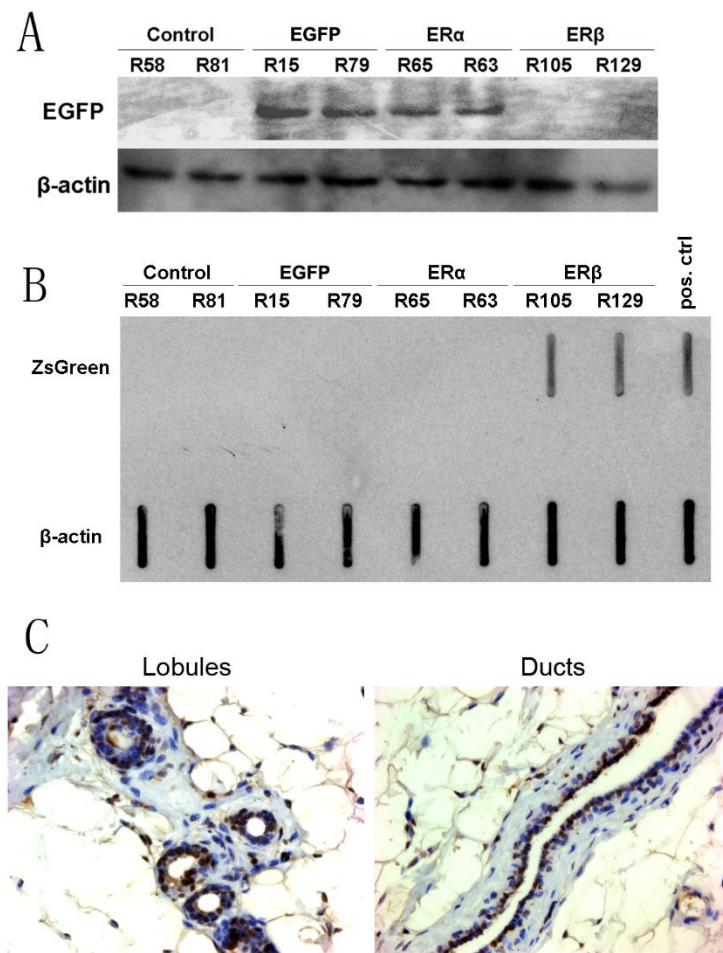


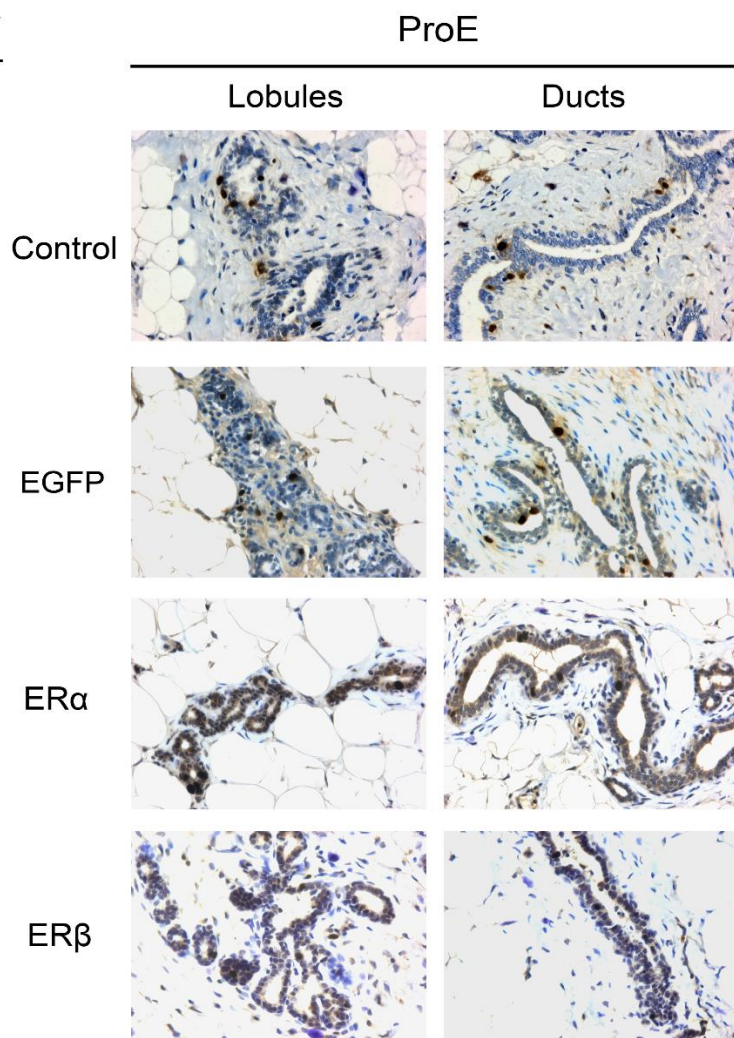
Figure 10. Immunostaining of EGFP and immunoblotting of EGFP and ZsGreen in the mammary gland infected by Lentivirus.

(A) Mammary gland tissues from rats in different groups were analyzed by SDS-PAGE and immunoblotting. Expression of EGFP using anti-EGFP antibody was detected in the EGFP and ER α groups but not in the uninfected control and ER β groups. (B) Mammary gland tissues from different groups were analyzed by slot-blot and immunoblotting. Protein lysate harvested from 293FT cells infected by ER β -ZsGreen

lentivirus was used as positive control (pos. ctrl). Expression of ZsGreen using anti-ZsGreen antibody was detected in the ER β group but not in any control and ER α groups.

(C) Representative EGFP IHC staining images of lobular and ductal structures in the mammary gland in the EGFP group. Over 50% of mammary epithelial cells showed intensive staining.

A



B

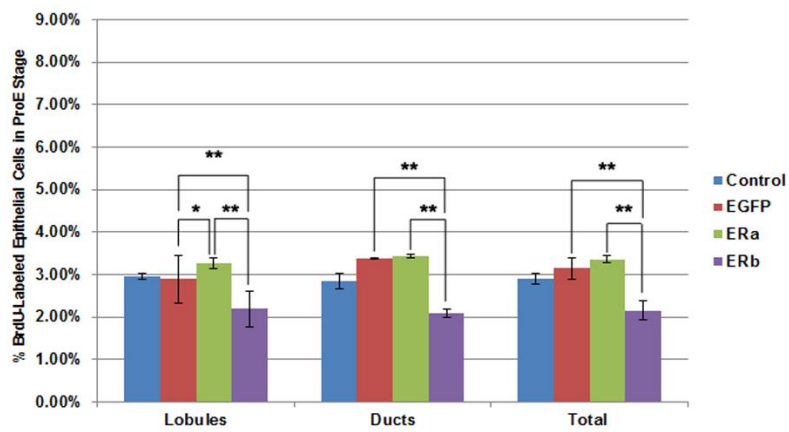
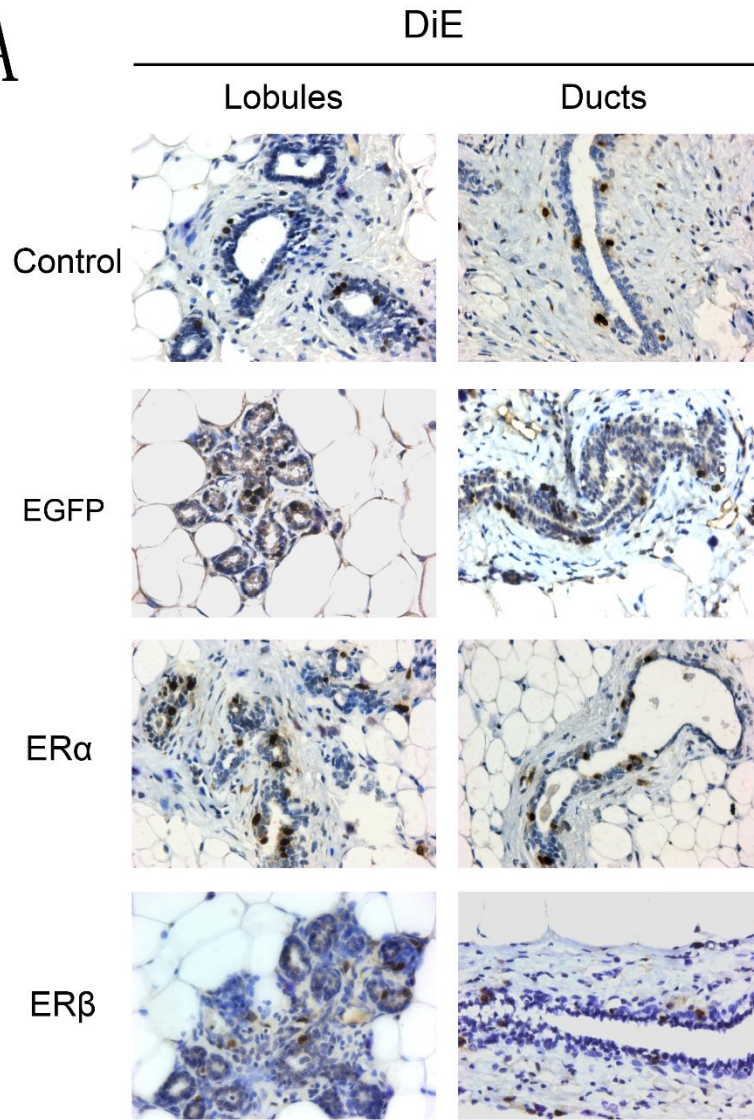


Figure 11. ER α stimulates and ER β inhibits mammary cell proliferation in the proestrus phase.

(A) Representative images showing BrdU-labeled proliferation cells in the mammary lobular and ductal structures of different groups of rats. BrdU-labeled cells were detected by IHC staining with DAB as substrate to give brown color. Magnification, 400 \times . (B) Percentage of BrdU-labeled proliferation cells in the mammary gland of different groups of rats. Mammary epithelial cells in lobular and ductal structures were counted separately, and the total is the combination of lobular and ductal cells. The p values of comparison between two groups for the lobular structure are in table below

P value	Lobular	Ductal	Total
ER α vs EGFP	0.012		
ER β vs EGFP	0.002	0.0083	0.0006
ER α vs ER β	<0.0001	0.0071	0.0002

A



B

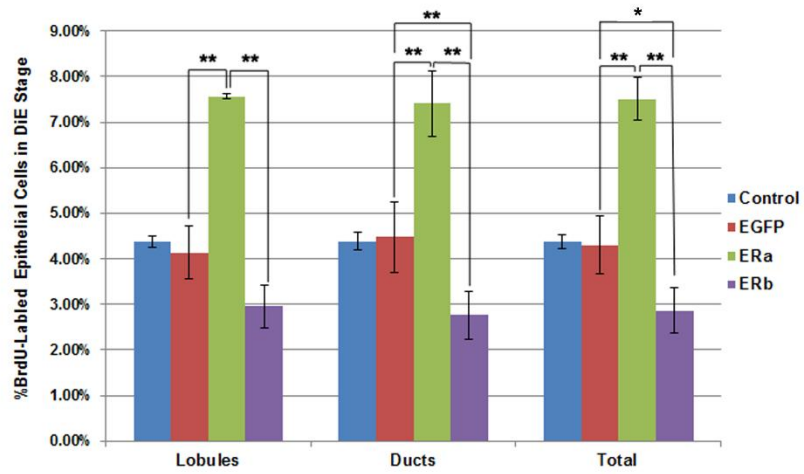


Figure 12. ER α stimulates and ER β inhibits mammary cell proliferation in the diestrus phase.

(A) Representative images showing BrdU-labeled proliferation cells in the mammary lobular and ductal structures of different groups of rats. BrdU-labeled cells were detected by IHC staining with DAB as substrate to give brown color. Magnification, 400 \times . (B) Percentage of BrdU-labeled proliferation cells in the mammary gland of different groups of rats. Mammary epithelial cells in lobular and ductal structures were counted separately, and the total is the combination of lobular and ductal cells. The p values of comparison between two groups for the lobular structure are in table below:

P value	Lobular	Ductal	Total
ER α vs EGFP	0.0005	<0.0001	0.0002
ER β vs EGFP		0.002	0.0225
ER α vs ER β	<0.0001	<0.0001	<0.0001

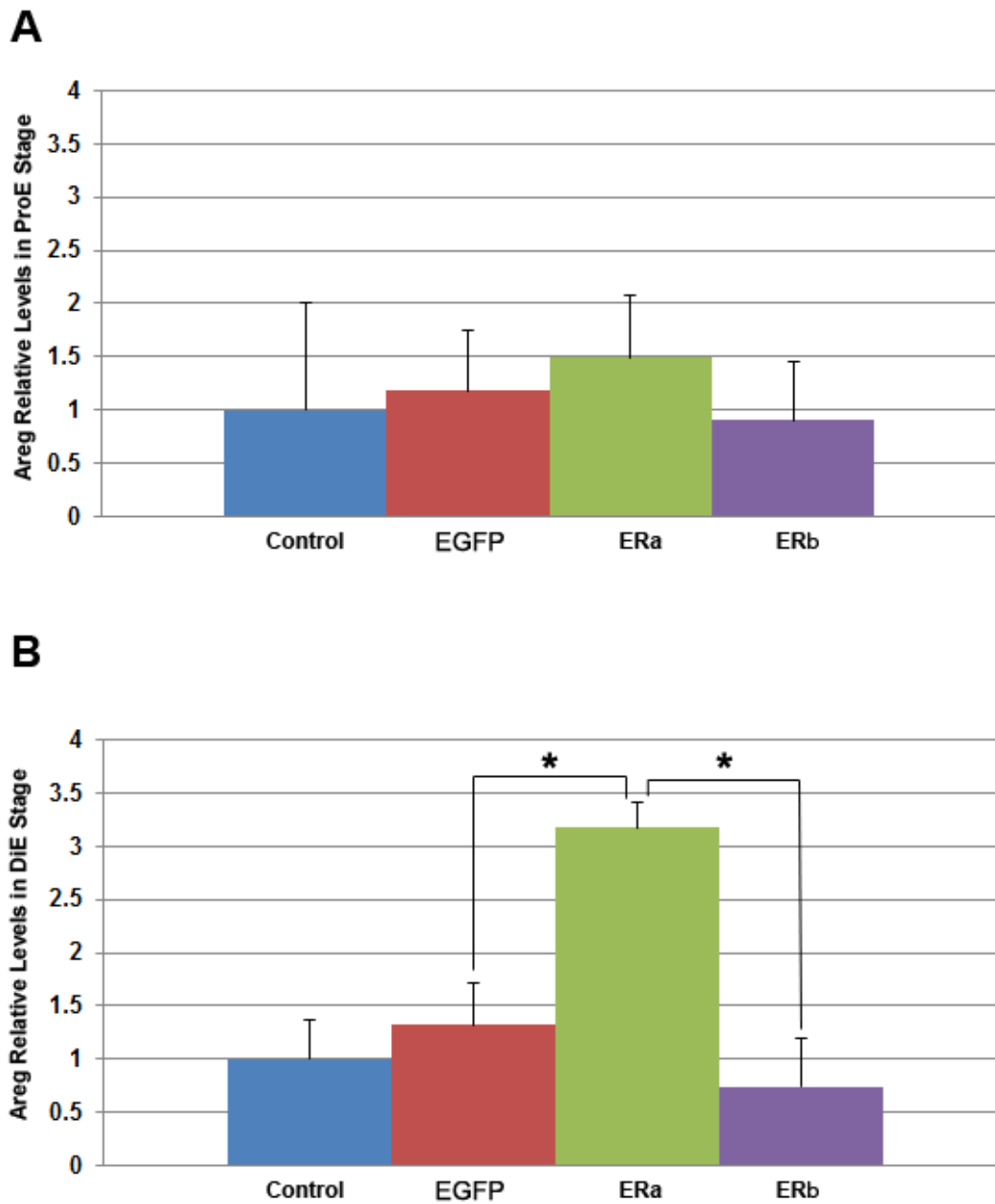


Figure 13. ER α increases amphiregulin expression in the mammary gland.

RNA levels of amphiregulin were determined by quantitative real-time RT-PCR. β -actin was used as internal control. In the proestrus phase, the expression levels of amphiregulin were similar in the Control, EGFP and ER β groups (figure 5 A). In the ER α

group the expression of amphiregulin was increased about 1.5 fold (figure 5 A). In the diestrus phase, amphiregulin expression in the ER α group was significantly higher than that in the Control and ER β group (figure 5 B). The p values are as follows: 0.013 for ER α vs. EGFP; 0.014 for ER α vs. ER β (*p < 0.05).

CHAPTER 4: Summary and Discussion

Expression of estrogen receptors in the Mammary Gland

The expression levels of estrogen receptors have been shown to be an important mechanism for regulating mammary cell proliferation under different physiological conditions (Clarke et al. 1997, Saji et al. 2000, Cheng et al. 2005). Abnormal expression of ER α and ER β may induce tumorigenesis (Frech et al. 2005, Regan et al. 2006). Clinical studies have demonstrated that about 70% of breast tumors are ER α positive and most of them have high level of ER α compared with normal breast tissues (Shoker et al. 1999, Shaaban et al. 2002). On the contrary, ER β expression is found decreased or lost in many primary breast tumors (Zhao et al. 2003, Girault et al. 2004, Gruvberger-Saal et al. 2007, Honma et al. 2008). Therefore it is important to characterize the expression of estrogen receptors in different stages and understand how the expression of estrogen receptors is regulated in different physiological environment.

The ovariectomised (OVX) animals are often used as a model for estrogen deficiency or postmenopausal research. The percentage of ER α positive cells increases in OVX rats, presumably due to decreased level of circulatory estrogens (Berry et al. 2003, Song et al. 2012). In chapter two of this dissertation, we investigated the expression of ER α and ER β in the mammary gland of OVX rats. ER α was detected in more than 50% of

luminal epithelial cells. Compared with that of the ovary-intact rats, the mammary gland of OVX rats showed more cells with ER α expression but the staining intensity was weaker. ER β was detected in almost all mammary epithelial cells with strong staining, and ER β staining was also detected in some stromal cells in the mammary gland. These results demonstrate that both ER α and ER β are expressed in significantly more cells in the low-estrogen *in vivo* environment and further support the downregulating effect of estrogen on the expression of estrogen receptors.

Estrous cycle is controlled by systemic hormones such as estrogen and progesterone (Jones et al. 2006). In the normal mammary gland, the expression of estrogen receptors is regulated by these hormones during estrous cycle (Clarke et al. 1997, Cheng et al. 2004). However, most of these studies have been focusing on the expression of ER α , the expression of ER β during estrous cycle is much less studied. It is generally believed that ER α is expressed in about 10-40% mammary epithelial cells but not expressed in stromal cells (Clarke et al. 1997, Clarke 2004). ER α reaches the peak of expression in the proestrus phase of the estrous cycle and is then quickly degraded due to peak level of estrogen in the estrus phase (Saji et al. 2000, Cheng et al. 2005). ER β is expressed not only in luminal epithelial cells but also in some stromal cells and myoepithelial cells (Palmieri et al. 2002). The studies by Schams et al. (2003) demonstrated that approximately 70% of epithelial cells express ER β throughout all developmental phases.

In chapter three of this dissertation, we evaluated the expression of ER α and ER β in normal rat mammary gland tissues during the estrus cycle. It is known that estrogen level gradually increases in the proestrus phase, reaches the peak in the estrus phase, remains at high level for most of the diestrus phase (Clarke et al. 1997, Cheng et al. 2004). Consistent with results from most previous studies, we found that in the proestrus phase, ER α was expressed in about 20% of mammary epithelial cells, and in the diestrus phase, no ER α staining was detected in mammary epithelial cells. ER β was expressed in more than 50% of mammary epithelial cells and some stromal cells in the proestrus phase. There were still 20% of epithelial cells and a fraction of stromal cells expressing ER β in the diestrus phase, although the percentage dropped significantly and the staining intensity of ER β was weaker compared to the proestrus phase. These results indicate that the expression of both ER α and ER β undergoes dynamic changes during the estrous cycle, higher before the peak of estrogen in the estrous phase and significantly decreased after estrogen spikes.

Function of Estrogen Receptors in Mammary Gland Development

Estrogen is involved in regulating the growth and differentiation of many tissues, including the mammary gland, the fallopian tube and the uterus; the function of estrogen is mediated by two types of estrogen receptors, ER α and ER β (Bocchinfuso et al. 1997, Cooke et al. 1997, Russo et al. 1999, Shao et al. 2007). In the mammary gland, ER α is known as the primary estrogen receptor and well characterized as a positive regulator of

cell proliferation (Bocchinfuso et al. 1997). In contrast to the well-established role of ER α , ER β is much less studied and the function of ER β in mammary cell proliferation is still debatable (Lazennec et al. 2001, Visvader et al. 2003, Cheng et al. 2004, Paruthiyil et al. 2004, Strom et al. 2004).

In this dissertation, we used two approaches to investigate the function of ER β in the mammary gland. In the first approach, endogenous ER α and/or ER β in OVX rats were selectively activated using ER α and/or ER β selective agonists (Chapter 2). We found that endogenous ER α activation stimulates cell proliferation whereas endogenous ER β activation counteracts the proliferative effect of ER α in the mammary gland (Chapter 2). In the second approach (Chapter 3), rat mammary gland was infected with lentiviruses to overexpress ER α or ER β to determine the effect of expression level of ER α or ER β on mammary cell proliferation. Overexpressing ER β in the mammary gland of mature virgin rats decreased cell proliferation rate in both proestrus and diestrus phases, further supporting that ER β , unlike ER α , is a negative regulator of mammary cell proliferation. The effects of estrogen receptors on cell proliferation are similar in the ductal and lobular structures in both OVX rats and mature virgin rats. Collectively, these data support that in contrast to ER α , ER β is able to inhibit mammary cell proliferation.

Both ER α and ER β belong to the nuclear receptor superfamily of transcription factors and share similar structures, accumulating evidence indicates that they have distinct functionalities (Heldring et al. 2007). Estrogen receptors regulate gene transcription by

binding to estrogen response elements (EREs) in the promoters of target genes and utilizing AF-1 and AF-2 domains to recruit coregulators. Therefore the trans-acting function of estrogen receptors is highly dependent on AF1 and AF2 domains in a cell-type and promoter-dependent manner (Matthews et al. 2003). The high degree of sequence divergence of AF domains suggests that ER α and ER β can recruit different co-regulators, hence regulate gene expression differently (Cowley et al. 1997, Hall et al. 1999). When comparing the activity of AFs in the two types of estrogen receptors, it has been noticed that AF-1 in ER α is very active, while under the same condition, the AF-1 domain of ER β only has negligible activities (Barkhem et al. 1998). The amphiregulin gene has a putative ERE and amphiregulin is an essential mediator of ER α mitogenic activity (Britton et al. 2006, Ciarloni et al. 2007, Kariagina et al. 2010). In chapter three of this dissertation, we observed that amphiregulin transcript level was induced by overexpression of ER α but not by ER β , which may be due to the relative lack of active AF-1 activity of ER β . It has been shown that alteration of the AF-1 domain can enable ER β to respond to anti-estrogens (McInerney et al. 1998).

Besides directly binding to ERE and recruiting coregulators to regulate gene transcription, estrogen receptors can modulate the activity of the AP-1 protein and stimulating protein 1 (Sp1) (Webb et al. 1999, Saville et al. 2000, Hall et al. 2001). Upon estrogen stimulation, ER β inhibits while ER α activates AP-1-mediated transcription (Webb et al. 1999, Kushner et al. 2000). The estrogen-ER α complex binds to sequences

containing the AP1 site or cAMP response element (CRE) to promote cyclin D transcription (Liu et al. 2002). In chapter two of this dissertation, we found that cyclin D1 was induced by ER α selective agonist PPT and PPT-induced cyclin D1 expression was suppressed by ER β selective agonist DPN.

In chapters two and three of this dissertation, we showed that that the level of estrogen and the expression level of ER α are critical for mammary cell proliferation. Under the condition of low level of estrogen in proestrus phase or in OVX rats, few ER α molecules are activated and the proliferation rate keeps at a lower level. After high level of estrogen stimulation in estrus stage or PPT activation, activated ER α stimulates target gene expression and promotes cell proliferation in diestrus phase and in the PPT group.

By investigating the relationship between the expression level of ER α , ER β and the proliferation rate, it is possible that the ratio of ER α /ER β might be involved in regulating mammary cell proliferation. ER α and ER β co-expressed in mammary cells could form homodimers or heterodimers to regulate gene expression in the nucleus upon estrogen activation, and the ratio of ER α /ER β might be critical for the formation of ER dimers. (Pettersson et al. 1997, Nilsson et al. 2001, Heldring et al. 2007). It is postulated that the higher ratio of ER α /ER β , the more ER α /ER α homodimers are formed to promote cell proliferation in the mammary gland. In contrast, more ER β /ER β homodimers will be formed when the ratio of ER α /ER β is lower, leading to reduced cell proliferation.

ER α /ER β heterodimer could also be formed in ER α and ER β co-expressing cells, and its binding affinity to ERE was shown similar to the homodimer (Cowley et al. 1997). However, the way it regulates cell proliferation is much more complicated. In the OVX rats, PPT and DPN activated ER α and ER β respectively. Treatment with PPT and DPN simultaneously activated both ER α and ER β and resulted in a proliferation rate higher than that in the untreated control group but lower than that in the PPT treated group, indicating that ER β activated by DPN is able to partially block the function of ER α via the formation of heterodimers (Chapter 2).

Chapter 3 shows that in the proestrus phase of normal mammary gland, although the ratio of ER α /ER β was relatively high, due to lack of estrogen stimulation, the proliferation rate was low. However, in the mammary gland overexpressing ER β , due to a lower ratio of ER α /ER β , the proliferation rate in the proestrus phase was even lower than that in the control group. In contrast, the mammary gland overexpressing ER α had a higher ER α /ER β ratio and also a higher proliferation rate. In the diestrus phase, the high ER α /ER β ratio might account for the high cell proliferation rate in the ER α overexpressing group; whereas the low ER α /ER β ratio might account for the reduced cell proliferation rate in the ER β overexpressing group. In addition, the high ratio of ER α /ER β in most breast tumors corresponds to a much higher proliferation rate than that in the normal breast tissues (Zhao et al. 2003, Girault et al. 2004, Gruvberger-Saal et al. 2007, Honma et al. 2008). All these

data support the hypothesis that the mammary cell proliferation rate is controlled largely by the ratio of ER α /ER β .

Function of ER α and ER β on cell proliferation in the uterus, colon, and lung

Besides the mammary gland, estrogens are also involved in the development of many other tissues. As one of the major classical estrogen target tissues, the uterine growth and differentiation is regulated by estrogens (Cooke et al. 1997, Tan et al. 1999). Increasing evidence suggested that estrogens are also important in lung and colon development, physiology, and even carcinogenesis (Adami et al. 1989, Arai et al. 2000, Omoto et al. 2001, Rossouw et al. 2002, Townsend et al. 2012). However the expression and effect of estrogen receptors in these tissue are not fully understood. In order to evaluate the effect of estrogen receptors in these organs, we used ER selective agonists to activate ER α and ER β in OVX rats.

The effects of estrogen in the uterus are mainly mediated by ER α which is the predominant type of estrogen receptor expressed in the uterus (Li 1994). We found that in the uterus of the OVX rats, ER α was detected in almost all endometrial luminal epithelial cells, glandular epithelial cells, and stromal cells and the staining intensity in the epithelial cells was much stronger than that in the stromal cells. Consistent with other studies, treatment with ER α -selective agonist PPT increased uterine diameter, uterine wet weight (UWW) and the cell proliferation rate (Couse et al. 1999, Persson et al. 1999, Harris et al.

2002, Frasor et al. 2003, Tilli et al. 2003, Hartman et al. 2006, Wegorzewska et al. 2008).

The uterus morphology and cell proliferation rate in the PPT group were very similar to that of the estrogen group, confirming that ER α predominately mediates estrogen action in the uterus. In contrast, the uterus morphology, weight and cell proliferation rate in the ER β -selective agonist DPN treated group were very similar to that of the control group, indicating that ER β had little effect on the estrogenic activity in the uterus. In addition, co-administration of DPN with PPT did not inhibit PPT's effect on uterine wet weight and cell proliferation rate, demonstrating that DPN had little effect on the estrogenic activity of PPT in the uterus. This is different from the suppressive effect of DPN on PPT in the mammary gland (Persson et al. 1999, Harris et al. 2002, Berry et al. 2003, Frasor et al. 2003, Sánchez-Criado et al. 2004, Wegorzewska et al. 2008, Bliedtner et al. 2010). The different effects of DPN on PPT in the mammary gland and the uterus may be due to differential expression levels of ER β or different ratios of ER α /ER β in these two types of tissues. ER β expression is more widespread than ER α in the mammary gland; whereas ER α expression is more prevalent in the uterine endometrium.

Conflicting results have been reported on the effects of estrogens on colon growth. Some studies showed that estrogens inhibit cell proliferation while others demonstrated that estrogens promote the proliferation of colon cells (Hoff et al. 1980, Xiaomeng et al. 1994, Calle et al. 1995, Newcomb et al. 1995, Di Domenico et al. 1996, Franceschi et al. 1998, Rossouw et al. 2002, Anderson et al. 2004). Although conflicting results have also

been obtained from studies on the expression of ER α , it has been widely agreed that ER β is the predominant estrogen receptor in the colon and can be detected in both the normal colon epithelium and colorectal carcinomas (Sutter et al. 1996, Waliszewski et al. 1997, Arai et al. 2000, Foley et al. 2000, Campbell-Thompson et al. 2001, Martinetti et al. 2005).

In the colon tissues of the OVX rats, we did detect the expression of ER β but we did not detect ER α staining. Our data demonstrated that activation of ER α and ER β by PPT and DPN respectively has no effect on the cell proliferation rate in the colon tissues of the OVX rats (Appendix A).

Estrogens have been illustrated to stimulate lung cell proliferation in normal lung fibroblasts, suggesting that estrogen may also be involved in lung development (Omoto et al. 2001, Townsend et al. 2012). Consistent with previous studies, we did not observe ER α expression in the lung tissues of the OVX rats (Matsuda et al. 1993, Ollayos et al. 1994, Brown et al. 1997, Nunno et al. 2000, Patrone et al. 2003, Morani et al. 2008). ER β staining was detected in the lungs of the OVX rats, indicating that ER β may be the predominant estrogen receptor in the lung. Many studies suggested that ER β is involved in the growth, development, or tumorigenesis of the lung (Omoto et al. 2001, Patrone et al. 2003). In Appendix B, we showed that the percentages of BrdU labeled cells were similar among different treatment groups, indicating that ER α and ER β activated by selective agonists have no effect on cell proliferation in lung tissues of the OVX rats.

Potential medical use of ER β in hormone replacement therapy and breast cancer treatment

In this dissertation, we evaluated the effect of ER β on cell proliferation in both mature virgin rats and OVX rats. Our data supports that ER β is a negative regulator of mammary gland growth and the ER α -mediated estrogenic activities in the mammary gland can be suppressed by ER β . These findings suggest that ER β could be served as the pharmaceutical target to design new hormone replacement therapy regimens for postmenopausal syndromes and new drugs to treat breast cancer. In chapter two of this dissertation, we found that PPT has similar activities as estrogen in the mammary gland and uterus and that the adverse effect of PPT mediated estrogenic activities in the mammary gland can be opposed by DPN, indicating that PPT can be used as alternative to estrogen and we can use PPT and DPN together to reduce the side effects in hormone replacement therapy.

In the OVX rats, we also evaluated the cell proliferation rate in the uterus, colon and lung tissues. We found that compared with the estrogen regimen, the PPT and DPN combined regimen is able to decrease the mammary cell proliferation rate, which reduces the risk of breast cancer. Moreover, the PPT and DPN regimen does not increase cell proliferation rate in lung and colon tissues, indicating that this regimen has no adverse effect on these tissues.

The estrogen and progesterone regimen has been observed to increase mammary cell proliferation much higher than estrogen alone, but the estrogenic activity of estrogen in the uterus can be opposed by progesterone (Grady et al. 1995, Persson et al. 1999, Ross et al. 2000). We showed that in the OVX postmenopausal rats, compared with the PPT and progesterone regimen, the PPT, DPN and progesterone regimen decreased cell proliferation in the mammary gland, which increased the risk of breast cancer. Moreover, the PPT, progesterone and DPN regimen does not increase cell proliferation rate in lung and colon tissues, indicating that this regimen has no adverse effect on these tissues. Thus, the PPT/DPN or PPT/DPN/Progesterone regimen may be a good option for hormone replacement therapy.

In this dissertation, we only assessed the effect of different estrogen alternatives on cell proliferation and hence the risk of cancer. There are still questions to be answered for the proposed PPT/DPN regimen for HRT. First, we have to show that the beneficial effects of estrogen or progesterone are not abolished by PPT or DPN. We have shown that in the uterus, DPN did not increase cell proliferation rate in the presence of progesterone, indicating that the beneficial effect of progesterone in the uterus was not abolished by DPN. PPT and DPN have been shown to prevent hot flashes (Harris et al. 2002, Bowe et al. 2006). PPT has also been proved to prevent loss of bone mineral density (Harris et al. 2002). Both estrogen and DPN are able to decrease depression and anxiety-like behaviors (Lund et al. 2005, Walf et al. 2008). These studies suggest that PPT and DPN preserve the beneficial

effects of estrogen on many aspects of postmenopausal symptoms. Another concern is that whether this new regimen has adverse effect in other tissues. Estrogen is an important systemic hormone, targeting on different systems and tissues including reproductive system, cardiovascular system, brain and bone (Massaro et al. 1996, McEwen et al. 1999, Kleerekoper 2001, Jones et al. 2006). Although we have demonstrated that this new regimen did not have adverse effect in the lung, colon and uterus tissues, further studies are needed to determine the effect of this regimen in other estrogen targeted tissues.

ER α selective antagonists such as tamoxifen and raloxifene have been used to treat breast cancer. According to the results of our study and other studies, ER β agonist DPN suppressed the estrogenic effect in the mammary gland and thus may also be potentially used to treat breast cancer (Paruthiyil et al. 2004, Ström et al. 2004, Honma et al. 2008, Song et al. 2012). Besides DPN, more ER β selective agonists have been discovered and all these agonists may be potential new drugs for the prevention and treatment of breast cancer (Harris et al. 2003, Malamas et al. 2004, Hughes et al. 2008).

Reference

- Adami, H. O., I. Persson, R. Hoover, C. Schairer and L. Bergkvist (1989). "Risk of cancer in women receiving hormone replacement therapy." International Journal of Cancer **44**(5): 833-839.
- Anderson, G. L., M. C. Limacher, A. R. Assaf, T. Bassford, S. A. Beresford, H. R. Black, D. E. Bonds, R. L. Brunner, R. G. Brzyski and B. Caan (2004). "Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial." Journal of the American Medical Association **291**(14).
- Arai, N., A. Ström, J. J. Rafter and J.-Å. Gustafsson (2000). "Estrogen receptor β mRNA in colon cancer cells: growth effects of estrogen and genistein." Biochemical and Biophysical Research Communications **270**(2): 425-431.
- Barkhem, T., B. Carlsson, Y. Nilsson, E. Enmark, J. Gustafsson and S. Nilsson (1998). "Differential response of estrogen receptor alpha and estrogen receptor beta to partial estrogen agonists/antagonists." Molecular Pharmacology **54**(1): 105-112.
- Berry, S., P. Jobst, S. Ellis, R. Howard, A. Capuco and R. Akers (2003). "Mammary Epithelial Proliferation and Estrogen Receptor α Expression in Prepubertal Heifers: Effects of Ovariectomy and Growth Hormone." Journal of Dairy Science **86**(6): 2098-2105.
- Berry, S. D., P. M. Jobst, S. E. Ellis, R. D. Howard, A. V. Capuco and R. M. Akers (2003). "Mammary epithelial proliferation and estrogen receptor alpha expression in prepubertal heifers: effects of ovariectomy and growth hormone." Journal Dairy Science **86**(6): 2098-2105.
- Bliedtner, A., O. Zierau, S. Albrecht, S. Liebhaber and G. Vollmer (2010). "Effects of genistein and estrogen receptor subtype-specific agonists in ArKO mice following different administration routes." Molecular and Cellular Endocrinology **314**(1): 41-52.
- Bocchinfuso, W. P. and K. S. Korach (1997). "Mammary gland development and tumorigenesis in estrogen receptor knockout mice." Journal of Mammary Gland Biology and Neoplasia **2**(4): 323-334.
- Bowe, J., X. F. Li, J. Kinsey-Jones, A. Heyerick, S. Brain, S. Milligan and K. O'Byrne (2006). "The hop phytoestrogen, 8-prenylnaringenin, reverses the ovariectomy-induced rise in skin temperature in an animal model of menopausal hot flashes." Journal of Endocrinology **191**(2): 399-405.
- Britton, D., I. Hutcheson, J. Knowlden, D. Barrow, M. Giles, R. McClelland, J. Gee and R. Nicholson (2006). "Bidirectional cross talk between ER α and EGFR signalling pathways regulates tamoxifen-resistant growth." Breast Cancer Research and Treatment **96**(2): 131-146.

Brown, R. W., L. Campagna, J. K. Dunn and P. T. Cagle (1997). "Immunohistochemical identification of tumor markers in metastatic adenocarcinoma: a diagnostic adjunct in the determination of primary site." American Journal of Clinical Pathology **107**(1): 12-19.

Calle, E. E., H. L. Miracle-McMahill, M. J. Thun and C. W. Heath (1995). "Estrogen replacement therapy and risk of fatal colon cancer in a prospective cohort of postmenopausal women." Journal of the National Cancer Institute **87**(7): 517-523.

Campbell-Thompson, M., I. J. Lynch and B. Bhardwaj (2001). "Expression of estrogen receptor (ER) subtypes and ER β isoforms in colon cancer." Cancer Research **61**(2): 632-640.

Cheng, G., Y. Li, Y. Omoto, Y. Wang, T. Berg, M. Nord, P. Vihko, M. Warner, Y.-S. Piao and J.-Å. Gustafsson (2005). "Differential regulation of estrogen receptor (ER) α and ER β in primate mammary gland." Journal of Clinical Endocrinology & Metabolism **90**(1): 435-444.

Cheng, G., Z. Weihua, M. Warner and J.-Å. Gustafsson (2004). "Estrogen receptors ER α and ER β in proliferation in the rodent mammary gland." Proceedings of the National Academy of Sciences USA **101**(11): 3739-3746.

Ciarloni, L., S. Mallepell and C. Brisken (2007). "Amphiregulin is an essential mediator of estrogen receptor alpha function in mammary gland development." Proceedings of the National Academy of Sciences USA **104**(13): 5455-5460.

Clarke, R. B. (2004). "Human breast cell proliferation and its relationship to steroid receptor expression." Climacteric **7**(2): 129-137.

Clarke, R. B., A. Howell, C. S. Potten and E. Anderson (1997). "Dissociation between steroid receptor expression and cell proliferation in the human breast." Cancer Research **57**(22): 4987-4991.

Cooke, P., D. Buchanan, P. Young, T. Setiawan, J. Brody, K. Korach, J. Taylor, D. Lubahn and G. Cunha (1997). "Stromal estrogen receptors mediate mitogenic effects of estradiol on uterine epithelium." Proceedings of the National Academy of Sciences USA **94**(12): 6535-6540.

Couse, J. F. and K. S. Korach (1999). "Estrogen receptor null mice: what have we learned and where will they lead us?" Endocrine Reviews **20**(3): 358.

Cowley, S. M., S. Hoare, S. Mosselman and M. G. Parker (1997). "Estrogen receptors α and β form heterodimers on DNA." Journal of Biological Chemistry **272**(32): 19858-19862.

Di Domenico, M., G. Castoria, A. Bilancio, A. Migliaccio and F. Auricchio (1996). "Estradiol activation of human colon carcinoma-derived Caco-2 cell growth." Cancer Research **56**(19): 4516-4521.

Foley, E. F., A. A. Jazaeri, M. A. Shupnik, O. Jazaeri and L. W. Rice (2000). "Selective loss of estrogen receptor β in malignant human colon." Cancer Research **60**(2): 245-248.

Franceschi, S. and C. La Vecchia (1998). "Colorectal cancer and hormone replacement therapy: an unexpected finding." European Journal of Cancer Prevention **7**(6): 427-438.

Frasor, J., D. H. Barnett, J. M. Danes, R. Hess, A. F. Parlow and B. S. Katzenellenbogen (2003). "Response-specific and ligand dose-dependent modulation of estrogen receptor (ER) α activity by ER β in the uterus." Endocrinology **144**(7): 3159-3166.

Frech, M. S., E. D. Halama, M. T. Tilli, B. Singh, E. J. Gunther, L. A. Chodosh, J. A. Flaws and P. A. Furth (2005). "Deregulated estrogen receptor α expression in mammary epithelial cells of transgenic mice results in the development of ductal carcinoma in situ." Cancer Research **65**(3): 681-685.

Girault, I., C. Andrieu, S. Tozlu, F. Spyrtos, I. Bieche and R. Lidereau (2004). "Altered expression pattern of alternatively spliced estrogen receptor beta transcripts in breast carcinoma." Cancer letters **215**(1): 101-112.

Grady, D., T. Gebretsadik, K. Kerlikowske, V. Ernster and D. Petitti (1995). "Hormone replacement therapy and endometrial cancer risk: a meta-analysis." Obstetrics & Gynecology **85**(2): 304-313.

Gruvberger-Saal, S. K., P. O. Bendahl, L. H. Saal, M. Laakso, C. Hegardt, P. Eden, C. Peterson, P. Malmstrom, J. Isola, A. Borg and M. Ferno (2007). "Estrogen receptor beta expression is associated with tamoxifen response in ERalpha-negative breast carcinoma." Clinical Cancer Research **13**(7): 1987-1994.

Hall, J. M., J. F. Couse and K. S. Korach (2001). "The multifaceted mechanisms of estradiol and estrogen receptor signaling." Journal of Biological Chemistry **276**(40): 36869-36872.

Hall, J. M. and D. P. McDonnell (1999). "The estrogen receptor β -isoform (ER β) of the human estrogen receptor modulates ER α transcriptional activity and is a key regulator of the cellular response to estrogens and antiestrogens." Endocrinology **140**(12): 5566-5578.

Harris, H. A., L. M. Albert, Y. Leathurby, M. S. Malamas, R. E. Mewshaw, C. P. Miller, Y. P. Kharode, J. Marzolf, B. S. Komm and R. C. Winneker (2003). "Evaluation of an estrogen receptor- β agonist in animal models of human disease." Endocrinology **144**(10): 4241-4249.

Harris, H. A., J. A. Katzenellenbogen and B. S. Katzenellenbogen (2002). "Characterization of the biological roles of the estrogen receptors, ER α and ER β , in estrogen target tissues in vivo through the use of an ER α -selective ligand." Endocrinology **143**(11): 4172-4177.

Hartman, J., K. Lindberg, A. Morani, J. Inzunza, A. Ström and J.-Å. Gustafsson (2006). "Estrogen receptor β inhibits angiogenesis and growth of T47D breast cancer xenografts." Cancer Research **66**(23): 11207-11213.

Heldring, N., A. Pike, S. Andersson, J. Matthews, G. Cheng, J. Hartman, M. Tujague, A. Ström, E. Treuter and M. Warner (2007). "Estrogen Receptors: how do they signal and what are their targets." Physiological Reviews **87**(3): 905-931.

Hoff, M. B., W. W. Chang and K. M. Mak (1980). "Effect of estrogen on cell proliferation in colonic mucosa of the mouse." Virchows Archiv B **35**(1): 263-273.

Honma, N., R. Horii, T. Iwase, S. Saji, M. Younes, K. Takubo, M. Matsuura, Y. Ito, F. Akiyama and G. Sakamoto (2008). "Clinical importance of estrogen receptor-beta evaluation in breast cancer patients treated with adjuvant tamoxifen therapy." Journal of Clinical Oncology **26**(22): 3727-3734.

Hughes, Z. A., F. Liu, B. J. Platt, J. M. Dwyer, C. M. Pulicicchio, G. Zhang, L. E. Schechter, S. Rosenzweig-Lipson and M. Day (2008). "WAY-200070, a selective agonist of estrogen receptor beta as a potential novel anxiolytic/antidepressant agent." Neuropharmacology **54**(7): 1136-1142.

Jones, R. E. and K. H. Lopez (2006). Human reproductive biology.

Kariagina, A., J. Xie, J. R. Leippardt and S. Z. Haslam (2010). "Amphiregulin mediates estrogen, progesterone, and EGFR signaling in the normal rat mammary gland and in hormone-dependent rat mammary cancers." Hormones and Cancer **1**(5): 229-244.

Kleerekoper, M. (2001). Estrogen and the Skeleton. Hormonal Carcinogenesis III, Springer: 357-362.

Kushner, P. J., D. A. Agard, G. L. Greene, T. S. Scanlan, A. K. Shiau, R. M. Uht and P. Webb (2000). "Estrogen receptor pathways to AP-1." The Journal of Steroid Biochemistry and Molecular Biology **74**(5): 311-317.

Lazennec, G., D. Bresson, A. Lucas, C. Chauveau and F. Vignon (2001). "ER β inhibits proliferation and invasion of breast cancer cells." Endocrinology **142**(9): 4120-4130.

Li, S. (1994). "Relationship between cellular DNA synthesis, PCNA expression and sex steroid hormone receptor status in the developing mouse ovary, uterus and oviduct." Histochemistry **102**(5): 405-413.

Liu, M.-M., C. Albanese, C. M. Anderson, K. Hilty, P. Webb, R. M. Uht, R. H. Price, R. G. Pestell and P. J. Kushner (2002). "Opposing action of estrogen receptors α and β on cyclin D1 gene expression." Journal of Biological Chemistry **277**(27): 24353-24360.

Lund, T. D., T. Rovis, W. C. Chung and R. J. Handa (2005). "Novel actions of estrogen receptor- β on anxiety-related behaviors." Endocrinology **146**(2): 797-807.

Malamas, M. S., E. S. Manas, R. E. McDevitt, I. Gunawan, Z. B. Xu, M. D. Collini, C. P. Miller, T. Dinh, R. A. Henderson and J. C. Keith (2004). "Design and synthesis of aryl diphenolic azoles as potent and selective estrogen receptor- β ligands." Journal of Medicinal Chemistry **47**(21): 5021-5040.

Martineti, V., L. Picariello, I. Tognarini, S. C. Sala, A. Gozzini, C. Azzari, C. Mavilia, A. Tanini, A. Falchetti and G. Fiorelli (2005). "ER β is a potent inhibitor of cell proliferation in the HCT8 human colon cancer cell line through regulation of cell cycle components." Endocrine-related Cancer **12**(2): 455-469.

Massaro, G. D., J. P. Mortola and D. Massaro (1996). "Estrogen modulates the dimensions of the lung's gas-exchange surface area and alveoli in female rats." American Journal of Physiology-Lung Cellular and Molecular Physiology **270**(1): L110-L114.

Matsuda, S., Y. Kadowaki, M. Ichino, T. Akiyama, K. Toyoshima and T. Yamamoto (1993). "17 beta-estradiol mimics ligand activity of the c-erbB2 protooncogene product." Proceedings of the National Academy of Sciences USA **90**(22): 10803-10807.

Matthews, J. and J.-Å. Gustafsson (2003). "Estrogen signaling: a subtle balance between ER α and ER β ." Molecular Interventions **3**(5): 281.

McEwen, B. S. and S. E. Alves (1999). "Estrogen actions in the central nervous system." Endocrine Reviews **20**(3): 279-307.

McInerney, E. M., K. E. Weis, J. Sun, S. Mosselman and B. S. Katzenellenbogen (1998). "Transcription activation by the human estrogen receptor subtype beta (ER beta) studied with ER beta and ER alpha receptor chimeras." Endocrinology **139**(11): 4513-4522.

Morani, A., M. Warner and J. Å. Gustafsson (2008). "Biological functions and clinical implications of oestrogen receptors alfa and beta in epithelial tissues." Journal of Internal Medicine **264**(2): 128-142.

Newcomb, P. A. and B. E. Storer (1995). "Postmenopausal hormone use and risk of large-bowel cancer." Journal of the National Cancer Institute **87**(14): 1067-1071.

Nilsson, S., S. Makela, E. Treuter, M. Tujague, J. Thomsen, G. Andersson, E. Enmark, K. Pettersson, M. Warner and J. A. Gustafsson (2001). "Mechanisms of estrogen action." Physiological Reviews **81**(4): 1535-1565.

Nunno, L. D., L. G. Larsson, J. J. Rinehart and R. S. Beissner (2000). "Estrogen and progesterone receptors in non-small cell lung cancer in 248 consecutive patients who underwent surgical resection." Archives of Pathology & Laboratory Medicine **124**(10): 1467-1470.

Ollayos, C., G. Riordan and J. Rushin (1994). "Estrogen receptor detection in paraffin sections of adenocarcinoma of the colon, pancreas, and lung." Archives of Pathology & Laboratory Medicine **118**(6): 630-632.

Omoto, Y., Y. Kobayashi, K. Nishida, E. Tsuchiya, H. Eguchi, K. Nakagawa, Y. Ishikawa, T. Yamori, H. Iwase and Y. Fujii (2001). "Expression, function, and clinical implications of the estrogen receptor β in human lung cancers." Biochemical and Biophysical Research Communications **285**(2): 340-347.

Palmieri, C., G. Cheng, S. Saji, M. Zelada-Hedman, Z. Weihua, S. Van Noorden, T. Wahlstrom, R. Coombes, M. Warner and J. Gustafsson (2002). "Estrogen receptor beta in breast cancer." Endocrine-related Cancer **9**(1): 1-13.

Paruthiyil, S., H. Parmar, V. Kerekatte, G. R. Cunha, G. L. Firestone and D. C. Leitman (2004). "Estrogen receptor β inhibits human breast cancer cell proliferation and tumor formation by causing a G2 cell cycle arrest." Cancer Research **64**(1): 423-428.

Patrone, C., T. N. Cassel, K. Pettersson, Y.-S. Piao, G. Cheng, P. Ciana, A. Maggi, M. Warner, J.-Å. Gustafsson and M. Nord (2003). "Regulation of postnatal lung development and homeostasis by estrogen receptor β ." Molecular and Cellular Biology **23**(23): 8542-8552.

- Persson, I., E. Weiderpass, L. Bergkvist, R. Bergström and C. Schairer (1999). "Risks of breast and endometrial cancer after estrogen and estrogen-progestin replacement." Cancer Causes & Control **10**(4): 253-260.
- Pettersson, K., K. Grandien, G. G. Kuiper and J.-Å. Gustafsson (1997). "Mouse estrogen receptor β forms estrogen response element-binding heterodimers with estrogen receptor α ." Molecular Endocrinology **11**(10): 1486-1496.
- Regan, M. M., G. Viale, M. G. Mastropasqua, E. Maiorano, R. Golouh, A. Carbone, B. Brown, M. Suurkula, G. Langman and L. Mazzucchelli (2006). "Re-evaluating adjuvant breast cancer trials: assessing hormone receptor status by immunohistochemical versus extraction assays." Journal of the National Cancer Institute **98**(21): 1571-1581.
- Ross, R. K., A. Paganini-Hill, P. C. Wan and M. C. Pike (2000). "Effect of hormone replacement therapy on breast cancer risk: estrogen versus estrogen plus progestin." Journal of the National Cancer Institute **92**(4): 328-332.
- Rossouw, J. E., G. L. Anderson, R. L. Prentice, A. Z. LaCroix, C. Kooperberg, M. Stefanick, R. D. Jackson, S. A. Beresford, B. V. Howard and K. C. Johnson (2002). "Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial." Journal of the American Medical Association **288**(3): 321-333.
- Russo, J., X. Ao, C. Grill and I. Russo (1999). "Pattern of distribution of cells positive for estrogen receptor α and progesterone receptor in relation to proliferating cells in the mammary gland." Breast Cancer Research and Treatment **53**(3): 217-227.
- Sánchez-Criado, J. E., C. Bellido, M. Tena-Sempere, R. Aguilar and A. Blanco (2004). "Biological role of pituitary estrogen receptors ER α and ER β on progesterone receptor expression and action and on gonadotropin and prolactin secretion in the rat." Neuroendocrinology **79**(5): 247-258.
- Saji, S., E. V. Jensen, S. Nilsson, T. Rylander, M. Warner and J. A. Gustafsson (2000). "Estrogen receptors alpha and beta in the rodent mammary gland." Proceedings of the National Academy of Sciences USA **97**(1): 337-342.
- Saville, B., M. Wormke, F. Wang, T. Nguyen, E. Enmark, G. Kuiper, J.-Å. Gustafsson and S. Safe (2000). "Ligand-, cell-, and estrogen receptor subtype (α/β)-dependent activation at GC-rich (Sp1) promoter elements." Journal of Biological Chemistry **275**(8): 5379-5387.
- Schams, D., S. Kohlenberg, W. Amselgruber, B. Berisha, M. Pfaffl and F. Sinowatz (2003). "Expression and localisation of oestrogen and progesterone receptors in the bovine mammary gland during development, function and involution." Journal of Endocrinology **177**(2): 305-317.
- Shaaban, A. M., J. P. Sloane, C. R. West and C. S. Foster (2002). "Breast cancer risk in usual ductal hyperplasia is defined by estrogen receptor- α and Ki-67 expression." The American Journal of Pathology **160**(2): 597-604.

Shao, R., E. Egecioglu, B. Weijdegård, J. J. Kopchick, J. Fernandez-Rodriguez, N. Andersson and H. Billig (2007). "Dynamic regulation of estrogen receptor- α isoform expression in the mouse fallopian tube: mechanistic insight into estrogen-dependent production and secretion of insulin-like growth factors." *American Journal of Physiology-Endocrinology And Metabolism* **293**(5): E1430-E1442.

Shoker, B. S., C. Jarvis, R. B. Clarke, E. Anderson, J. Hewlett, M. Davies, D. R. Sibson and J. P. Sloane (1999). "Estrogen receptor-positive proliferating cells in the normal and precancerous breast." *The American Journal of Pathology* **155**(6): 1811-1815.

Song, X. and Z.-Z. Pan (2012). "Estrogen receptor-beta agonist diarylpropionitrile counteracts the estrogenic activity of estrogen receptor-alpha agonist propylpyrazole-triol in the mammary gland of ovariectomized Sprague Dawley rats." *The Journal of Steroid Biochemistry and Molecular Biology* **130**(1-2): 26-35.

Ström, A., J. Hartman, J. S. Foster, S. Kietz, J. Wimalasena and J.-Å. Gustafsson (2004). "Estrogen receptor β inhibits 17 β -estradiol-stimulated proliferation of the breast cancer cell line T47D." *Proceedings of the National Academy of Sciences USA* **101**(6): 1566-1571.

Strom, A., J. Hartman, J. S. Foster, S. Kietz, J. Wimalasena and J. A. Gustafsson (2004). "Estrogen receptor beta inhibits 17beta-estradiol-stimulated proliferation of the breast cancer cell line T47D." *Proceedings of the National Academy of Sciences USA* **101**(6): 1566-1571.

Sutter, T., K. Carnevale, N. Arber and I. Weinstein (1996). "Expression of cyclins D1 and E in human colon adenocarcinomas." *Journal of Medicine* **28**(5-6): 285-309.

Tan, J., B. C. Paria, S. K. Dey and S. K. Das (1999). "Differential uterine expression of estrogen and progesterone receptors correlates with uterine preparation for implantation and decidualization in the mouse." *Endocrinology* **140**(11): 5310-5321.

Tilli, M. T., M. S. Frech, M. E. Steed, K. S. Hruska, M. D. Johnson, J. A. Flaws and P. A. Furth (2003). "Introduction of estrogen receptor- α into the tTA/TAg conditional mouse model precipitates the development of estrogen-responsive mammary adenocarcinoma." *The American Journal of Pathology* **163**(5): 1713-1719.

Townsend, E. A., V. M. Miller and Y. Prakash (2012). "Sex differences and sex steroids in lung health and disease." *Endocrine reviews* **33**(1): 1-47.

Visvader, J. E. and G. J. Lindeman (2003). "Transcriptional regulators in mammary gland development and cancer." *The International Journal of Biochemistry & Cell Biology* **35**(7): 1034-1051.

Walf, A. A., C. J. Koonce and C. A. Frye (2008). "Estradiol or diarylpropionitrile decrease anxiety-like behavior of wildtype, but not estrogen receptor beta knockout, mice." *Behavioral Neuroscience* **122**(5): 974.

Waliszewski, P., M. Blaszczyk, E. Wolinska - Witort, M. Drews, M. Snochowski and R. E. Hurst (1997). "Molecular study of sex steroid receptor gene expression in human colon and in colorectal carcinomas." *Journal of Surgical Oncology* **64**(1): 3-11.

- Webb, P., P. Nguyen, C. Valentine, G. N. Lopez, G. R. Kwok, E. McInerney, B. S. Katzenellenbogen, E. Enmark, J. A. Gustafsson, S. Nilsson and P. J. Kushner (1999). "The estrogen receptor enhances AP-1 activity by two distinct mechanisms with different requirements for receptor transactivation functions." Molecular Endocrinology **13**(10): 1672-1685.
- Wegorzewska, I. N., K. Walters, M. J. Weiser, D. F. Cruthirds, E. Ewell, D. O. Larco, R. J. Handa and T. J. Wu (2008). "Postovariectomy weight gain in female rats is reversed by estrogen receptor α agonist, propylpyrazoletriol." American Journal of Obstetrics and Gynecology **199**(1): 67. e61-67. e65.
- Xiaomeng, X. and M. L. Thomas (1994). "Estrogen receptor-mediated direct stimulation of colon cancer cell growth in vitro." Molecular and Cellular Endocrinology **105**(2): 197-201.
- Zhao, C., E. W. Lam, A. Sunters, E. Enmark, M. T. De Bella, R. C. Coombes, J. A. Gustafsson and K. Dahlman-Wright (2003). "Expression of estrogen receptor beta isoforms in normal breast epithelial cells and breast cancer: regulation by methylation." Oncogene **22**(48): 7600-7606.

COMPREHENSIVE BIBLIOGRAPHY

- Abney, T. O. and R. B. Myers (1991). "17 β - Estradiol Inhibition of Leydig Cell Regeneration in the Ethane Dimethylsulfonate - Treated Mature Rat." Journal of Andrology **12**(5): 295-304.
- Adami, H. O., I. Persson, R. Hoover, C. Schairer and L. Bergkvist (1989). "Risk of cancer in women receiving hormone replacement therapy." International Journal of Cancer **44**(5): 833-839.
- Alanko, A., E. Heinonen, T. Scheinin, E. M. Tolppanen and R. Vihko (1985). "Significance of estrogen and progesterone receptors, disease - free interval, and site of first metastasis on survival of breast cancer patients." Cancer **56**(7): 1696-1700.
- Alberts, B., A. Johnson, J. Lewis, M. Raff and K. Roberts (2002). Molecular biology of the cell 4th edition, National Center for Biotechnology Informations Bookshelf.
- Anderson, D. C. (1974). "Sex - hormone - binding globulin." Clinical Endocrinology **3**(1): 69-96.
- Anderson, G. L., M. C. Limacher, A. R. Assaf, T. Bassford, S. A. Beresford, H. R. Black, D. E. Bonds, R. L. Brunner, R. G. Brzyski and B. Caan (2004). "Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial." Journal of the American Medical Association **291**(14).
- Anderson, R. (1978). "Embryonic and fetal development of the mammary apparatus." Lactation **4**: 3-40.
- Anderson, R. and I. Wahab (1990). "Changes in parenchyma and stroma of goat udders during pregnancy, lactation and involution." Small Ruminant Research **3**(6): 605-615.
- Ankrapp, D. P., J. M. Bennett and S. Z. Haslam (1998). "Role of epidermal growth factor in the acquisition of ovarian steroid hormone responsiveness in the normal mouse mammary gland." Journal of Cellular Physiology **174**(2): 251-260.
- Antal, M. C., A. Krust, P. Chambon and M. Mark (2008). "Sterility and absence of histopathological defects in nonreproductive organs of a mouse ER β -null mutant." Proceedings of the National Academy of Sciences USA **105**(7): 2433-2438.
- Anwar, A., P. McTernan, L. Anderson, J. Askaa, C. Moody, A. Barnett, M. Eggo and S. Kumar (2001). "Site - specific regulation of oestrogen receptor α and β by oestradiol in human adipose tissue." Diabetes, Obesity and Metabolism **3**(5): 338-349.
- Arai, N., A. Ström, J. J. Rafter and J.-Å. Gustafsson (2000). "Estrogen receptor β mRNA in colon cancer cells: growth effects of estrogen and genistein." Biochemical and Biophysical Research Communications **270**(2): 425-431.

- Arimatsu, Y. and H. Hatanaka (1986). "Estrogen treatment enhances survival of cultured fetal rat amygdala neurons in a defined medium." Developmental Brain Research **26**(1): 151-159.
- Arnold, S. F., J. D. Obourn, H. Jaffe and A. C. Notides (1995). "Phosphorylation of the human estrogen receptor by mitogen-activated protein kinase and casein kinase II: consequence on DNA binding." The Journal of Steroid Biochemistry and Molecular Biology **55**(2): 163-172.
- Aronica, S. and B. Katzenellenbogen (1993). "Stimulation of estrogen receptor-mediated transcription and alteration in the phosphorylation state of the rat uterine estrogen receptor by estrogen, cyclic adenosine monophosphate, and insulin-like growth factor-I." Molecular Endocrinology **7**(6): 743-752.
- Atanassova, N., C. McKinnell, J. Fisher and R. M. Sharpe (2005). "Neonatal treatment of rats with diethylstilboestrol (DES) induces stromal-epithelial abnormalities of the vas deferens and cauda epididymis in adulthood following delayed basal cell development." Reproduction **129**(5): 589-601.
- Azuma, K., T. Urano, K. Horie-Inoue, S.-i. Hayashi, R. Sakai, Y. Ouchi and S. Inoue (2009). "Association of estrogen receptor α and histone deacetylase 6 causes rapid deacetylation of tubulin in breast cancer cells." Cancer Research **69**(7): 2935-2940.
- Ball, P. and R. Knuppen (1980). "Catecholestrogens (2- and 4-hydroxyoestrogens): chemistry, biogenesis, metabolism, occurrence and physiological significance." Acta Endocrinol Suppl (Copenh) **232**: 1-127.
- Ballard, P. L. (1989). "Hormonal regulation of pulmonary surfactant." Endocrine Reviews **10**(2): 165-181.
- Banks, E., V. Beral, D. Bull, G. Reeves, J. Austoker and R. English (2003). "Breast cancer and hormone-replacement therapy in the Million Women Study." Lancet **362**(9382): 419-427.
- Barkhem, T., B. Carlsson, Y. Nilsson, E. Enmark, J.-Å. Gustafsson and S. Nilsson (1998). "Differential response of estrogen receptor α and estrogen receptor β to partial estrogen agonists/antagonists." Molecular Pharmacology **54**(1): 105-112.
- Barkhem, T., B. Carlsson, Y. Nilsson, E. Enmark, J. Gustafsson and S. Nilsson (1998). "Differential response of estrogen receptor alpha and estrogen receptor beta to partial estrogen agonists/antagonists." Molecular Pharmacology **54**(1): 105-112.
- Barrett-Connor, E., N. K. Wenger, D. Grady, L. Mosca, P. Collins, M. Kornitzer, D. A. Cox, E. Moscarelli and P. W. Anderson (1998). "Hormone and nonhormone therapy for the maintenance of postmenopausal health: the need for randomized controlled trials of estrogen and raloxifene." Journal of Women's Health **7**(7): 839-847.
- Beattie, C. W., N. W. Hansen and P. A. Thomas (1985). "Steroid receptors in human lung cancer." Cancer Research **45**(9): 4206-4214.

- Beral, V. (2003). "Breast cancer and hormone-replacement therapy in the Million Women Study." Lancet **362**(9382): 419-427.
- Bergman, L., M. L. Beelen, M. P. Gallee, H. Hollema, J. Benraadt and F. E. van Leeuwen (2000). "Risk and prognosis of endometrial cancer after tamoxifen for breast cancer." The Lancet **356**(9233): 881-887.
- Bernstein, L. (2002). "Epidemiology of endocrine-related risk factors for breast cancer." Journal of Mammary Gland biology and Neoplasia **7**(1): 3-15.
- Berry, S., P. Jobst, S. Ellis, R. Howard, A. Capuco and R. Akers (2003). "Mammary Epithelial Proliferation and Estrogen Receptor α Expression in Prepubertal Heifers: Effects of Ovariectomy and Growth Hormone." Journal of Dairy Science **86**(6): 2098-2105.
- Berry, S. D., P. M. Jobst, S. E. Ellis, R. D. Howard, A. V. Capuco and R. M. Akers (2003). "Mammary epithelial proliferation and estrogen receptor alpha expression in prepubertal heifers: effects of ovariectomy and growth hormone." Journal Dairy Science **86**(6): 2098-2105.
- Bhat, R. A., D. C. Harnish, P. E. Stevis, C. R. Lyttle and B. S. Komm (1998). "A novel human estrogen receptor β : identification and functional analysis of additional N-terminal amino acids." The Journal of Steroid Biochemistry and Molecular Biology **67**(3): 233-240.
- Bièche, I., I. Laurendeau, S. Tozlu, M. Olivi, D. Vidaud, R. Lidereau and M. Vidaud (1999). "Quantitation of MYC gene expression in sporadic breast tumors with a real-time reverse transcription-PCR assay." Cancer Research **59**(12): 2759-2765.
- Birge, S. J. (2000). "HRT and cognition: what the evidence shows." OBG Manage **12**(10): 40-59.
- Bliedtner, A., O. Zierau, S. Albrecht, S. Liebhaber and G. Vollmer (2010). "Effects of genistein and estrogen receptor subtype-specific agonists in ArKO mice following different administration routes." Molecular and Cellular Endocrinology **314**(1): 41-52.
- Bocchinfuso, W. P. and K. S. Korach (1997). "Mammary gland development and tumorigenesis in estrogen receptor knockout mice." J Mammary Gland Biol Neoplasia **2**(4): 323-334.
- Bocchinfuso, W. P. and K. S. Korach (1997). "Mammary gland development and tumorigenesis in estrogen receptor knockout mice." Journal of Mammary Gland Biology and Neoplasia **2**(4): 323-334.
- Bonneterre, J., A. Buzdar, J. M. A. Nabholz, J. F. Robertson, B. Thürlimann, M. von Euler, T. Sahmoud, A. Webster and M. Steinberg (2001). "Anastrozole is superior to tamoxifen as first - line therapy in hormone receptor positive advanced breast carcinoma." Cancer **92**(9): 2247-2258.
- Bonneterre, J., B. Thürlimann, J. Robertson, M. Krzakowski, L. Mauriac, P. Koralewski, I. Vergote, A. Webster, M. Steinberg and M. Von Euler (2000). "Anastrozole versus tamoxifen as first-line therapy for advanced breast cancer in 668 postmenopausal women:

results of the Tamoxifen or Arimidex Randomized Group Efficacy and Tolerability study." Journal of Clinical Oncology **18**(22): 3748-3757.

Bowe, J., X. F. Li, J. Kinsey-Jones, A. Heyerick, S. Brain, S. Milligan and K. O'Byrne (2006). "The hop phytoestrogen, 8-prenylnaringenin, reverses the ovariectomy-induced rise in skin temperature in an animal model of menopausal hot flushes." Journal of Endocrinology **191**(2): 399-405.

Brisken, C., S. Park, T. Vass, J. P. Lydon, B. W. O'Malley and R. A. Weinberg (1998). "A paracrine role for the epithelial progesterone receptor in mammary gland development." Proceedings of the National Academy of Sciences USA **95**(9): 5076-5081.

Britt, K., A. Ashworth and M. Smalley (2007). "Pregnancy and the risk of breast cancer." Endocrine-related Cancer **14**(4): 907-933.

Britt, K. and J. Findlay (2002). "Estrogen actions in the ovary revisited." Journal of Endocrinology **175**(2): 269-276.

Britton, D., I. Hutcheson, J. Knowlden, D. Barrow, M. Giles, R. McClelland, J. Gee and R. Nicholson (2006). "Bidirectional cross talk between ER α and EGFR signalling pathways regulates tamoxifen-resistant growth." Breast Cancer Research and Treatment **96**(2): 131-146.

Britton, D., I. R. Hutcheson, J. Knowlden, D. Barrow, M. Giles, R. McClelland, J. Gee and R. Nicholson (2006). "Bidirectional cross talk between ER α and EGFR signalling pathways regulates tamoxifen-resistant growth." Breast Cancer Research and Treatment **96**(2): 131-146.

Brooks, S. C. and L. Horn (1971). "Hepatic sulfation of estrogen metabolites." Biochim Biophys Acta **231**(1): 233-241.

Brown, J. R., E. Nigh, R. J. Lee, H. Ye, M. A. Thompson, F. Saudou, R. G. Pestell and M. E. Greenberg (1998). "Fos family members induce cell cycle entry by activating cyclin D1." Molecular and Cellular Biology **18**(9): 5609-5619.

Brown, R. W., L. Campagna, J. K. Dunn and P. T. Cagle (1997). "Immunohistochemical identification of tumor markers in metastatic adenocarcinoma: a diagnostic adjunct in the determination of primary site." American Journal of Clinical Pathology **107**(1): 12-19.

Brueggemeier, R. W., P.-K. Li, H.-H. Chen, P. P. Moh and N. E. Katlic (1990). "Biochemical and pharmacological development of steroidal inhibitors of aromatase." The Journal of Steroid Biochemistry and Molecular Biology **37**(3): 379-385.

Brzozowski, A. M., A. C. Pike, Z. Dauter, R. E. Hubbard, T. Bonn, O. Engström, L. Öhman, G. L. Greene, J.-Å. Gustafsson and M. Carlquist (1997). "Molecular basis of agonism and antagonism in the oestrogen receptor." Nature **389**(6652): 753-758.

Brzozowski, A. M., A. C. W. Pike, Z. Dauter, R. E. Hubbard, T. Bonn, O. Engstrom, L. Ohman, G. L. Greene, J. A. Gustafsson and M. Carlquist (1997). "Molecular basis of agonism and antagonism in the oestrogen receptor." Nature **389**(6652): 753-757.

- Buchanan, D. L., T. Kurita, J. A. Taylor, D. B. Lubahn, G. R. Cunha and P. S. Cooke (1998). "Role of stromal and epithelial estrogen receptors in vaginal epithelial proliferation, stratification, and cornification." Endocrinology **139**(10): 4345-4352.
- Buhi, W., I. Alvarez and A. Kouba (2008). "Secreted proteins of the oviduct." Cells Tissues Organs **166**(2): 165-179.
- Bunone, G., P. Briand, R. Miksicek and D. Picard (1996). "Activation of the unliganded estrogen receptor by EGF involves the MAP kinase pathway and direct phosphorylation." The EMBO journal **15**(9): 2174.
- Butler, W. B., W. L. Kirkland and T. L. Jorgensen (1979). "Induction of plasminogen activator by estrogen in a human breast cancer cell line (MCF-7)." Biochemical and Biophysical Research Communications **90**(4): 1328-1334.
- Calle, E. E., H. L. Miracle-McMahill, M. J. Thun and C. W. Heath (1995). "Estrogen replacement therapy and risk of fatal colon cancer in a prospective cohort of postmenopausal women." Journal of the National Cancer Institute **87**(7): 517-523.
- Campbell-Thompson, M., I. J. Lynch and B. Bhardwaj (2001). "Expression of estrogen receptor (ER) subtypes and ER β isoforms in colon cancer." Cancer Research **61**(2): 632-640.
- Chambliss, K. L., I. S. Yuhanna, R. G. Anderson, M. E. Mendelsohn and P. W. Shaul (2002). "ER β has nongenomic action in caveolae." Molecular Endocrinology **16**(5): 938-946.
- Chan, C. M., A. E. Lykkesfeldt, M. G. Parker and M. Dowsett (1999). "Expression of nuclear receptor interacting proteins TIF-1, SUG-1, receptor interacting protein 140, and corepressor SMRT in tamoxifen-resistant breast cancer." Clinical Cancer Research **5**(11): 3460-3467.
- Chan, H., W. Zhou, W. Fu, W. Ko and P. Wong (1995). "Different regulatory pathways involved in ATP - stimulated chloride secretion in rat epididymal epithelium." Journal of Cellular Physiology **164**(2): 271-276.
- Chang, E. C., J. Frasor, B. Komm and B. S. Katzenellenbogen (2006). "Impact of estrogen receptor β on gene networks regulated by estrogen receptor α in breast cancer cells." Endocrinology **147**(10): 4831-4842.
- Chen, D., H. Ma, H. Hong, S. S. Koh, S.-M. Huang, B. T. Schurter, D. W. Aswad and M. R. Stallcup (1999). "Regulation of transcription by a protein methyltransferase." Science **284**(5423): 2174-2177.
- Chen, H., T. R. Tritton, N. Kenny, M. Absher and J. F. Chiu (1996). "Tamoxifen induces TGF - β 1 activity and apoptosis of human MCF - 7 breast cancer cells in vitro." Journal of Cellular Biochemistry **61**(1): 9-17.
- Chen, J.-Q., P. A. Russo, C. Cooke, I. H. Russo and J. Russo (2007). "ER β shifts from mitochondria to nucleus during estrogen-induced neoplastic transformation of human breast epithelial cells and is involved in estrogen-induced synthesis of mitochondrial

respiratory chain proteins." Biochimica et Biophysica Acta (BBA)-Molecular Cell Research **1773**(12): 1732-1746.

Cheng, G., Y. Li, Y. Omoto, Y. Wang, T. Berg, M. Nord, P. Vihko, M. Warner, Y.-S. Piao and J.-Å. Gustafsson (2005). "Differential regulation of estrogen receptor (ER) α and ER β in primate mammary gland." Journal of Clinical Endocrinology & Metabolism **90**(1): 435-444.

Cheng, G., Y. Li, Y. Omoto, Y. Wang, T. Berg, M. Nord, P. Vihko, M. Warner, Y. S. Piao and J. A. Gustafsson (2005). "Differential regulation of estrogen receptor (ER) α and ER β in primate mammary gland." The Journal of Clinical Endocrinology & Metabolism **90**(1): 435-444.

Cheng, G., Z. Weihua, S. Mäkinen, S. Mäkelä S. Saji, M. Warner, J.-Å. Gustafsson and O. Hovatta (2002). "A role for the androgen receptor in follicular atresia of estrogen receptor beta knockout mouse ovary." Biology of Reproduction **66**(1): 77-84.

Cheng, G., Z. Weihua, M. Warner and J.-Å. Gustafsson (2004). "Estrogen receptors ER α and ER β in proliferation in the rodent mammary gland." Proceedings of the National Academy of Sciences USA **101**(11): 3739-3746.

Cheng, G., Z. Weihua, M. Warner and J. A. Gustafsson (2004). "Estrogen receptors ER α and ER β in proliferation in the rodent mammary gland." Proceedings of the National Academy of Sciences USA **101**(11): 3739-3746.

Cho, H. W., R. Nie, K. Carnes, Q. Zhou, N. Sharief and R. A. Hess (2003). "The antiestrogen ICI 182,780 induces early effects on the adult male mouse reproductive tract and long-term decreased fertility without testicular atrophy." Reproductive Biology and Endocrinology **1**(1): 57.

Ciarloni, L., S. Mallepell and C. Brisken (2007). "Amphiregulin is an essential mediator of estrogen receptor α function in mammary gland development." Proceedings of the National Academy of Sciences USA **104**(13): 5455-5460.

Ciarloni, L., S. Mallepell and C. Brisken (2007). "Amphiregulin is an essential mediator of estrogen receptor α function in mammary gland development." Proceedings of the National Academy of Sciences USA **104**(13): 5455-5460.

Clarke, R. B. (2004). "Human breast cell proliferation and its relationship to steroid receptor expression." Climacteric **7**(2): 129-137.

Clarke, R. B., E. Anderson, A. Howell and C. S. Potten (2003). "Regulation of human breast epithelial stem cells." Cell Proliferation **36**(s1): 45-58.

Clarke, R. B., A. Howell and E. Anderson (1997). "Estrogen sensitivity of normal human breast tissue in vivo and implanted into athymic nude mice: analysis of the relationship between estrogen-induced proliferation and progesterone receptor expression." Breast Cancer Research and Treatment **45**(2): 121-133.

Clarke, R. B., A. Howell, C. S. Potten and E. Anderson (1997). "Dissociation between steroid receptor expression and cell proliferation in the human breast." Cancer Research **57**(22): 4987-4991.

Cleland, W. H., C. R. Mendelson and E. R. Simpson (1985). "Effects of aging and obesity on aromatase activity of human adipose cells." Journal of Clinical Endocrinology & Metabolism **60**(1): 174-177.

Clemons, M. and P. Goss (2001). "Estrogen and the risk of breast cancer." New England Journal of Medicine **344**(4): 276-285.

Cobellis, G., R. Pierantoni, S. Minucci, R. Pernas-Alonso, R. Meccariello and S. Fasano (1999). "c-fos activity in *Rana esculenta* testis: seasonal and estradiol-induced changes." Endocrinology **140**(7): 3238-3244.

Cobellis, L., F. M. Reis, L. Driul, G. Vultaggio, I. Ferretti, E. Villa and F. Petraglia (2002). "Estrogen receptor α mRNA variant lacking exon 5 is co-expressed with the wild-type in endometrial adenocarcinoma." European Journal of Obstetrics & Gynecology and Reproductive Biology **102**(1): 92-95.

Cohen, D. and T. Curran (1989). "The structure and function of the fos proto-oncogene." Critical Reviews in Oncogenesis **1**(1): 65.

Collins, P. and C. Webb (1999). "Estrogen hits the surface." Nature Medicine **5**(10): 1130-1131.

Conrad, K. P., G. M. Joffe, H. Kruszyna, R. Kruszyna, L. Rochelle, R. Smith, J. Chavez and M. Mosher (1993). "Identification of increased nitric oxide biosynthesis during pregnancy in rats." The FASEB Journal **7**(6): 566-571.

Cooke, P., D. Buchanan, P. Young, T. Setiawan, J. Brody, K. Korach, J. Taylor, D. Lubahn and G. Cunha (1997). "Stromal estrogen receptors mediate mitogenic effects of estradiol on uterine epithelium." Proceedings of the National Academy of Sciences USA **94**(12): 6535-6540.

Cooke, P. S., F. Uchima, D. K. Fujii, H. A. Bern and G. R. Cunha (1986). "Restoration of normal morphology and estrogen responsiveness in cultured vaginal and uterine epithelia transplanted with stroma." Proceedings of the National Academy of Sciences USA **83**(7): 2109-2113.

Cotrim, C., V. Fabris, M. Doria, K. Lindberg, J.-Å. Gustafsson, F. Amado, C. Lanari and L. Helguero (2012). "Estrogen receptor beta growth-inhibitory effects are repressed through activation of MAPK and PI3K signalling in mammary epithelial and breast cancer cells." Oncogene **32**(19): 2390-2402.

Couse, J., S. C. Hewitt, D. Bunch, M. Sar, V. Walker, B. Davis and K. Korach (1999). "Postnatal sex reversal of the ovaries in mice lacking estrogen receptors and." Science Signaling **286**(5448): 2328.

Couse, J. F., V. L. Davis, R. B. Hanson, W. N. Jefferson, J. A. McLachlan, B. C. Bullock, R. R. Newbold and K. S. Korach (1997). "Accelerated onset of uterine tumors in transgenic

mice with aberrant expression of the estrogen receptor after neonatal exposure to diethylstilbestrol." Molecular Carcinogenesis **19**(4): 236-242.

Couse, J. F. and K. S. Korach (1999). "Estrogen receptor null mice: what have we learned and where will they lead us?" Endocrine Reviews **20**(3): 358.

Covalada, A. M. S., H. van den Berg, J. Vervoort, P. van der Saag, A. Ström, J.-Å. Gustafsson, I. Rietjens and A. J. Murk (2008). "Influence of cellular ER α /ER β ratio on the ER α -agonist induced proliferation of human T47D breast cancer cells." Toxicological Sciences **105**(2): 303-311.

Cowley, S. M., S. Hoare, S. Mosselman and M. G. Parker (1997). "Estrogen receptors α and β form heterodimers on DNA." Journal of Biological Chemistry **272**(32): 19858-19862.

Cowley, S. M. and M. G. Parker (1999). "A comparison of transcriptional activation by ER α and ER β ." The Journal of Steroid Biochemistry and Molecular Biology **69**(1): 165-175.

Crabtree, J. S., X. Zhang, B. J. Peano, Z. Zhang, R. C. Winneker and H. A. Harris (2006). "Development of a mouse model of mammary gland versus uterus tissue selectivity using estrogen-and progesterone-regulated gene markers." The Journal of Steroid Biochemistry and Molecular Biology **101**(1): 11-21.

Curtis, S. W., T. Washburn, C. Sewall, R. DiAugustine, J. Lindzey, J. F. Couse and K. S. Korach (1996). "Physiological coupling of growth factor and steroid receptor signaling pathways: estrogen receptor knockout mice lack estrogen-like response to epidermal growth factor." Proceedings of the National Academy of Sciences USA **93**(22): 12626-12630.

Danielian, P., R. White, J. Lees and M. Parker (1992). "Identification of a conserved region required for hormone dependent transcriptional activation by steroid hormone receptors." The EMBO journal **11**(3): 1025.

Darimont, B. D., R. L. Wagner, J. W. Apriletti, M. R. Stallcup, P. J. Kushner, J. D. Baxter, R. J. Fletterick and K. R. Yamamoto (1998). "Structure and specificity of nuclear receptor-coactivator interactions." Genes & development **12**(21): 3343-3356.

Davis, R. A., F. Kern, R. Showalter, E. Sutherland, M. Sinensky and F. R. Simon (1978). "Alterations of hepatic Na⁺, K⁺-ATPase and bile flow by estrogen: effects on liver surface membrane lipid structure and function." Proceedings of the National Academy of Sciences USA **75**(9): 4130-4134.

Delage-Mourroux, R., P. G. Martini, I. Choi, D. M. Kraichely, J. Hoeksema and B. S. Katzenellenbogen (2000). "Analysis of estrogen receptor interaction with a repressor of estrogen receptor activity (REA) and the regulation of estrogen receptor transcriptional activity by REA." Journal of Biological Chemistry **275**(46): 35848-35856.

Delmas, P. D., N. H. Bjarnason, B. H. Mitlak, A.-C. Ravoux, A. S. Shah, W. J. Huster, M. Draper and C. Christiansen (1997). "Effects of raloxifene on bone mineral density, serum

cholesterol concentrations, and uterine endometrium in postmenopausal women." New England Journal of Medicine **337**(23): 1641-1647.

DeMichele, A., A. B. Troxel, J. A. Berlin, A. L. Weber, G. R. Bunin, E. Turzo, R. Schinnar, D. Burgh, M. Berlin and S. C. Rubin (2008). "Impact of raloxifene or tamoxifen use on endometrial cancer risk: a population-based case-control study." Journal of Clinical Oncology **26**(25): 4151-4159.

Deming, S., S. Nass, R. Dickson and B. Trock (2000). "C-myc amplification in breast cancer: a meta-analysis of its occurrence and prognostic relevance." British Journal of Cancer **83**(12): 1688.

Deng, C.-X. and S. G. Brodie (2000). "Roles of BRCA1 and its interacting proteins." Bioessays **22**: 728-737.

Deroo, B. J. and A. V. Buensuceso (2010). "Minireview: Estrogen receptor- β : mechanistic insights from recent studies." Molecular Endocrinology **24**(9): 1703-1714.

Deroo, B. J. and K. S. Korach (2006). "Estrogen receptors and human disease." Journal of Clinical Investigation **116**(3): 561-570.

Dey, S. and H. Lim (1996). "Implantation." Reproductive Endocrinology, Surgery and Technology **1**: 421-435.

Di Domenico, M., G. Castoria, A. Bilancio, A. Migliaccio and F. Auricchio (1996). "Estradiol activation of human colon carcinoma-derived Caco-2 cell growth." Cancer Research **56**(19): 4516-4521.

Doershuk, C., B. Fisher and L. Matthews (1974). "Specific airway resistance from the perinatal period into adulthood. Alterations in childhood pulmonary disease." The American Review of Respiratory Disease **109**(4): 452-457.

Donovan, J. and J. Slingerland (2000). "Transforming growth factor-beta and breast cancer: Cell cycle arrest by transforming growth factor- β and its disruption in cancer." Breast Cancer Research **2**(2): 116.

Dubik, D., T. C. Dembinski and R. P. Shiu (1987). "Stimulation of c-myc oncogene expression associated with estrogen-induced proliferation of human breast cancer cells." Cancer Research **47**(24 Part 1): 6517-6521.

Dubik, D. and R. Shiu (1992). "Mechanism of estrogen activation of c-myc oncogene expression." Oncogene **7**(8): 1587-1594.

Dunbar, M. E., P. R. Dann, G. W. Robinson, L. Hennighausen, J.-P. Zhang and J. J. Wysolmerski (1999). "Parathyroid hormone-related protein signaling is necessary for sexual dimorphism during embryonic mammary development." Development **126**(16): 3485-3493.

Dupont, S., A. Krust, A. Gansmuller, A. Dierich, P. Chambon and M. Mark (2000). "Effect of single and compound knockouts of estrogen receptors alpha (ERalpha) and beta (ERbeta) on mouse reproductive phenotypes." Development **127**(19): 4277-4291.

Eghbali-Fatourehchi, G., S. Khosla, A. Sanyal, W. J. Boyle, D. L. Lacey and B. L. Riggs (2003). "Role of RANK ligand in mediating increased bone resorption in early postmenopausal women." Journal of Clinical Investigation **111**(8): 1221-1230.

El-Tanani, M. K. and C. D. Green (1997). "Two separate mechanisms for ligand-independent activation of the estrogen receptor." Molecular Endocrinology **11**(7): 928-937.

Endoh, H., K. Maruyama, Y. Masuhiro, Y. Kobayashi, M. Goto, H. Tai, J. Yanagisawa, D. Metzger, S. Hashimoto and S. Kato (1999). "Purification and identification of p68 RNA helicase acting as a transcriptional coactivator specific for the activation function 1 of human estrogen receptor α ." Molecular and Cellular Biology **19**(8): 5363-5372.

Engdahl, C., C. Jochems, S. H. Windahl, A. E. Bärjesson, C. Ohlsson, H. Carlsten and M. K. Lagerquist (2010). "Amelioration of collagen - induced arthritis and immune - associated bone loss through signaling via estrogen receptor α , and not estrogen receptor β or G protein - coupled receptor 30." Arthritis & Rheumatism **62**(2): 524-533.

Ewan, K. B., H. A. Oketch-Rabah, S. A. Ravani, G. Shyamala, H. L. Moses and M. H. Barcellos-Hoff (2005). "Proliferation of estrogen receptor- α -positive mammary epithelial cells is restrained by transforming growth factor- β 1 in adult mice." The American Journal of Pathology **167**(2): 409-417.

Ewan, K. B., G. Shyamala, S. A. Ravani, Y. Tang, R. Akhurst, L. Wakefield and M. H. Barcellos-Hoff (2002). "Latent transforming growth factor- β activation in mammary gland: regulation by ovarian hormones affects ductal and alveolar proliferation." The American Journal of Pathology **160**(6): 2081-2093.

Förster, C., S. Mäkela, A. Wäri, S. Kietz, D. Becker, K. Hultenby, M. Warner and J.-Å. Gustafsson (2002). "Involvement of estrogen receptor β in terminal differentiation of mammary gland epithelium." Proceedings of the National Academy of Sciences USA **99**(24): 15578-15583.

Fan, S., Y. X. Ma, C. Wang, R.-Q. Yuan, Q. Meng, J.-A. Wang, M. Erdos, I. D. Goldberg, P. Webb and P. J. Kushner (2002). "p300 Modulates the BRCA1 inhibition of estrogen receptor activity." Cancer Research **62**(1): 141-151.

Fan, S., J.-A. Wang, R. Yuan, Y. Ma, Q. Meng, M. Erdos, R. Pestell, F. Yuan, K. Auburn and I. Goldberg (1999). "BRCA1 inhibition of estrogen receptor signaling in transfected cells." Science **284**(5418): 1354-1356.

Fata, J. E., V. Chaudhary and R. Khokha (2001). "Cellular turnover in the mammary gland is correlated with systemic levels of progesterone and not 17 β -estradiol during the estrous cycle." Biology of Reproduction **65**(3): 680-688.

Feinleib, M. (1968). "Breast cancer and artificial menopause: a cohort study." Journal of the National Cancer Institute **41**(2): 315-329.

Ferguson, D. and T. Anderson (1981). "Morphological evaluation of cell turnover in relation to the menstrual cycle in the "resting" human breast." British Journal of Cancer **44**(2): 177.

- Ferin, M., R. Jewelewicz and M. P. Warren (1993). The menstrual cycle: Physiology, reproductive disorders, and infertility, Oxford University Press.
- Fernandez, E., S. Gallus, C. Bosetti, S. Franceschi, E. Negri and C. La Vecchia (2003). "Hormone replacement therapy and cancer risk: A systematic analysis from a network of case - control studies." International Journal of Cancer **105**(3): 408-412.
- Ferrandina, G., F. O. Ranelletti, V. Gallotta, E. Martinelli, G. F. Zannoni, M. Gessi and G. Scambia (2005). "Expression of cyclooxygenase-2 (COX-2), receptors for estrogen (ER), and progesterone (PR), p53, ki67, and neu protein in endometrial cancer." Gynecologic Oncology **98**(3): 383-389.
- Filardo, E. J., J. A. Quinn, K. I. Bland and A. R. Frackelton (2000). "Estrogen-induced activation of Erk-1 and Erk-2 requires the G protein-coupled receptor homolog, GPR30, and occurs via trans-activation of the epidermal growth factor receptor through release of HB-EGF." Molecular Endocrinology **14**(10): 1649-1660.
- Filardo, E. J., J. A. Quinn, A. R. Frackelton and K. I. Bland (2002). "Estrogen action via the G protein-coupled receptor, GPR30: stimulation of adenylyl cyclase and cAMP-mediated attenuation of the epidermal growth factor receptor-to-MAPK signaling axis." Molecular Endocrinology **16**(1): 70-84.
- Findlay, J. K., K. Britt, J. B. Kerr, L. O'Donnell, M. E. Jones, A. E. Drummond and E. R. Simpson (2001). "The road to ovulation: the role of oestrogens." Reproduction, Fertility and Development **13**(7-8): 543-547.
- Fiorelli, G., L. Picariello, V. Martineti, F. Tonelli and M. L. Brandi (1999). "Functional estrogen receptor β in colon cancer cells." Biochemical and Biophysical Research Communications **261**(2): 521-527.
- Fischer-Dzoga, K., R. W. Wissler and D. Vesselinovitch (1983). "The effect of estradiol on the proliferation of rabbit aortic medial tissue culture cells induced by hyperlipemic serum." Experimental and Molecular Pathology **39**(3): 355-363.
- Fisher, C. R., K. H. Graves, A. F. Parlow and E. R. Simpson (1998). "Characterization of mice deficient in aromatase (ArKO) because of targeted disruption of the cyp19 gene." Proceedings of the National Academy of Sciences USA **95**(12): 6965-6970.
- Foley, E. F., A. A. Jazaeri, M. A. Shupnik, O. Jazaeri and L. W. Rice (2000). "Selective loss of estrogen receptor β in malignant human colon." Cancer Research **60**(2): 245-248.
- Foley, J., P. Dann, J. Hong, J. Cosgrove, B. Dreyer, D. Rimm, M. Dunbar, W. Philbrick and J. Wysolmerski (2001). "Parathyroid hormone-related protein maintains mammary epithelial fate and triggers nipple skin differentiation during embryonic breast development." Development **128**(4): 513-525.
- Food, U. (2011). Drug Administration. Orange book: approved drug products with therapeutic equivalence evaluations.
- Franceschi, S. and C. La Vecchia (1998). "Colorectal cancer and hormone replacement therapy: an unexpected finding." European Journal of Cancer Prevention **7**(6): 427-438.

Frasor, J., D. H. Barnett, J. M. Danes, R. Hess, A. F. Parlow and B. S. Katzenellenbogen (2003). "Response-specific and ligand dose-dependent modulation of estrogen receptor (ER) α activity by ER β in the uterus." Endocrinology **144**(7): 3159-3166.

Frech, M. S., E. D. Halama, M. T. Tilli, B. Singh, E. J. Gunther, L. A. Chodosh, J. A. Flaws and P. A. Furth (2005). "Deregulated estrogen receptor α expression in mammary epithelial cells of transgenic mice results in the development of ductal carcinoma in situ." Cancer Research **65**(3): 681-685.

Freedman, L. P. (1999). "Increasing the complexity of coactivation in nuclear receptor signaling." Cell **97**(1): 5-8.

Frenette, G., P. Leclerc, O. D'amours and R. Sullivan (2009). "Estrogen sulfotransferase is highly expressed along the bovine epididymis and is secreted into the intraluminal environment." Journal of Andrology **30**(5): 580-589.

Galien, R. and T. Garcia (1997). "Estrogen receptor impairs interleukin-6 expression by preventing protein binding on the NF- κ B site." Nucleic Acids Research **25**(12): 2424-2429.

Ganjam, v. k. and r. p. Amann (1976). "Steroids in fluids and sperm entering and leaving the bovine epididymis, epididymal tissue, and accessory sex gland secretions." Endocrinology **99**(6): 1618-1630.

Gehin, M., M. Mark, C. Dennefeld, A. Dierich, H. Gronemeyer and P. Chambon (2002). "The function of TIF2/GRIP1 in mouse reproduction is distinct from those of SRC-1 and p/CIP." Molecular and Cellular Biology **22**(16): 5923-5937.

Genazzani, A. R. (2001). Hormone replacement therapy and cardiovascular disease. New York, Parthenon Pub. Group.

Georgescu, S. P., J. H. Li, Q. Lu, R. H. Karas, M. Brown and M. E. Mendelsohn (2005). "Modulator recognition factor 1, an AT-rich interaction domain family member, is a novel corepressor for estrogen receptor α ." Molecular Endocrinology **19**(10): 2491-2501.

Gethins, M. (2012). "Breast cancer in men." Journal of the National Cancer Institute **104**(6): 436-438.

Ghosh, D., J. Griswold, M. Erman and W. Pangborn (2009). "Structural basis for androgen specificity and oestrogen synthesis in human aromatase." Nature **457**(7226): 219-223.

Girault, I., C. Andrieu, S. Tozlu, F. Spyrtos, I. Bièche and R. Lidereau (2004). "Altered expression pattern of alternatively spliced estrogen receptor β transcripts in breast carcinoma." Cancer Letters **215**(1): 101-112.

Girault, I., C. Andrieu, S. Tozlu, F. Spyrtos, I. Bieche and R. Lidereau (2004). "Altered expression pattern of alternatively spliced estrogen receptor beta transcripts in breast carcinoma." Cancer letters **215**(1): 101-112.

Glasier, A. and A. S. McNeilly (1990). "Physiology of lactation." Baillière's Clinical Endocrinology and Metabolism **4**(2): 379-395.

Goldstein, S. R. (1998). "Selective estrogen receptor modulators: a new category of therapeutic agents for extending the health of postmenopausal women." American Journal of Obstetrics and Gynecology **179**(6): 1479-1484.

Gorodeski, G. (1996). "The cervical cycle." Reproductive Endocrinology, Surgery, and Technology **1**: 302-324.

Gorodeski, G. I. (1998). "Estrogen increases the permeability of the cultured human cervical epithelium by modulating cell deformability." American Journal of Physiology-Cell Physiology **275**(3): C888-C899.

Goss, P. E., J. N. Ingle, J. E. Alés-Martínez, A. M. Cheung, R. T. Chlebowski, J. Wactawski-Wende, A. McTiernan, J. Robbins, K. C. Johnson and L. W. Martin (2011). "Exemestane for breast-cancer prevention in postmenopausal women." New England Journal of Medicine **364**(25): 2381-2391.

Grady, D., T. Gebretsadik, K. Kerlikowske, V. Ernster and D. Petitti (1995). "Hormone replacement therapy and endometrial cancer risk: a meta-analysis." Obstetrics & Gynecology **85**(2): 304-313.

Green, P. S., J. Bishop and J. W. Simpkins (1997). "17 α -Estradiol exerts neuroprotective effects on SK-N-SH cells." The Journal of Neuroscience **17**(2): 511-515.

Green, S., P. Walter, V. Kumar, A. Krust, J.-M. Bornert, P. Argos and P. Chambon (1986). "Human oestrogen receptor cDNA: sequence, expression and homology to v-erb-A."

Grey, A., J. Stapleton, M. Evans and I. Reid (1995). "The effect of the anti-estrogen tamoxifen on cardiovascular risk factors in normal postmenopausal women." Journal of Clinical Endocrinology & Metabolism **80**(11): 3191-3195.

Gruber, C. J., W. Tschugguel, C. Schneeberger and J. C. Huber (2002). "Production and actions of estrogens." New England Journal of Medicine **346**(5): 340-352.

Gruvberger-Saal, S. K., P. O. Bendahl, L. H. Saal, M. Laakso, C. Hegardt, P. Eden, C. Peterson, P. Malmstrom, J. Isola, A. Borg and M. Ferno (2007). "Estrogen receptor beta expression is associated with tamoxifen response in ERalpha-negative breast carcinoma." Clinical Cancer Research **13**(7): 1987-1994.

Gudelsky, G. A., D. D. Nansel and J. C. Porter (1981). "Role of estrogen in the dopaminergic control of prolactin secretion." Endocrinology **108**(2): 440-444.

Gustafsson, J.-A. (1999). "Estrogen receptor beta--a new dimension in estrogen mechanism of action." Journal of Endocrinology **163**(3): 379-383.

Hadley, M. and J. E. Levine (2006). Endocrinology, 6/e, Pearson Education India.

Hall, J. M., J. F. Couse and K. S. Korach (2001). "The multifaceted mechanisms of estradiol and estrogen receptor signaling." Journal of Biological Chemistry **276**(40): 36869-36872.

Hall, J. M. and D. P. McDonnell (1999). "The estrogen receptor β -isoform (ER β) of the human estrogen receptor modulates ER α transcriptional activity and is a key regulator of the cellular response to estrogens and antiestrogens." Endocrinology **140**(12): 5566-5578.

Hanahan, D. and R. A. Weinberg (2000). "The hallmarks of cancer." Cell **100**(1): 57-70.

Hanstein, B., R. Eckner, J. DiRenzo, S. Halachmi, H. Liu, B. Searcy, R. Kurokawa and M. Brown (1996). "p300 is a component of an estrogen receptor coactivator complex." Proceedings of the National Academy of Sciences USA **93**(21): 11540-11545.

Harris, H. A. (2006). "The unexpected science of estrogen receptor- β selective agonists: a new class of anti-inflammatory agents?" Nuclear Receptor Signaling **4**.

Harris, H. A. (2007). "Estrogen receptor-beta: recent lessons from in vivo studies." Molecular Endocrinology **21**(1): 1-13.

Harris, H. A., L. M. Albert, Y. Leathurby, M. S. Malamas, R. E. Mewshaw, C. P. Miller, Y. P. Kharode, J. Marzolf, B. S. Komm and R. C. Winneker (2003). "Evaluation of an estrogen receptor- β agonist in animal models of human disease." Endocrinology **144**(10): 4241-4249.

Harris, H. A., J. A. Katzenellenbogen and B. S. Katzenellenbogen (2002). "Characterization of the biological roles of the estrogen receptors, ER α and ER β , in estrogen target tissues in vivo through the use of an ER α -selective ligand." Endocrinology **143**(11): 4172-4177.

Hartman, J., K. Lindberg, A. Morani, J. Inzunza, A. Ström and J.-Å. Gustafsson (2006). "Estrogen receptor β inhibits angiogenesis and growth of T47D breast cancer xenografts." Cancer Research **66**(23): 11207-11213.

Hartman, J., A. Strom and J. A. Gustafsson (2009). "Estrogen receptor beta in breast cancer--diagnostic and therapeutic implications." Steroids **74**(8): 635-641.

Haskell, S. G. (2003). "Selective estrogen receptor modulators." Southern medical journal **96**(5): 469.

Hatcher, R. A. (2007). Contraceptive technology, Physicians Desk Reference Incorporated.

Hayes, D. F., J. Van Zyl, A. Hacking, L. Goedhals, W. Bezwoda, J. A. Mailliard, S. E. Jones, C. L. Vogel, R. F. Berris and I. Shemano (1995). "Randomized comparison of tamoxifen and two separate doses of toremifene in postmenopausal patients with metastatic breast cancer." Journal of clinical oncology **13**(10): 2556.

Heiss, G., R. Wallace, G. L. Anderson, A. Aragaki, S. A. Beresford, R. Brzyski, R. T. Chlebowski, M. Gass, A. LaCroix and J. E. Manson (2008). "Health risks and benefits 3 years after stopping randomized treatment with estrogen and progestin." Journal of the American Medical Association **299**(9): 1036-1045.

Heldin, C.-H., M. Landström and A. Moustakas (2009). "Mechanism of TGF- β signaling to growth arrest, apoptosis, and epithelial-mesenchymal transition." Current Opinion in Cell Biology **21**(2): 166-176.

Heldring, N., A. Pike, S. Andersson, J. Matthews, G. Cheng, J. Hartman, M. Tujague, A. Ström, E. Treuter and M. Warner (2007). "Estrogen Receptors: how do they signal and what are their targets." Physiological Reviews **87**(3): 905-931.

Helguero, L. A., M. H. Faulds, J.-Å. Gustafsson and L.-A. Haldosén (2005). "Estrogen receptors alfa (ER α) and beta (ER β) differentially regulate proliferation and apoptosis of the normal murine mammary epithelial cell line HC11." Oncogene **24**(44): 6605-6616.

Helguero, L. A., M. H. Faulds, J. A. Gustafsson and L. A. Haldosen (2005). "Estrogen receptors alfa (ERalpha) and beta (ERbeta) differentially regulate proliferation and apoptosis of the normal murine mammary epithelial cell line HC11." Oncogene **24**(44): 6605-6616.

Hemsell, D. L., J. Grodin, P. Brenner, P. Siiteri and P. MacDonald (1974). "Plasma precursors of estrogen. II. Correlation of the extent of conversion of plasma androstenedione to estrone with age." Journal of Clinical Endocrinology & Metabolism **38**(3): 476-479.

Henderson, V. W. (2000). Estrogen for Alzheimer's disease in women Randomized, double-blind, placebo-controlled trial.

Hennighausen, L. and G. W. Robinson (2001). "Signaling pathways in mammary gland development." Developmental Cell **1**(4): 467-475.

Hens, J. R., P. Dann, J.-P. Zhang, S. Harris, G. W. Robinson and J. Wysolmerski (2007). "BMP4 and PTHrP interact to stimulate ductal outgrowth during embryonic mammary development and to inhibit hair follicle induction." Development **134**(6): 1221-1230.

Henttu, P., E. Kalkhoven and M. G. Parker (1997). "AF-2 activity and recruitment of steroid receptor coactivator 1 to the estrogen receptor depend on a lysine residue conserved in nuclear receptors." Molecular and Cellular Biology **17**(4): 1832-1839.

Herber, B., M. Truss, M. Beato and R. Müller (1994). "Inducible regulatory elements in the human cyclin D1 promoter." Oncogene **9**(4): 1295-1304.

Hernán, M. A., A. Alonso, R. Logan, F. Grodstein, K. B. Michels, W. C. Willett, J. E. Manson and J. M. Robins (2008). "Observational studies analyzed like randomized experiments: an application to postmenopausal hormone therapy and coronary heart disease." Epidemiology **19**(6): 766-779.

Hernandez, J. S., R. W. Watson, T. C. Wood and R. M. Weinshilboum (1992). "Sulfation of estrone and 17 beta-estradiol in human liver. Catalysis by thermostable phenol sulfotransferase and by dehydroepiandrosterone sulfotransferase." Drug Metabolism and Disposition **20**(3): 413-422.

Hershberger, P. A., A. C. Vasquez, B. Kanterewicz, S. Land, J. M. Siegfried and M. Nichols (2005). "Regulation of endogenous gene expression in human non-small cell lung cancer cells by estrogen receptor ligands." Cancer Research **65**(4): 1598-1605.

Herynk, M. H. and S. A. Fuqua (2004). "Estrogen receptor mutations in human disease." Endocrine Reviews **25**(6): 869-898.

Hess, R. and K. Carnes (2004). "The role of estrogen in testis and the male reproductive tract: a review and species comparison." Animal Reproduction **1**(1): 5-30.

Hess, R. A. (2003). "Estrogen in the adult male reproductive tract: a review." Reproductive Biology and Endocrinology **1**(1): 52.

Hess, R. A., D. Bunick, K.-H. Lee, J. Bahr, J. A. Taylor, K. S. Korach and D. B. Lubahn (1997). "A role for oestrogens in the male reproductive system." Nature **390**(6659): 509-512.

Hess, R. A., D. H. Gist, D. Bunick, D. B. Lubahn, A. Farrell, J. Bahr, P. S. Cooke and G. L. Greene (1997). "Estrogen receptor (α and β) expression in the excurrent ducts of the adult male rat reproductive tract." Journal of Andrology **18**(6): 602-611.

Hewitt, S. C., E. H. Goulding, E. Eddy and K. S. Korach (2002). "Studies using the estrogen receptor α knockout uterus demonstrate that implantation but not decidualization-associated signaling is estrogen dependent." Biology of Reproduction **67**(4): 1268-1277.

Hewitt, S. C. and K. S. Korach (2002). "Estrogen receptors: structure, mechanisms and function." Reviews in Endocrine and Metabolic Disorders **3**(3): 193-200.

Hickey, M., J. Elliott and S. L. Davison (2012). "Hormone replacement therapy." British Medical Journal **344**.

Hodges-Gallagher, L., C. D. Valentine, S. El Bader and P. J. Kushner (2008). "Estrogen receptor beta increases the efficacy of antiestrogens by effects on apoptosis and cell cycling in breast cancer cells." Breast Cancer Research and Treatment **109**(2): 241-250.

Hoff, M. B. and W. W. Chang (1979). "The effect of estrogen on epithelial cell proliferation and differentiation in the crypts of the descending colon of the mouse: A radioautographic study." American Journal of Anatomy **155**(4): 507-516.

Hoff, M. B., W. W. Chang and K. M. Mak (1980). "Effect of estrogen on cell proliferation in colonic mucosa of the mouse." Virchows Archiv B **35**(1): 263-273.

Hofseth, L. J., A. M. Raafat, J. R. Osuch, D. R. Pathak, C. A. Slomski and S. Z. Haslam (1999). "Hormone replacement therapy with estrogen or estrogen plus medroxyprogesterone acetate is associated with increased epithelial proliferation in the normal postmenopausal breast." Journal of Clinical Endocrinology & Metabolism **84**(12): 4559-4565.

Honma, N., R. Horii, T. Iwase, S. Saji, M. Younes, K. Takubo, M. Matsuura, Y. Ito, F. Akiyama and G. Sakamoto (2008). "Clinical importance of estrogen receptor-beta evaluation in breast cancer patients treated with adjuvant tamoxifen therapy." Journal of Clinical Oncology **26**(22): 3727-3734.

Honma, N., R. Horii, T. Iwase, S. Saji, M. Younes, K. Takubo, M. Matsuura, Y. Ito, F. Akiyama and G. Sakamoto (2008). "Clinical importance of estrogen receptor- β evaluation in breast cancer patients treated with adjuvant tamoxifen therapy." Journal of Clinical Oncology **26**(22): 3727-3734.

Hou, Y. F., S. T. Yuan, H. C. Li, J. Wu, J. S. Lu, G. Liu, L. J. Lu, Z. Z. Shen, J. Ding and Z. M. Shao (2004). "ERbeta exerts multiple stimulative effects on human breast carcinoma cells." Oncogene **23**(34): 5799-5806.

Hughes, Z. A., F. Liu, B. J. Platt, J. M. Dwyer, C. M. Pulicicchio, G. Zhang, L. E. Schechter, S. Rosenzweig-Lipson and M. Day (2008). "WAY-200070, a selective agonist of estrogen receptor beta as a potential novel anxiolytic/antidepressant agent." Neuropharmacology **54**(7): 1136-1142.

Hulley, S., D. Grady, T. Bush, C. Furberg, D. Herrington, B. Riggs and E. Vittinghoff (1998). "Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women." Journal of the American Medical Association **280**(7): 605-613.

Hunter, D. J., G. A. Colditz, S. E. Hankinson, S. Malspeis, D. Spiegelman, W. Chen, M. J. Stampfer and W. C. Willett (2010). "Oral contraceptive use and breast cancer: a prospective study of young women." Cancer Epidemiology Biomarkers & Prevention **19**(10): 2496-2502.

Ibarra de Palacios, P., G. Schmidt, T. Sergejew, E. Quebe-Fehling, L. Lockhart and L. Krinsky (2002). "Comparative study to evaluate skin irritation and adhesion of Estradot® and Climara® in healthy postmenopausal women." Climacteric **5**(4): 383-389.

Ignar-Trowbridge, D., M. Pimentel, M. Parker, J. McLachlan and K. Korach (1996). "Peptide growth factor cross-talk with the estrogen receptor requires the A/B domain and occurs independently of protein kinase C or estradiol." Endocrinology **137**(5): 1735-1744.

Ignar-Trowbridge, D. M., K. G. Nelson, M. C. Bidwell, S. W. Curtis, T. F. Washburn, J. A. McLachlan and K. S. Korach (1992). "Coupling of dual signaling pathways: epidermal growth factor action involves the estrogen receptor." Proceedings of the National Academy of Sciences USA **89**(10): 4658-4662.

Ing, N. H. and M. B. Tornesi (1997). "Estradiol up-regulates estrogen receptor and progesterone receptor gene expression in specific ovine uterine cells." Biology of Reproduction **56**(5): 1205-1215.

Issa, J.-P. J., Y. L. Ottaviano, P. Celano, S. R. Hamilton, N. E. Davidson and S. B. Baylin (1994). "Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon." Nature Genetics **7**(4): 536-540.

Järvinen, T. A., M. Peltö-Huikko, K. Holli and J. Isola (2000). "Estrogen receptor β is coexpressed with ER α and PR and associated with nodal status, grade, and proliferation rate in breast cancer." The American Journal of Pathology **156**(1): 29-35.

Jameson, J. L. and L. J. De Groot (2010). Endocrinology: adult and pediatric, Saunders.

Jansen, R. P. (1984). "Endocrine response in the fallopian tube." Endocrine Reviews **5**(4): 525-551.

Jensen, E. (1962). "On the mechanism of estrogen action." Perspectives in Biology and Medicine **6**: 47.

Jensen, E. V., G. Cheng, C. Palmieri, S. Saji, S. Mäkelä, S. Van Noorden, T. Wahlström, M. Warner, R. C. Coombes and J.-Å. Gustafsson (2001). "Estrogen receptors and

proliferation markers in primary and recurrent breast cancer." Proceedings of the National Academy of Sciences USA **98**(26): 15197-15202.

Jick, H., J. A. Kaye, C. Vasilakis-Scaramozza and S. S. Jick (2000). "Risk of venous thromboembolism among users of third generation oral contraceptives compared with users of oral contraceptives with levonorgestrel before and after 1995: cohort and case-control analysis." British Medical Journal **321**(7270): 1190-1195.

Jilka, R. (1998). "Cytokines, bone remodeling, and estrogen deficiency: a 1998 update." Bone **23**(2): 75.

Joel, P. B., J. Smith, T. W. Sturgill, T. L. Fisher, J. Blenis and D. A. Lannigan (1998). "pp90rsk1 regulates estrogen receptor-mediated transcription through phosphorylation of Ser-167." Molecular and Cellular Biology **18**(4): 1978-1984.

Jones, R. E. and K. H. Lopez (2006). Human reproductive biology.

Jordan, V. C. and A. M. Brodie (2007). "Development and evolution of therapies targeted to the estrogen receptor for the treatment and prevention of breast cancer." Steroids **72**(1): 7-25.

Jordan, V. C. and B. W. O'Malley (2007). "Selective estrogen-receptor modulators and antihormonal resistance in breast cancer." Journal of Clinical Oncology **25**(36): 5815-5824.

Kacsoh, B. (2000). Endocrine physiology, McGraw-Hill.

Kahlert, S., S. Nuedling, M. van Eickels, H. Vetter, R. Meyer and C. Grohé (2000). "Estrogen receptor α rapidly activates the IGF-1 receptor pathway." Journal of Biological Chemistry **275**(24): 18447-18453.

Karas, R. H., B. L. Patterson and M. E. Mendelsohn (1994). "Human vascular smooth muscle cells contain functional estrogen receptor." Circulation **89**(5): 1943-1950.

Kariagina, A., J. Xie, J. R. Leippardt and S. Z. Haslam (2010). "Amphiregulin mediates estrogen, progesterone, and EGFR signaling in the normal rat mammary gland and in hormone-dependent rat mammary cancers." Hormones and Cancer **1**(5): 229-244.

Karlsson, S. (2006). "Histopathology and histomorphometry of the urogenital tract in 15-month old male and female rats treated neonatally with SERMs and estrogens." Experimental and Toxicologic Pathology **58**(1): 1-12.

Kato, S. (2001). "Estrogen receptor-mediated cross-talk with growth factor signaling pathways." Breast Cancer **8**(1): 3-9.

Kato, S., H. Endoh, Y. Masuhiro, T. Kitamoto, S. Uchiyama, H. Sasaki, S. Masushige, Y. Gotoh, E. Nishida and H. Kawashima (1995). "Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase." Science **270**(5241): 1491-1494.

Keating, N. L., P. D. Cleary, A. S. Rossi, A. M. Zaslavsky and J. Z. Ayanian (1999). "Use of hormone replacement therapy by postmenopausal women in the United States." Annals of Internal Medicine **130**(7): 545-553.

Kennecke, H., R. Yerushalmi, R. Woods, M. C. U. Cheang, D. Voduc, C. H. Speers, T. O. Nielsen and K. Gelmon (2010). "Metastatic behavior of breast cancer subtypes." Journal of Clinical Oncology **28**(20): 3271-3277.

Kenney, N. J., G. H. Smith, K. Rosenberg, M. L. Cutler and R. B. Dickson (1996). "Induction of ductal morphogenesis and lobular hyperplasia by amphiregulin in the mouse mammary gland." Cell Growth & Differentiation **7**(12): 1769-1781.

Khachaturian, Z. S. (1985). "Diagnosis of Alzheimer's disease." Archives of Neurology **42**(11): 1097.

Khan, S. A., M. A. Rogers, K. K. Khurana, M. M. Meguid and P. J. Numann (1998). "Estrogen receptor expression in benign breast epithelium and breast cancer risk." Journal of the National Cancer Institute **90**(1): 37-42.

Khurana, S., S. Ranmal and N. Ben-Jonathan (2000). "Exposure of newborn male and female rats to environmental estrogens: delayed and sustained hyperprolactinemia and alterations in estrogen receptor expression." Endocrinology **141**(12): 4512-4517.

Kim, N., K. Min, M. Pessina, R. Munarriz, I. Goldstein and A. Traish (2004). "Effects of ovariectomy and steroid hormones on vaginal smooth muscle contractility." International Journal of Impotence Research **16**(1): 43-50.

Kleerekoper, M. (2001). Estrogen and the Skeleton. Hormonal Carcinogenesis III, Springer: 357-362.

Kleuser, B., D. Malek, R. Gust, H. H. Pertz and H. Potteck (2008). "17- β -Estradiol inhibits transforming growth factor- β signaling and function in breast cancer cells via activation of extracellular signal-regulated kinase through the G protein-coupled receptor 30." Molecular Pharmacology **74**(6): 1533-1543.

Kobayashi, Y., T. Kitamoto, Y. Masuhiro, M. Watanabe, T. Kase, D. Metzger, J. Yanagisawa and S. Kato (2000). "p300 mediates functional synergism between AF-1 and AF-2 of estrogen receptor α and β by interacting directly with the N-terminal A/B domains." Journal of Biological Chemistry **275**(21): 15645-15651.

Kouros-Mehr, H., E. M. Slorach, M. D. Sternlicht and Z. Werb (2006). "GATA-3 maintains the differentiation of the luminal cell fate in the mammary gland." Cell **127**(5): 1041-1055.

Kraus, W. and B. Katzenellenbogen (1993). "Regulation of progesterone receptor gene expression and growth in the rat uterus: modulation of estrogen actions by progesterone and sex steroid hormone antagonists." Endocrinology **132**(6): 2371-2379.

Kraus, W. L. and J. T. Kadonaga (1998). "p300 and estrogen receptor cooperatively activate transcription via differential enhancement of initiation and reinitiation." Genes & development **12**(3): 331-342.

Krege, J. H., J. B. Hodgin, J. F. Couse, E. Enmark, M. Warner, J. F. Mahler, M. Sar, K. S. Korach, J.-Å. Gustafsson and O. Smithies (1998). "Generation and reproductive

phenotypes of mice lacking estrogen receptor β ." Proceedings of the National Academy of Sciences USA **95**(26): 15677-15682.

Kronenberg, M. S. and J. H. Clark (1985). "Changes in keratin expression during the estrogen-mediated differentiation of rat vaginal epithelium." Endocrinology **117**(4): 1480-1489.

Kuiper (1997). "Carlsson B. Grandien K. Enmark E. Häggblad J. Nilsson S. Gustafsson JA. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta." Endocrinology **138**(3): 863-870.

Kuiper, G., E. Enmark, M. Peltö-Huikko, S. Nilsson and J.-A. Gustafsson (1996). "Cloning of a novel receptor expressed in rat prostate and ovary." Proceedings of the National Academy of Sciences USA **93**(12): 5925-5930.

Kuiper, G. G., B. Carlsson, K. Grandien, E. Enmark, J. Häggblad, S. Nilsson and J.-Å. Gustafsson (1997). "Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β ." Endocrinology **138**(3): 863-870.

Kuiper, G. G., J. G. Lemmen, B. Carlsson, J. C. Corton, S. H. Safe, P. T. van der Saag, B. van der Burg and J.-Å. Gustafsson (1998). "Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β ." Endocrinology **139**(10): 4252-4263.

Kuiper, G. G., J. G. Lemmen, B. Carlsson, J. C. Corton, S. H. Safe, P. T. van der Saag, B. van der Burg and J. A. Gustafsson (1998). "Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta." Endocrinology **139**(10): 4252-4263.

Kumle, M., E. Weiderpass, T. Braaten, I. Persson, H.-O. Adami and E. Lund (2002). "Use of Oral Contraceptives and Breast Cancer Risk The Norwegian-Swedish Women's Lifestyle and Health Cohort Study." Cancer Epidemiology Biomarkers & Prevention **11**(11): 1375-1381.

Kushner, P. J., D. A. Agard, G. L. Greene, T. S. Scanlan, A. K. Shiau, R. M. Uht and P. Webb (2000). "Estrogen receptor pathways to AP-1." The Journal of Steroid Biochemistry and Molecular Biology **74**(5): 311-317.

LaCroix, A. Z., T. Powles, C. K. Osborne, K. Wolter, J. R. Thompson, D. D. Thompson, D. C. Allred, R. Armstrong, S. R. Cummings and R. Eastell (2010). "Breast cancer incidence in the randomized PEARL trial of lasofoxifene in postmenopausal osteoporotic women." Journal of the National Cancer Institute **102**(22): 1706-1715.

Laherty, C. D., A. N. Billin, R. M. Lavinsky, G. S. Yochum, A. C. Bush, J.-M. Sun, T.-M. Mullen, J. R. Davie, D. W. Rose and C. K. Glass (1998). "SAP30, a component of the mSin3 corepressor complex involved in N-CoR-mediated repression by specific transcription factors." Molecular Cell **2**(1): 33-42.

Lannigan, D. A. (2003). "Estrogen receptor phosphorylation." Steroids **68**(1): 1-9.

Larner, J. M. and R. B. Hochberg (1985). "The clearance and metabolism of estradiol and estradiol-17-esters in the rat." Endocrinology **117**(3): 1209-1214.

- Lavinsky, R. M., K. Jepsen, T. Heinzel, J. Torchia, T.-M. Mullen, R. Schiff, A. L. Del-Rio, M. Ricote, S. Ngo and J. Gemsch (1998). "Diverse signaling pathways modulate nuclear receptor recruitment of N-CoR and SMRT complexes." Proceedings of the National Academy of Sciences USA **95**(6): 2920-2925.
- Lazari, M. F. M., T. F. G. Lucas, F. Yasuhara, G. R. O. Gomes, E. R. Siu, C. Royer, S. A. F. Fernandes and C. S. Porto (2009). "Estrogen receptors and function in the male reproductive system." Arquivos Brasileiros de Endocrinologia & Metabologia **53**(8): 923-933.
- Lazennec, G., D. Bresson, A. Lucas, C. Chauveau and F. Vignon (2001). "ER beta inhibits proliferation and invasion of breast cancer cells." Endocrinology **142**(9): 4120-4130.
- Lazennec, G., D. Bresson, A. Lucas, C. Chauveau and F. Vignon (2001). "ER β inhibits proliferation and invasion of breast cancer cells." Endocrinology **142**(9): 4120-4130.
- Lecce, G., G. Meduri, M. ANCELIN, C. BERGERON and M. PERROT-APPLANAT (2001). "Presence of estrogen receptor β in the human endometrium through the cycle: expression in glandular, stromal, and vascular cells." Journal of Clinical Endocrinology & Metabolism **86**(3): 1379-1386.
- Lee, K.-H., C. Finnigan-Bunick, J. Bahr and D. Bunick (2001). "Estrogen regulation of ion transporter messenger RNA levels in mouse efferent ductules are mediated differentially through estrogen receptor (ER) α and ER β ." Biology of Reproduction **65**(5): 1534-1541.
- Leers, J., E. Treuter and J.-Å. Gustafsson (1998). "Mechanistic principles in NR box-dependent interaction between nuclear hormone receptors and the coactivator TIF2." Molecular and Cellular Biology **18**(10): 6001-6013.
- Leese, H. (1988). "The formation and function of oviduct fluid." Journal of Reproduction and Fertility **82**(2): 843-856.
- Lemmen, J. G., J. L. Broekhof, G. G. Kuiper, J.-Å. Gustafsson, P. T. van der Saag and B. van der Burg (1999). "Expression of estrogen receptor alpha and beta during mouse embryogenesis." Mechanisms of Development **81**(1): 163-167.
- Leon, R. L., J. D. Huber and C. L. Rosen (2011). "Potential age-dependent effects of estrogen on neural injury." The American Journal of Pathology **178**(6): 2450-2460.
- Lewis-Wambi, J. S. and V. C. Jordan (2009). "Estrogen regulation of apoptosis: how can one hormone stimulate and inhibit." Breast Cancer Research **11**(3): 206.
- Li, J. and R. W. McMurray (2006). "Effects of estrogen receptor subtype-selective agonists on immune functions in ovariectomized mice." International Immunopharmacology **6**(9): 1413-1423.
- Li, S. (1994). "Relationship between cellular DNA synthesis, PCNA expression and sex steroid hormone receptor status in the developing mouse ovary, uterus and oviduct." Histochemistry **102**(5): 405-413.
- Liu, H., K. Liu and D. L. Bodenner (2005). "Estrogen receptor inhibits interleukin-6 gene expression by disruption of nuclear factor κ B transactivation." Cytokine **31**(4): 251-257.

Liu, M.-M., C. Albanese, C. M. Anderson, K. Hilty, P. Webb, R. M. Uht, R. H. Price, R. G. Pestell and P. J. Kushner (2002). "Opposing action of estrogen receptors α and β on cyclin D1 gene expression." Journal of Biological Chemistry **277**(27): 24353-24360.

Liu, M. M., C. Albanese, C. M. Anderson, K. Hilty, P. Webb, R. M. Uht, R. H. Price, Jr., R. G. Pestell and P. J. Kushner (2002). "Opposing action of estrogen receptors alpha and beta on cyclin D1 gene expression." The Journal of Biological Chemistry **277**(27): 24353-24360.

Liu, S., G. Dontu, I. D. Mantle, S. Patel, N.-s. Ahn, K. W. Jackson, P. Suri and M. S. Wicha (2006). "Hedgehog signaling and Bmi-1 regulate self-renewal of normal and malignant human mammary stem cells." Cancer Research **66**(12): 6063-6071.

Liu, S. and F. Mauvais-Jarvis (2009). "Rapid, nongenomic estrogen actions protect pancreatic islet survival." Islets **1**(3): 273-275.

Livak, K. J. and T. D. Schmittgen (2001). "Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method." Methods **25**(4): 402-408.

Love, R. R., R. B. Mazess, H. S. Barden, S. Epstein, P. A. Newcomb, V. C. Jordan, P. P. Carbone and D. L. DeMets (1992). "Effects of tamoxifen on bone mineral density in postmenopausal women with breast cancer." New England Journal of Medicine **326**(13): 852-856.

Lubahn, D. B., J. S. Moyer, T. S. Golding, J. F. Couse, K. S. Korach and O. Smithies (1993). "Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene." Proceedings of the National Academy of Sciences USA **90**(23): 11162-11166.

Lucas, T. F., E. R. Siu, C. A. Esteves, H. P. Monteiro, C. A. Oliveira, C. S. Porto and M. F. M. Lazari (2008). "17beta-estradiol induces the translocation of the estrogen receptors ESR1 and ESR2 to the cell membrane, MAPK3/1 phosphorylation and proliferation of cultured immature rat Sertoli cells." Biology of Reproduction **78**(1): 101-114.

Lund, T. D., T. Rovis, W. C. Chung and R. J. Handa (2005). "Novel actions of estrogen receptor- β on anxiety-related behaviors." Endocrinology **146**(2): 797-807.

Ma, Z., S. Santagati, C. Patrone, G. Pollio, E. Vegeto and A. Maggi (1994). "Insulin-like growth factors activate estrogen receptor to control the growth and differentiation of the human neuroblastoma cell line SK-ER3." Molecular Endocrinology **8**(7): 910-918.

MacCalman, C. D., S. Getsios, R. Farookhi and O. W. Blaschuk (1997). "Estrogens potentiate the stimulatory effects of follicle-stimulating hormone on N-cadherin messenger ribonucleic acid levels in cultured mouse Sertoli cells." Endocrinology **138**(1): 41-48.

Macgregor, J. I. and V. C. Jordan (1998). "Basic guide to the mechanisms of antiestrogen action." Pharmacological Reviews **50**(2): 151-196.

Mahato, D., E. H. Goulding, K. S. Korach and E. M. Eddy (2000). "Spermatogenic cells do not require estrogen receptor- α for development or function." Endocrinology **141**(3): 1273-1273.

- Maki, D. D. and R. I. Grossman (2000). "Patterns of disease spread in metastatic breast carcinoma: influence of estrogen and progesterone receptor status." American Journal of Neuroradiology **21**(6): 1064-1066.
- Malamas, M. S., E. S. Manas, R. E. McDevitt, I. Gunawan, Z. B. Xu, M. D. Collini, C. P. Miller, T. Dinh, R. A. Henderson and J. C. Keith (2004). "Design and synthesis of aryl diphenolic azoles as potent and selective estrogen receptor- β ligands." Journal of Medicinal Chemistry **47**(21): 5021-5040.
- Mallepell, S., A. Krust, P. Chambon and C. Briskin (2006). "Paracrine signaling through the epithelial estrogen receptor α is required for proliferation and morphogenesis in the mammary gland." Proceedings of the National Academy of Sciences USA **103**(7): 2196-2201.
- Mallepell, S., A. Krust, P. Chambon and C. Briskin (2006). "Paracrine signaling through the epithelial estrogen receptor α is required for proliferation and morphogenesis in the mammary gland." Proceedings of the National Academy of Sciences USA **103**(7): 2196-2201.
- Manolagas, S. C. and R. L. Jilka (1995). "Bone marrow, cytokines, and bone remodeling. Emerging insights into the pathophysiology of osteoporosis." The New England Journal of Medicine **332**(5): 305.
- Marchbanks, P. A., K. M. Curtis, M. G. Mandel, H. G. Wilson, G. Jeng, S. G. Folger, J. A. McDonald, J. R. Daling, L. Bernstein and K. E. Malone (2012). "Oral contraceptive formulation and risk of breast cancer." Contraception **85**(4): 342-350.
- Marieb, E. N. and K. Hoehn (2007). Human anatomy & physiology, Pearson Education.
- Marsh, D. A., H. J. Brodie, W. Garrett, C. H. Tsai-Morris and A. M. Brodie (1985). "Aromatase inhibitors. Synthesis and biological activity of androstenedione derivatives." Journal of Medicinal Chemistry **28**(6): 788-795.
- Martin, L.-A. and M. Dowsett (2013). "BCL-2: A New Therapeutic Target in Estrogen Receptor-Positive Breast Cancer?" Cancer Cell **24**(1): 7-9.
- Martin, L., C. Finn and G. Trinder (1973). "Hypertrophy and hyperplasia in the mouse uterus after oestrogen treatment: an autoradiographic study." Journal of Endocrinology **56**(1): 133-NP.
- Martineti, V., L. Picariello, I. Tognarini, S. C. Sala, A. Gozzini, C. Azzari, C. Mavilia, A. Tanini, A. Falchetti and G. Fiorelli (2005). "ER β is a potent inhibitor of cell proliferation in the HCT8 human colon cancer cell line through regulation of cell cycle components." Endocrine-related Cancer **12**(2): 455-469.
- Marttunen, M. B., P. Hietanen, A. Tiitinen and O. Ylikorkala (1998). "Comparison of effects of tamoxifen and toremifene on bone biochemistry and bone mineral density in postmenopausal breast cancer patients." Journal of Clinical Endocrinology & Metabolism **83**(4): 1158.

- Martucci, C. and J. Fishman (1993). "P450 enzymes of estrogen metabolism." Pharmacology & Therapeutics **57**(2-3): 237.
- Massaro, D., L. B. Clerch and G. D. Massaro (2007). "Estrogen receptor- α regulates pulmonary alveolar loss and regeneration in female mice: morphometric and gene expression studies." American Journal of Physiology-Lung Cellular and Molecular Physiology **293**(1): L222-L228.
- Massaro, G. D., J. P. Mortola and D. Massaro (1996). "Estrogen modulates the dimensions of the lung's gas-exchange surface area and alveoli in female rats." American Journal of Physiology-Lung Cellular and Molecular Physiology **270**(1): L110-L114.
- Matsuda, S., Y. Kadowaki, M. Ichino, T. Akiyama, K. Toyoshima and T. Yamamoto (1993). "17 beta-estradiol mimics ligand activity of the c-erbB2 protooncogene product." Proceedings of the National Academy of Sciences USA **90**(22): 10803-10807.
- Matsuda, T., T. Yamamoto, A. Muraguchi and F. Saatcioglu (2001). "Cross-talk between transforming growth factor- β and estrogen receptor signaling through Smad3." Journal of Biological Chemistry **276**(46): 42908-42914.
- Matsuzaki, S., T. Fukaya, T. Suzuki, T. Murakami, H. Sasano and A. Yajima (1999). "Oestrogen receptor α and β mRNA expression in human endometrium throughout the menstrual cycle." Molecular Human Reproduction **5**(6): 559-564.
- Matthews, J. and J.-Å. Gustafsson (2003). "Estrogen signaling: a subtle balance between ER α and ER β ." Molecular Interventions **3**(5): 281.
- Matthews, J. and J. Gustafsson (2003). "Estrogen signaling: a subtle balance between ER alpha and ER beta." Molecular Interventions **3**(5): 281.
- Mawson, A., A. Lai, J. S. Carroll, C. M. Sergio, C. J. Mitchell and B. Sarcevic (2005). "Estrogen and insulin/IGF-1 cooperatively stimulate cell cycle progression in MCF-7 breast cancer cells through differential regulation of c-Myc and cyclin D1." Molecular and Cellular Endocrinology **229**(1): 161-173.
- Mazumdar, A., R.-A. Wang, S. K. Mishra, L. Adam, R. Bagheri-Yarmand, M. Mandal, R. K. Vadlamudi and R. Kumar (2000). "Transcriptional repression of oestrogen receptor by metastasis-associated protein 1 corepressor." Nature Cell Biology **3**(1): 30-37.
- McDonnell, D. P., D. L. Clemm, T. Hermann, M. E. Goldman and J. Pike (1995). "Analysis of estrogen receptor function in vitro reveals three distinct classes of antiestrogens." Molecular Endocrinology **9**(6): 659-669.
- McDonnell, D. P. and J. D. Norris (2002). "Connections and regulation of the human estrogen receptor." Science **296**(5573): 1642-1644.
- McEwen, B. S. and S. E. Alves (1999). "Estrogen actions in the central nervous system." Endocrine Reviews **20**(3): 279-307.
- McInerney, E. M., M.-J. Tsai, B. W. O'Malley and B. S. Katzenellenbogen (1996). "Analysis of estrogen receptor transcriptional enhancement by a nuclear hormone receptor coactivator." Proceedings of the National Academy of Sciences USA **93**(19): 10069-10073.

McInerney, E. M., K. E. Weis, J. Sun, S. Mosselman and B. S. Katzenellenbogen (1998). "Transcription activation by the human estrogen receptor subtype beta (ER beta) studied with ER beta and ER alpha receptor chimeras." Endocrinology **139**(11): 4513-4522.

McKenna, N. J., R. B. Lanz and B. W. O'Malley (1999). "Nuclear receptor coregulators: cellular and molecular biology." Endocrine Reviews **20**(3): 321-344.

McKinnell, C., N. Atanassova, K. Williams, J. Fisher, M. Walker, K. Turner, P. Saunders and R. Sharpe (2001). "Suppression of androgen action and the induction of gross abnormalities of the reproductive tract in male rats treated neonatally with diethylstilbestrol." Journal of Andrology **22**(2): 323-338.

Meisel, R. and B. Sachs (1994). "The physiology of male sexual behavior." The Physiology of Reproduction **2**: 3-105.

Meistrich, M., T. Hughes and W. Bruce (1975). "Alteration of epididymal sperm transport and maturation in mice by oestrogen and testosterone."

Meyers, M. J., J. Sun, K. E. Carlson, G. A. Marriner, B. S. Katzenellenbogen and J. A. Katzenellenbogen (2001). "Estrogen receptor-beta potency-selective ligands: structure-activity relationship studies of diarylpropionitriles and their acetylene and polar analogues." Journal of Medicinal Chemistry **44**(24): 4230-4251.

Meyers, M. J., J. Sun, K. E. Carlson, G. A. Marriner, B. S. Katzenellenbogen and J. A. Katzenellenbogen (2001). "Estrogen receptor- β potency-selective ligands: structure-activity relationship studies of diarylpropionitriles and their acetylene and polar analogues." Journal of Medicinal Chemistry **44**(24): 4230-4251.

Meyers, R. R. A. (2005). Encyclopedia of molecular cell biology and molecular medicine, Wiley Online Library.

Migliaccio, A., G. Castoria, M. Di Domenico, A. de Falco, A. Bilancio, M. Lombardi, M. V. Barone, D. Ametrano, M. S. Zannini and C. Abbondanza (2000). "Steroid-induced androgen receptor-oestradiol receptor β -Src complex triggers prostate cancer cell proliferation." The EMBO journal **19**(20): 5406-5417.

Migliaccio, A., M. Di Domenico, G. Castoria, A. De Falco, P. Bontempo, E. Nola and F. Auricchio (1996). "Tyrosine kinase/p21ras/MAP-kinase pathway activation by estradiol-receptor complex in MCF-7 cells." The EMBO journal **15**(6): 1292.

Millier, S. G., P. F. Whitelaw and C. D. Smyth (1994). "Follicular oestrogen synthesis: the 'two-cell, two-gonadotrophin' model revisited." Molecular and Cellular Endocrinology **100**(1): 51-54.

Minutolo, F., M. Macchia, B. S. Katzenellenbogen and J. A. Katzenellenbogen (2011). "Estrogen receptor β ligands: Recent advances and biomedical applications." Medicinal Research Reviews **31**(3): 364-442.

Mollerup, S., K. Jørgensen, G. Berge and A. Haugen (2002). "Expression of estrogen receptors α and β in human lung tissue and cell lines." Lung Cancer **37**(2): 153-159.

- Mooradian, A. D. (1993). "Antioxidant properties of steroids." The Journal of Steroid Biochemistry and Molecular Biology **45**(6): 509-511.
- Morani, A., M. Warner and J. Å. Gustafsson (2008). "Biological functions and clinical implications of oestrogen receptors alfa and beta in epithelial tissues." Journal of Internal Medicine **264**(2): 128-142.
- Mosher, W. D., G. M. Martinez, A. Chandra, J. C. Abma and S. J. Willson (2004). Use of contraception and use of family planning services in the United States, 1982-2002, US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics.
- Mosselman, S., J. Polman and R. Dijkema (1996). "ER β : identification and characterization of a novel human estrogen receptor." FEBS letters **392**(1): 49-53.
- Motrich, R. D., A. A. Ponce and V. E. Rivero (2007). "Effect of tamoxifen treatment on the semen quality and fertility of the male rat." Fertility and Sterility **88**(2): 452-461.
- Mowa, C. and T. Iwanaga (2000). "Developmental changes of the oestrogen receptor-alpha and-beta mRNAs in the female reproductive organ of the rat--an analysis by in situ hybridization." Journal of Endocrinology **167**(3): 363-369.
- Mulac-Jericevic, B., J. P. Lydon, F. J. DeMayo and O. M. Conneely (2003). "Defective mammary gland morphogenesis in mice lacking the progesterone receptor B isoform." Proceedings of the National Academy of Sciences USA **100**(17): 9744-9749.
- Musey, P., K. Wright, J. Preedy and D. Collins (1997). "Formation and metabolism of steroid conjugates: effect of conjugation on excretion and tissue distribution." Steroid Biochemistry **2**: 81-132.
- Musgrove, E. A., C. Lee, M. F. Buckley and R. L. Sutherland (1994). "Cyclin D1 induction in breast cancer cells shortens G1 and is sufficient for cells arrested in G1 to complete the cell cycle." Proceedings of the National Academy of Sciences USA **91**(17): 8022-8026.
- Nabholtz, J., A. Buzdar, M. Pollak, W. Harwin, G. Burton, A. Mangalik, M. Steinberg, A. Webster and M. Von Euler (2000). "Anastrozole is superior to tamoxifen as first-line therapy for advanced breast cancer in postmenopausal women: results of a North American multicenter randomized trial." Journal of Clinical Oncology **18**(22): 3758-3767.
- Navarrete, M., C. M. Maier, R. Falzoni, L. Quadros, G. R. Lima, E. C. Baracat and A. Nazario (2005). "Assessment of the proliferative, apoptotic and cellular renovation indices of the human mammary epithelium during the follicular and luteal phases of the menstrual cycle." Breast Cancer Research **7**(3): R306-313.
- Nelson, H. D., B. Zakher, A. Cantor, R. Fu, J. Griffin, E. S. O'Meara, D. S. Buist, K. Kerlikowske, N. T. van Ravesteyn and A. Trentham-Dietz (2012). "Risk Factors for Breast Cancer for Women Aged 40 to 49 Years A Systematic Review and Meta-analysis." Annals of Internal Medicine **156**(9): 635-648.
- Nelson, L. R. and S. E. Bulun (2001). "Estrogen production and action." Journal of the American Academy of Dermatology **45**(3): S116-S124.

Nephew, K. P., X. Long, E. Osborne, K. A. Burke, A. Ahluwalia and R. M. Bigsby (2000). "Effect of estradiol on estrogen receptor expression in rat uterine cell types." Biology of Reproduction **62**(1): 168-177.

Newcomb, P. A. and B. E. Storer (1995). "Postmenopausal hormone use and risk of large-bowel cancer." Journal of the National Cancer Institute **87**(14): 1067-1071.

Newton, C. J., R. Buric, T. Trapp, S. Brockmeier, U. Pagotto and G. K. Stalla (1994). "The unliganded estrogen receptor (ER) transduces growth factor signals." The Journal of steroid biochemistry and molecular biology **48**(5): 481-486.

Nikolic, I., D. Liu, J. A. Bell, J. Collins, C. Steenbergen and E. Murphy (2007). "Treatment with an estrogen receptor-beta-selective agonist is cardioprotective." Journal of Molecular and Cellular Cardiology **42**(4): 769-780.

Nilsson, O. and S. Reinius (1969). "Light and electron microscopic structure of the oviduct." The Mammalian Oviduct: 57-84.

Nilsson, S., S. Makela, E. Treuter, M. Tujague, J. Thomsen, G. Andersson, E. Enmark, K. Pettersson, M. Warner and J. A. Gustafsson (2001). "Mechanisms of estrogen action." Physiological Reviews **81**(4): 1535-1565.

Norris, J. D., D. Fan, A. Sherk and D. P. McDonnell (2002). "A negative coregulator for the human ER." Molecular Endocrinology **16**(3): 459-468.

Nunno, L. D., L. G. Larsson, J. J. Rinehart and R. S. Beissner (2000). "Estrogen and progesterone receptors in non-small cell lung cancer in 248 consecutive patients who underwent surgical resection." Archives of Pathology & Laboratory Medicine **124**(10): 1467-1470.

O'donnell, L., K. M. Robertson, M. E. Jones and E. R. Simpson (2001). "Estrogen and spermatogenesis." Endocrine Reviews **22**(3): 289-318.

Ogawa, S., S. Inoue, T. Watanabe, H. Hiroi, A. Orimo, T. Hosoi, Y. Ouchi and M. Muramatsu (1998). "The Complete Primary Structure of Human Estrogen Receptor β (hER β) and Its Heterodimerization with ER α in Vivo and in Vitro." Biochemical and Biophysical Research Communications **243**(1): 122-126.

Ojeda, S. and S. McCann (1974). "Development of dopaminergic and estrogenic control of prolactin release in the female rat." Endocrinology **95**(6): 1499-1505.

Oliveira, C. A., G. A. Mahecha, K. Carnes, G. S. Prins, P. T. Saunders, L. R. França and R. A. Hess (2004). "Differential hormonal regulation of estrogen receptors ER α and ER β and androgen receptor expression in rat efferent ductules." Reproduction **128**(1): 73-86.

Oliveira, C. A., Q. Zhou, K. Carnes, R. Nie, D. E. Kuehl, G. L. Jackson, L. R. Franca, M. Nakai and R. A. Hess (2002). "ER function in the adult male rat: short-and long-term effects of the antiestrogen ICI 182,780 on the testis and efferent ductules, without changes in testosterone." Endocrinology **143**(6): 2399-2409.

Ollayos, C., G. Riordan and J. Rushin (1994). "Estrogen receptor detection in paraffin sections of adenocarcinoma of the colon, pancreas, and lung." Archives of Pathology & Laboratory Medicine **118**(6): 630-632.

Omoto, Y., Y. Kobayashi, K. Nishida, E. Tsuchiya, H. Eguchi, K. Nakagawa, Y. Ishikawa, T. Yamori, H. Iwase and Y. Fujii (2001). "Expression, function, and clinical implications of the estrogen receptor β in human lung cancers." Biochemical and Biophysical Research Communications **285**(2): 340-347.

Onate, S. A., V. Boonyaratanakornkit, T. E. Spencer, S. Y. Tsai, M.-J. Tsai, D. P. Edwards and B. W. O'Malley (1998). "The steroid receptor coactivator-1 contains multiple receptor interacting and activation domains that cooperatively enhance the activation function 1 (AF1) and AF2 domains of steroid receptors." Journal of Biological Chemistry **273**(20): 12101-12108.

Overpeck, J. G., S. H. Colson, J. R. Hohmann, M. S. Applestine and J. F. Reilly (1978). "Concentrations of circulating steroids in normal prepubertal and adult male and female humans, chimpanzees, rhesus monkeys, rats, mice, and hamsters: a literature survey." Journal of Toxicology and Environmental Health, Part A Current Issues **4**(5-6): 785-803.

Paech, K., P. Webb, G. G. Kuiper, S. Nilsson, J.-Å. Gustafsson, P. J. Kushner and T. S. Scanlan (1997). "Differential ligand activation of estrogen receptors ER α and ER β at AP1 sites." Science **277**(5331): 1508-1510.

Palijan, A., I. Fernandes, M. Verway, M. Kourelis, Y. Bastien, L. E. Tavera-Mendoza, A. Sacheli, V. Bourdeau, S. Mader and J. H. White (2009). "Ligand-dependent corepressor LCoR is an attenuator of progesterone-regulated gene expression." Journal of Biological Chemistry **284**(44): 30275-30287.

Palmieri, C., G. Cheng, S. Saji, M. Zelada-Hedman, Z. Weihua, S. Van Noorden, T. Wahlstrom, R. Coombes, M. Warner and J. Gustafsson (2002). "Estrogen receptor beta in breast cancer." Endocrine-related Cancer **9**(1): 1-13.

Parakkal, P. F. (1974). "Cyclical changes in the vaginal epithelium of the rat seen by scanning electron microscopy." Anat Rec **178**(3): 529-537.

Parakkal, P. F. and A. Gregoire (1972). "Differentiation of vaginal epithelium in the normal and hormone-treated rhesus monkey." Biology of Reproduction **6**(1): 117-130.

Paruthiyil, S., A. Cvorov, X. Zhao, Z. Wu, Y. Sui, R. E. Staub, S. Baggett, C. B. Herber, C. Griffin and M. Tagliaferri (2009). "Drug and cell type-specific regulation of genes with different classes of estrogen receptor β -selective agonists." PloS one **4**(7): e6271.

Paruthiyil, S., H. Parmar, V. Kerekatte, G. R. Cunha, G. L. Firestone and D. C. Leitman (2004). "Estrogen receptor beta inhibits human breast cancer cell proliferation and tumor formation by causing a G2 cell cycle arrest." Cancer Research **64**(1): 423-428.

Paruthiyil, S., H. Parmar, V. Kerekatte, G. R. Cunha, G. L. Firestone and D. C. Leitman (2004). "Estrogen receptor β inhibits human breast cancer cell proliferation and tumor formation by causing a G2 cell cycle arrest." Cancer Research **64**(1): 423-428.

- Pascoe, P. A. (1996). *Endocrinology*: By Mac E. Hadley. Upper Saddle River, NJ, Prentice Hall, 1996, \$80.00 (xiii+ 518 Pages), ISBN 0-13-317926-5, Elsevier Current Trends.
- Patrone, C., T. N. Cassel, K. Pettersson, Y.-S. Piao, G. Cheng, P. Ciana, A. Maggi, M. Warner, J.-Å. Gustafsson and M. Nord (2003). "Regulation of postnatal lung development and homeostasis by estrogen receptor β ." *Molecular and Cellular Biology* **23**(23): 8542-8552.
- Pelletier, G. and M. El-Alfy (2000). "Immunocytochemical localization of estrogen receptors α and β in the human reproductive organs." *Journal of Clinical Endocrinology & Metabolism* **85**(12): 4835-4840.
- Pentikäinen, V., K. Erkkilä L. Suomalainen, M. Parvinen and L. Dunkel (2000). "Estradiol acts as a germ cell survival factor in the human testis in vitro." *Journal of Clinical Endocrinology & Metabolism* **85**(5): 2057-2067.
- Persson, I., E. Weiderpass, L. Bergkvist, R. Bergström and C. Schairer (1999). "Risks of breast and endometrial cancer after estrogen and estrogen-progestin replacement." *Cancer Causes & Control* **10**(4): 253-260.
- Persson, I., E. Weiderpass, L. Bergkvist, R. Bergstrom and C. Schairer (1999). "Risks of breast and endometrial cancer after estrogen and estrogen-progestin replacement." *Cancer Causes Control* **10**(4): 253-260.
- Pettersson, K., K. Grandien, G. G. Kuiper and J.-Å. Gustafsson (1997). "Mouse estrogen receptor β forms estrogen response element-binding heterodimers with estrogen receptor α ." *Molecular Endocrinology* **11**(10): 1486-1496.
- Pettersson, K. and J.-Å. Gustafsson (2001). "Role of estrogen receptor beta in estrogen action." *Annual Review of Physiology* **63**(1): 165-192.
- Pezzi, V., J. Mathis, W. E. Rainey and B. R. Carr (2003). "Profiling transcript levels for steroidogenic enzymes in fetal tissues." *The Journal of Steroid Biochemistry and Molecular Biology* **87**(2): 181-189.
- Pfaff, D., S. Schwartz-Giblin, M. McCarthy, L. Kow, E. Knobil and J. Neill (1994). *Physiology of reproduction*.
- Pietras, R. J., D. C. Márquez, H.-W. Chen, E. Tsai, O. Weinberg and M. Fishbein (2005). "Estrogen and growth factor receptor interactions in human breast and non-small cell lung cancer cells." *Steroids* **70**(5): 372-381.
- Pike, M. C. and R. K. Ross (2000). "Progestins and menopause: epidemiological studies of risks of endometrial and breast cancer." *Steroids* **65**(10): 659-664.
- Platet, N., S. Cunat, D. Chalbos, H. Rochefort and M. Garcia (2000). "Unliganded and liganded estrogen receptors protect against cancer invasion via different mechanisms." *Molecular Endocrinology* **14**(7): 999-1009.
- Potten, C. S., R. Watson, G. Williams, S. Tickle, S. A. Roberts, M. Harris and A. Howell (1988). "The effect of age and menstrual cycle upon proliferative activity of the normal human breast." *British Journal of Cancer* **58**(2): 163.

- Power, R. F., S. K. Mani, J. Codina, O. M. Conneely and B. W. O'Malley (1991). "Dopaminergic and ligand-independent activation of steroid hormone receptors." Science **254**(5038): 1636-1639.
- Pragnell, M., K. Snay, J. Trimmer, N. MacLusky, F. Naftolin, L. Kaczmarek and M. Boyle (1990). "Estrogen induction of a small, putative K⁺ channel mRNA in rat uterus." Neuron **4**(5): 807-812.
- Prall, O. W., B. Sarcevic, E. A. Musgrove, C. K. Watts and R. L. Sutherland (1997). "Estrogen-induced activation of Cdk4 and Cdk2 during G1-S phase progression is accompanied by increased cyclin D1 expression and decreased cyclin-dependent kinase inhibitor association with cyclin E-Cdk2." Journal of Biological Chemistry **272**(16): 10882-10894.
- Prescott, E., A. M. Bjerg, P. K. Andersen, P. Lange and J. Vestbo (1997). "Gender difference in smoking effects on lung function and risk of hospitalization for COPD: results from a Danish longitudinal population study." European Respiratory Journal **10**(4): 822-827.
- Pyo Kim, H., J. Young Lee, J. Kim Jeong, S. Won Bae, H. Kyu Lee and I. Jo (1999). "Nongenomic stimulation of nitric oxide release by estrogen is mediated by estrogen receptor α localized in caveolae." Biochemical and Biophysical Research Communications **263**(1): 257-262.
- Qing, Z., R. NIE, G. S. PRINS, P. T. SAUNDERS, B. S. KATZENELLENBOGEN and R. A. HESS (2002). "Localization of androgen and estrogen receptors in adult male mouse reproductive tract." Journal of Andrology **23**(6): 870-881.
- Quirk, S., R. Cowan, R. Harman, C.-L. Hu and D. Porter (2004). "Ovarian follicular growth and atresia: the relationship between cell proliferation and survival." Journal of Animal Science **82**(13 suppl): E40-E52.
- Regan, M. M., G. Viale, M. G. Mastropasqua, E. Maiorano, R. Golouh, A. Carbone, B. Brown, M. Suurkõla, G. Langman and L. Mazzucchelli (2006). "Re-evaluating adjuvant breast cancer trials: assessing hormone receptor status by immunohistochemical versus extraction assays." Journal of the National Cancer Institute **98**(21): 1571-1581.
- Ripoll, C., A. B. Ropero, P. Alonso-Magdalena, I. Quesada, E. Fuentes and A. Nadal (2008). "Rapid Regulation of Pancreatic-and-Cell Signalling Systems by Estrogens." Infectious Disorders-Drug Targets **8**(1): 61-64.
- Ritte, R., A. Lukanova, A. Tjønneland, A. Olsen, K. Overvad, S. Mesrine, G. Fagherazzi, L. Dossus, B. Teucher and K. Steindorf (2012). "Height, age at menarche and risk of hormone receptor - positive and - negative breast cancer: A cohort study." International Journal of Cancer.
- Rody, A., U. Holtrich, C. Solbach, K. Kourtis, G. Von Minckwitz, K. Engels, S. Kissler, R. Gätje, T. Karn and M. Kaufmann (2005). "Methylation of estrogen receptor β promoter

correlates with loss of ER- β expression in mammary carcinoma and is an early indication marker in premalignant lesions." Endocrine-related Cancer **12**(4): 903-916.

Roger, P., M. E. Sahla, S. Mäkelä J. Å. Gustafsson, P. Baldet and H. Rochefort (2001). "Decreased expression of estrogen receptor β protein in proliferative preinvasive mammary tumors." Cancer Research **61**(6): 2537-2541.

Rosenfeld, M. G., V. V. Lunyak and C. K. Glass (2006). "Sensors and signals: a coactivator/corepressor/epigenetic code for integrating signal-dependent programs of transcriptional response." Genes & Development **20**(11): 1405-1428.

Ross, R. K., A. Paganini-Hill, P. C. Wan and M. C. Pike (2000). "Effect of hormone replacement therapy on breast cancer risk: estrogen versus estrogen plus progestin." Journal of the National Cancer Institute **92**(4): 328-332.

Rossouw, J. E., G. L. Anderson, R. L. Prentice, A. Z. LaCroix, C. Kooperberg, M. Stefanick, R. D. Jackson, S. A. Beresford, B. V. Howard and K. C. Johnson (2002). "Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial." Journal of the American Medical Association **288**(3): 321-333.

Ruan, W., V. Catanese, R. Wiczorek, M. Feldman and D. Kleinberg (1995). "Estradiol enhances the stimulatory effect of insulin-like growth factor-I (IGF-I) on mammary development and growth hormone-induced IGF-I messenger ribonucleic acid." Endocrinology **136**(3): 1296-1302.

Russo, I. H. and J. Russo (1998). "Role of hormones in mammary cancer initiation and progression." Journal of Mammary Gland Biology and Neoplasia **3**(1): 49-61.

Russo, J., X. Ao, C. Grill and I. Russo (1999). "Pattern of distribution of cells positive for estrogen receptor α and progesterone receptor in relation to proliferating cells in the mammary gland." Breast Cancer Research and Treatment **53**(3): 217-227.

Söderqvist, G., E. Isaksson, B. von Schoultz, K. Carlström, E. Tani and L. Skoog (1997). "Proliferation of breast epithelial cells in healthy women during the menstrual cycle." American Journal of Obstetrics and Gynecology **176**(1): 123-128.

Sánchez-Criado, J. E., C. Bellido, M. Tena-Sempere, R. Aguilar and A. Blanco (2004). "Biological role of pituitary estrogen receptors ER α and ER β on progesterone receptor expression and action and on gonadotropin and prolactin secretion in the rat." Neuroendocrinology **79**(5): 247-258.

Sabourin, J., A. Martin, J. Baruch, J. Truc, A. Gompel and P. Poitout (1994). "bcl - 2 expression in normal breast tissue during the menstrual cycle." International Journal of Cancer **59**(1): 1-6.

Saji, S., E. V. Jensen, S. Nilsson, T. Rylander, M. Warner and J.-Å. Gustafsson (2000). "Estrogen receptors α and β in the rodent mammary gland." Breast Cancer Research **2**(Suppl 1): S. 11.

- Saji, S., E. V. Jensen, S. Nilsson, T. Rylander, M. Warner and J. A. Gustafsson (2000). "Estrogen receptors alpha and beta in the rodent mammary gland." Proceedings of the National Academy of Sciences USA **97**(1): 337-342.
- Santen, R. J., P. Fan, Z. Zhang, Y. Bao, R. X. Song and W. Yue (2009). "Estrogen signals via an extra-nuclear pathway involving IGF-1R and EGFR in tamoxifen-sensitive and -resistant breast cancer cells." Steroids **74**(7): 586-594.
- Sar, M. and F. Welsch (1999). "Differential expression of estrogen receptor-beta and estrogen receptor-alpha in the rat ovary." Endocrinology **140**(2): 963-971.
- Saville, B., M. Wormke, F. Wang, T. Nguyen, E. Enmark, G. Kuiper, J.-Å. Gustafsson and S. Safe (2000). "Ligand-, cell-, and estrogen receptor subtype (α/β)-dependent activation at GC-rich (Sp1) promoter elements." Journal of Biological Chemistry **275**(8): 5379-5387.
- Saville, B., M. Wormke, F. Wang, T. Nguyen, E. Enmark, G. Kuiper, J. A. Gustafsson and S. Safe (2000). "Ligand-, cell-, and estrogen receptor subtype (alpha/beta)-dependent activation at GC-rich (Sp1) promoter elements." Journal of Biological Chemistry **275**(8): 5379-5387.
- Schairer, C., J. Lubin, R. Troisi, S. Sturgeon, L. Brinton and R. Hoover (2000). "Menopausal estrogen and estrogen-progestin replacement therapy and breast cancer risk." Journal of the American Medical Association **283**(4): 485-491.
- Schams, D., S. Kohlenberg, W. Amselgruber, B. Berisha, M. Pfaffl and F. Sinowatz (2003). "Expression and localisation of oestrogen and progesterone receptors in the bovine mammary gland during development, function and involution." Journal of Endocrinology **177**(2): 305-317.
- Schedin, P., T. Mitrenga and M. Kaeck (2000). "Estrous cycle regulation of mammary epithelial cell proliferation, differentiation, and death in the Sprague-Dawley rat: a model for investigating the role of estrous cycling in mammary carcinogenesis." Journal of Mammary Gland Biology and Neoplasia **5**(2): 211-225.
- Schramek, D., V. Sigl and J. M. Penninger (2011). "RANKL and RANK in sex hormone-induced breast cancer and breast cancer metastasis." Trends in Endocrinology & Metabolism **22**(5): 188-194.
- Schwabe, J. W., L. Chapman, J. T. Finch and D. Rhodes (1993). "The crystal structure of the estrogen receptor DNA-binding domain bound to DNA: how receptors discriminate between their response elements." Cell **75**(3): 567-578.
- Scully, K. M., A. S. Gleiberman, J. Lindzey, D. B. Lubahn, K. S. Korach and M. G. Rosenfeld (1997). "Role of estrogen receptor- α in the anterior pituitary gland." Molecular Endocrinology **11**(6): 674-681.
- Shaaban, A. M., P. A. O'Neill, M. P. Davies, R. Sibson, C. R. West, P. H. Smith and C. S. Foster (2003). "Declining Estrogen Receptor-[beta] Expression Defines Malignant Progression of Human Breast Neoplasia." The American Journal of Surgical Pathology **27**(12): 1502-1512.

Shaaban, A. M., J. P. Sloane, C. R. West and C. S. Foster (2002). "Breast cancer risk in usual ductal hyperplasia is defined by estrogen receptor- α and Ki-67 expression." The American Journal of Pathology **160**(2): 597-604.

Shang, Y. and M. Brown (2002). "Molecular determinants for the tissue specificity of SERMs." Science **295**(5564): 2465-2468.

Shanle, E. K. and W. Xu (2010). "Endocrine disrupting chemicals targeting estrogen receptor signaling: identification and mechanisms of action." Chemical Research in Toxicology **24**(1): 6-19.

Shao, R., E. Egecioglu, B. Weijdegård, J. J. Kopchick, J. Fernandez-Rodriguez, N. Andersson and H. Billig (2007). "Dynamic regulation of estrogen receptor- α isoform expression in the mouse fallopian tube: mechanistic insight into estrogen-dependent production and secretion of insulin-like growth factors." American Journal of Physiology-Endocrinology And Metabolism **293**(5): E1430-E1442.

Shao, R., M. Nutu, L. Karlsson-Lindahl, A. Benrick, B. Weijdegård, S. Lager, E. Egecioglu, J. Fernandez-Rodriguez, K. Gemzell-Danielsson and C. Ohlsson (2009). "Downregulation of cilia-localized Il-6R α by 17 β -estradiol in mouse and human fallopian tubes." American Journal of Physiology-Cell Physiology **297**(1): C140-C151.

Shao, R., B. Weijdegård, J. Fernandez-Rodriguez, E. Egecioglu, C. Zhu, N. Andersson, A. Thurin-Kjellberg, C. Bergh and H. Billig (2007). "Ciliated epithelial-specific and regional-specific expression and regulation of the estrogen receptor- β 2 in the fallopian tubes of immature rats: a possible mechanism for estrogen-mediated transport process in vivo." American Journal of Physiology-Endocrinology And Metabolism **293**(1): E147-E158.

Shayu, D., C. Kesava, R. Soundarajan and A. J. Rao (2005). "Effects of ICI 182780 on estrogen receptor expression, fluid absorption and sperm motility in the epididymis of the bonnet monkey." Reproductive Biology and Endocrinology **3**(10).

Sherwood, O., E. Knobil and J. Neill (1994). "The physiology of reproduction." Relaxin, 2nd ed, Raven Press, New York: 861-1009.

Shevde, N. K., A. C. Bendixen, K. M. Dienger and J. Pike (2000). "Estrogens suppress RANK ligand-induced osteoclast differentiation via a stromal cell independent mechanism involving c-Jun repression." Proceedings of the National Academy of Sciences USA **97**(14): 7829-7834.

Shi, Y., M. Downes, W. Xie, H.-Y. Kao, P. Ordentlich, C.-C. Tsai, M. Hon and R. M. Evans (2001). "Sharp, an inducible cofactor that integrates nuclear receptor repression and activation." Genes & Development **15**(9): 1140-1151.

Shiau, A. K., D. Barstad, P. M. Loria, L. Cheng, P. J. Kushner, D. A. Agard and G. L. Greene (1998). "The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen." Cell **95**(7): 927-937.

Shoker, B. S., C. Jarvis, R. B. Clarke, E. Anderson, J. Hewlett, M. Davies, D. R. Sibson and J. P. Sloane (1999). "Estrogen receptor-positive proliferating cells in the normal and precancerous breast." The American Journal of Pathology **155**(6): 1811-1815.

Shoker, B. S., C. Jarvis, D. R. Sibson, C. Walker and J. P. Sloane (1999). "Oestrogen receptor expression in the normal and pre - cancerous breast." The Journal of Pathology **188**(3): 237-244.

Shyamala, G., Y.-C. Chou, S. Louie, R. Guzman, G. Smith and S. Nandi (2002). "Cellular expression of estrogen and progesterone receptors in mammary glands: regulation by hormones, development and aging." The Journal of Steroid Biochemistry and Molecular Biology **80**(2): 137-148.

Sicinski, P. and R. A. Weinberg (1997). "A specific role for cyclin D1 in mammary gland development." Journal of Mammary Gland Biology and Neoplasia **2**(4): 335-342.

Siiteri, P. K. and P. C. MacDonald (1966). "Placental estrogen biosynthesis during human pregnancy." Journal of Clinical Endocrinology & Metabolism **26**(7): 751-761.

Silberstein, G., K. Van Horn, G. Shyamala and C. Daniel (1994). "Essential role of endogenous estrogen in directly stimulating mammary growth demonstrated by implants containing pure antiestrogens." Endocrinology **134**(1): 84-90.

Silberstein, G. B. (2001). "Postnatal mammary gland morphogenesis." Microscopy Research and Technique **52**(2): 155-162.

Silberstein, G. B., K. Van Horn, G. Shyamala and C. W. Daniel (1994). "Essential role of endogenous estrogen in directly stimulating mammary growth demonstrated by implants containing pure antiestrogens." Endocrinology **134**(1): 84-90.

Simoncini, T., P. Mannella, L. Fornari, A. Caruso, G. Varone and A. R. Genazzani (2004). "Genomic and non-genomic effects of estrogens on endothelial cells." Steroids **69**(8): 537-542.

Simpson, J. F., D. E. Quan, F. O'Malley, T. Odom-Maryon and P. E. Clarke (1997). "Amplification of CCND1 and expression of its protein product, cyclin D1, in ductal carcinoma in situ of the breast." The American Journal of Pathology **151**(1): 161.

Sinha, Y. and H. A. Tucker (1969). "Mammary development and pituitary prolactin level of heifers from birth through puberty and during the estrous cycle." Journal of Dairy Science **52**(4): 507-512.

Siwko, S. K., J. Dong, M. T. Lewis, H. Liu, S. G. Hilsenbeck and Y. Li (2008). "Evidence That an Early Pregnancy Causes a Persistent Decrease in the Number of Functional Mammary Epithelial Stem Cells — Implications for Pregnancy - Induced Protection Against Breast Cancer." Stem Cells **26**(12): 3205-3209.

Slayden, O. D. and R. M. Brenner (2004). "Hormonal regulation and localization of estrogen, progestin and androgen receptors in the endometrium of nonhuman primates: effects of progesterone receptor antagonists." Archives of Histology and Cytology **67**(5): 393-409.

Smith, C. L., O. M. Conneely and B. W. O'Malley (1993). "Modulation of the ligand-independent activation of the human estrogen receptor by hormone and antihormone." Proceedings of the National Academy of Sciences USA **90**(13): 6120-6124.

Snedeker, S. M., C. F. Brown and R. P. DiAugustine (1991). "Expression and functional properties of transforming growth factor alpha and epidermal growth factor during mouse mammary gland ductal morphogenesis." Proceedings of the National Academy of Sciences USA **88**(1): 276-280.

Song, X. and Z.-Z. Pan (2012). "Estrogen receptor-beta agonist diarylpropionitrile counteracts the estrogenic activity of estrogen receptor-alpha agonist propylpyrazole-triol in the mammary gland of ovariectomized Sprague Dawley rats." The Journal of Steroid Biochemistry and Molecular Biology **130**(1-2): 26-35.

Speirs, V., P. J. Carder, S. Lane, D. Dodwell, M. R. Lansdown and A. M. Hanby (2004). "Oestrogen receptor β : what it means for patients with breast cancer." The Lancet Oncology **5**(3): 174-181.

Speirs, V., G. Skliris, S. Burdall and P. Carder (2002). "Distinct expression patterns of ER α and ER β in normal human mammary gland." Journal of Clinical Pathology **55**(5): 371-374.

Speroff, L. and P. D. Darney (2010). Clinical guide for contraception, Lippincott Williams & Wilkins.

Spyridopoulos, I., A. B. Sullivan, M. Kearney, J. M. Isner and D. W. Losordo (1997). "Estrogen-receptor-mediated inhibition of human endothelial cell apoptosis: estradiol as a survival factor." Circulation **95**(6): 1505-1514.

Srivastava, S., G. Toraldo, M. N. Weitzmann, S. Cenci, F. P. Ross and R. Pacifici (2001). "Estrogen decreases osteoclast formation by down-regulating receptor activator of NF-kappa B ligand (RANKL)-induced JNK activation." Journal of Biological Chemistry **276**(12): 8836-8840.

Stabile, L. P., A. L. G. Davis, C. T. Gubish, T. M. Hopkins, J. D. Luketich, N. Christie, S. Finkelstein and J. M. Siegfried (2002). "Human non-small cell lung tumors and cells derived from normal lung express both estrogen receptor α and β and show biological responses to estrogen." Cancer Research **62**(7): 2141-2150.

Stabile, L. P. and J. M. Siegfried (2004). "Estrogen receptor pathways in lung cancer." Current Oncology Reports **6**: 259-267.

Stauffer, S. R., C. J. Coletta, R. Tedesco, G. Nishiguchi, K. Carlson, J. Sun, B. S. Katzenellenbogen and J. A. Katzenellenbogen (2000). "Pyrazole ligands: structure-affinity/activity relationships and estrogen receptor-alpha-selective agonists." Journal of Medicinal Chemistry **43**(26): 4934-4947.

Stauffer, S. R., C. J. Coletta, R. Tedesco, G. Nishiguchi, K. Carlson, J. Sun, B. S. Katzenellenbogen and J. A. Katzenellenbogen (2000). "Pyrazole ligands: structure-affinity/activity relationships and estrogen receptor- α -selective agonists." Journal of Medicinal Chemistry **43**(26): 4934-4947.

Steeg, P. S. and Q. Zhou (1998). Cyclins and breast cancer. Prognostic variables in node-negative and node-positive breast cancer, Springer: 107-118.

Ström, A., J. Hartman, J. S. Foster, S. Kietz, J. Wimalasena and J.-Å. Gustafsson (2004). "Estrogen receptor β inhibits 17 β -estradiol-stimulated proliferation of the breast cancer cell line T47D." Proceedings of the National Academy of Sciences USA **101**(6): 1566-1571.

Strauss, L., J. Kallio, N. Desai, P. Pakarinen, T. Miettinen, H. Gylling, M. Albrecht, S. Mäkelä, A. Mayerhofer and M. Poutanen (2009). "Increased exposure to estrogens disturbs maturation, steroidogenesis, and cholesterol homeostasis via estrogen receptor α in adult mouse Leydig cells." Endocrinology **150**(6): 2865-2872.

Strom, A., J. Hartman, J. S. Foster, S. Kietz, J. Wimalasena and J. A. Gustafsson (2004). "Estrogen receptor beta inhibits 17 β -estradiol-stimulated proliferation of the breast cancer cell line T47D." Proceedings of the National Academy of Sciences USA **101**(6): 1566-1571.

Sun, G., W. Porter and S. Safe (1998). "Estrogen-induced retinoic acid receptor α 1 gene expression: role of estrogen receptor-Sp1 complex." Molecular Endocrinology **12**(6): 882-890.

Sun, M., J. E. Paciga, R. I. Feldman, Z.-q. Yuan, D. Coppola, Y. Y. Lu, S. A. Shelley, S. V. Nicosia and J. Q. Cheng (2001). "Phosphatidylinositol-3-OH kinase (PI3K)/AKT2, activated in breast cancer, regulates and is induced by estrogen receptor α (ER α) via interaction between ER α and PI3K." Cancer Research **61**(16): 5985-5991.

Sutter, T., K. Carnevale, N. Arber and I. Weinstein (1996). "Expression of cyclins D1 and E in human colon adenocarcinomas." Journal of Medicine **28**(5-6): 285-309.

Swerdlow, A. J. and M. E. Jones (2005). "Tamoxifen treatment for breast cancer and risk of endometrial cancer: a case-control study." Journal of the National Cancer Institute **97**(5): 375-384.

Sylvia, V., B. Boyan, D. Dean and Z. Schwartz (2000). "The membrane effects of 17 β estradiol on chondrocyte phenotypic expression are mediated by activation of protein kinase C through phospholipase C and G-proteins." The Journal of Steroid Biochemistry and Molecular Biology **73**(5): 211-224.

Taioli, E. and E. L. WYNDER (1994). "Endocrine factors and adenocarcinoma of the lung in women." Journal of the National Cancer Institute **86**(11): 869-870.

Tan, H., Y. Zhong and Z. Pan (2009). "Autocrine regulation of cell proliferation by estrogen receptor-alpha in estrogen receptor-alpha-positive breast cancer cell lines." BMC Cancer **9**(1): 31.

Tan, J., B. C. Paria, S. K. Dey and S. K. Das (1999). "Differential uterine expression of estrogen and progesterone receptors correlates with uterine preparation for implantation and decidualization in the mouse." Endocrinology **140**(11): 5310-5321.

Tena-Sempere, M., L. Gonzalez, L. Pinilla, I. Huhtaniemi and E. Aguilar (2001). "Neonatal imprinting and regulation of estrogen receptor alpha and beta mRNA expression by

estrogen in the pituitary and hypothalamus of the male rat." Neuroendocrinology **73**(1): 12-25.

Thompson Jr, E. and P. Siiteri (1979). "Subcellular distribution of aromatase in human placenta and ovary." Hormone Research in Paediatrics **11**(4): 179-185.

Tilli, M. T., M. S. Frech, M. E. Steed, K. S. Hruska, M. D. Johnson, J. A. Flaws and P. A. Furth (2003). "Introduction of estrogen receptor-alpha into the tTA/TAg conditional mouse model precipitates the development of estrogen-responsive mammary adenocarcinoma." American Journal of Pathology **163**(5): 1713-1719.

Tilli, M. T., M. S. Frech, M. E. Steed, K. S. Hruska, M. D. Johnson, J. A. Flaws and P. A. Furth (2003). "Introduction of estrogen receptor- α into the tTA/TAg conditional mouse model precipitates the development of estrogen-responsive mammary adenocarcinoma." The American Journal of Pathology **163**(5): 1713-1719.

Tomkinson, A., J. Reeve, R. Shaw and B. Noble (1997). "The death of osteocytes via apoptosis accompanies estrogen withdrawal in human bone." Journal of Clinical Endocrinology & Metabolism **82**(9): 3128-3135.

Townsend, E. A., V. M. Miller and Y. Prakash (2012). "Sex differences and sex steroids in lung health and disease." Endocrine Reviews **33**(1): 1-47.

Treeck, O., I. Juhasz-Boess, C. Lattrich, F. Horn, R. Goerse and O. Ortmann (2008). "Effects of exon-deleted estrogen receptor β transcript variants on growth, apoptosis and gene expression of human breast cancer cell lines." Breast Cancer Research and Treatment **110**(3): 507-520.

Tucker, H. A. (1987). "Quantitative estimates of mammary growth during various physiological states: a review." Journal of Dairy Science **70**(9): 1958-1966.

Tulchinsky, D., C. Hobel, E. Yeager and J. Marshall (1972). "Plasma estrone, estradiol, estriol, progesterone, and 17-hydroxyprogesterone in human pregnancy. I. Normal pregnancy." American Journal of Obstetrics and Gynecology **112**(8): 1095.

Umayahara, Y., R. Kawamori, H. Watada, E. Imano, N. Iwama, T. Morishima, Y. Yamasaki, Y. Kajimoto and T. Kamada (1994). "Estrogen regulation of the insulin-like growth factor I gene transcription involves an AP-1 enhancer." Journal of Biological Chemistry **269**(23): 16433-16442.

van Amerongen, R. and R. Nusse (2009). "Towards an integrated view of Wnt signaling in development." Development **136**(19): 3205-3214.

Vandenberg, G., G. DeVane and S. Yen (1974). "Effects of exogenous estrogen and progestin on pituitary responsiveness to synthetic luteinizing hormone-releasing factor." Journal of Clinical Investigation **53**(6): 1750.

Vazquez-Alcantara, M. A., M. Menjivar, G. A. Garcia, J. C. Diaz-Zagoya and J. Garza-Flores (1989). "Long-acting estrogenic responses of estradiol fatty acid esters." Journal of Steroid Biochemistry **33**(6): 1111-1118.

- Veltmaat, J. M., F. Relaix, L. T. Le, K. Kratochwil, F. G. Sala, W. van Veelen, R. Rice, B. Spencer-Dene, A. A. Mailleux and D. P. Rice (2006). "Gli3-mediated somitic Fgf10 expression gradients are required for the induction and patterning of mammary epithelium along the embryonic axes." Development **133**(12): 2325-2335.
- Venkov, C. D., A. B. Rankin and D. E. Vaughan (1996). "Identification of authentic estrogen receptor in cultured endothelial cells. A potential mechanism for steroid hormone regulation of endothelial function." Circulation **94**(4): 727-733.
- Visvader, J. E. and G. J. Lindeman (2003). "Transcriptional regulators in mammary gland development and cancer." The International Journal of Biochemistry & Cell Biology **35**(7): 1034-1051.
- Visvader, J. E. and G. J. Lindeman (2003). "Transcriptional regulators in mammary gland development and cancer." Int J Biochem Cell Biol **35**(7): 1034-1051.
- Vladusic, E., A. Hornby, F. Guerra-Vladusic, J. Lakins and R. Lupu (2000). "Expression and regulation of estrogen receptor beta in human breast tumors and cell lines." Oncology Reports **7**(1): 157-224.
- Vo, N., C. Fjeld and R. H. Goodman (2001). "Acetylation of nuclear hormone receptor-interacting protein RIP140 regulates binding of the transcriptional corepressor CtBP." Molecular and Cellular Biology **21**(18): 6181-6188.
- Vogel, P., N. Georgiade, B. Fetter, F. Vogel and K. McCarty Jr (1981). "The correlation of histologic changes in the human breast with the menstrual cycle." The American Journal of Pathology **104**(1): 23.
- Waard, F. d. and J. Thijssen (2005). "Hormonal aspects in the causation of human breast cancer: epidemiological hypotheses reviewed, with special reference to nutritional status and first pregnancy." The Journal of Steroid Biochemistry and Molecular Biology **97**(5): 451-458.
- Walf, A. A., C. J. Koonce and C. A. Frye (2008). "Estradiol or diarylpropionitrile decrease anxiety-like behavior of wildtype, but not estrogen receptor beta knockout, mice." Behavioral Neuroscience **122**(5): 974.
- Waliszewski, P., M. Blaszczyk, E. Wolinska - Witort, M. Drews, M. Snochowski and R. E. Hurst (1997). "Molecular study of sex steroid receptor gene expression in human colon and in colorectal carcinomas." Journal of Surgical Oncology **64**(1): 3-11.
- Walker, V. R. and K. S. Korach (2004). "Estrogen receptor knockout mice as a model for endocrine research." ILAR journal **45**(4): 455-461.
- Warner, M. and J.-Å. Gustafsson (2010). "The role of estrogen receptor β (ER β) in malignant diseases—A new potential target for antiproliferative drugs in prevention and treatment of cancer." Biochemical and Biophysical Research Communications **396**(1): 63-66.
- Webb, P., P. Nguyen, J. Shinsako, C. Anderson, W. Feng, M. P. Nguyen, D. Chen, S.-M. Huang, S. Subramanian and E. McKinerney (1998). "Estrogen receptor activation function

1 works by binding p160 coactivator proteins." Molecular Endocrinology **12**(10): 1605-1618.

Webb, P., P. Nguyen, C. Valentine, G. N. Lopez, G. R. Kwok, E. McInerney, B. S. Katzenellenbogen, E. Enmark, J. A. Gustafsson, S. Nilsson and P. J. Kushner (1999). "The estrogen receptor enhances AP-1 activity by two distinct mechanisms with different requirements for receptor transactivation functions." Molecular Endocrinology **13**(10): 1672-1685.

Wegorzewska, I. N., K. Walters, M. J. Weiser, D. F. Cruthirds, E. Ewell, D. O. Larco, R. J. Handa and T. J. Wu (2008). "Postovariectomy weight gain in female rats is reversed by estrogen receptor α agonist, propylpyrazoletriol." American Journal of Obstetrics and Gynecology **199**(1): 67. e61-67. e65.

Wen-Chang, C., N. Junko, O. Hajime and M. Sei-Itsu (1980). "Stimulation of prostaglandin cyclooxygenase and prostacyclin synthetase activities by estradiol in rat aortic smooth muscle cells." Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism **620**(3): 472-482.

Weyant, M. J., A. M. Carothers, N. N. Mahmoud, H. L. Bradlow, H. Remotti, R. T. Bilinski and M. M. Bertagnolli (2001). "Regular Articles-Epidemiology and Prevention-Reciprocal Expression of ER α and ER β Is Associated with Estrogen-mediated Modulation of Intestinal Tumorigenesis." Cancer Research **61**(6): 2547-2551.

Wijayarathne, A. L., S. C. Nagel, L. A. Paige, D. J. Christensen, J. D. Norris, D. M. Fowlkes and D. P. McDonnell (1999). "Comparative analyses of mechanistic differences among antiestrogens." Endocrinology **140**(12): 5828.

Willert, K., J. D. Brown, E. Danenberg, A. W. Duncan, I. L. Weissman, T. Reya, J. R. Yates and R. Nusse (2003). "Wnt proteins are lipid-modified and can act as stem cell growth factors." Nature **423**(6938): 448-452.

Witte, D., M. Chirala, A. Younes, Y. Li and M. Younes (2001). "Estrogen receptor β is expressed in human colorectal adenocarcinoma." Human pathology **32**(9): 940-944.

Wong, C.-W., C. McNally, E. Nickbarg, B. S. Komm and B. J. Cheskis (2002). "Estrogen receptor-interacting protein that modulates its nongenomic activity-crosstalk with Src/Erk phosphorylation cascade." Proceedings of the National Academy of Sciences USA **99**(23): 14783-14788.

Wysolmerski, J. J., S. Cormier, W. M. Philbrick, P. Dann, J.-P. Zhang, J. Roume, A.-L. Delezoide and C. Silve (2001). "Absence of functional type 1 parathyroid hormone (PTH)/PTH-related protein receptors in humans is associated with abnormal breast development and tooth impaction." Journal of Clinical Endocrinology & Metabolism **86**(4): 1788-1794.

Wysolmerski, J. J., W. M. Philbrick, M. E. Dunbar, B. Lanske, H. Kronenberg and A. Broadus (1998). "Rescue of the parathyroid hormone-related protein knockout mouse

demonstrates that parathyroid hormone-related protein is essential for mammary gland development." Development **125**(7): 1285-1294.

Xiaomeng, X. and M. L. Thomas (1994). "Estrogen receptor-mediated direct stimulation of colon cancer cell growth in vitro." Molecular and Cellular Endocrinology **105**(2): 197-201.

Xu, J., Y. Qiu, F. J. DeMayo, S. Y. Tsai, M.-J. Tsai and B. W. O'Malley (1998). "Partial hormone resistance in mice with disruption of the steroid receptor coactivator-1 (SRC-1) gene." Science **279**(5358): 1922-1925.

Xu, L., C. K. Glass and M. G. Rosenfeld (1999). "Coactivator and corepressor complexes in nuclear receptor function." Current Opinion in Genetics & Development **9**(2): 140-147.

Yang, E., J. Zha, J. Jockel, L. H. Boise, C. B. Thompson and S. J. Korsmeyer (1995). "Bad, a heterodimeric partner for Bcl-x and Bcl-2, displaces bax and promotes cell death." Cell **80**(2): 285-291.

Yoshida, K. and Y. Miki (2004). "Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage." Cancer Science **95**(11): 866-871.

Yuan, C.-X., M. Ito, J. D. Fondell, Z.-Y. Fu and R. G. Roeder (1998). "The TRAP220 component of a thyroid hormone receptor-associated protein (TRAP) coactivator complex interacts directly with nuclear receptors in a ligand-dependent fashion." Proceedings of the National Academy of Sciences USA **95**(14): 7939-7944.

Yue, W., J. D. Yager, J.-P. Wang, E. R. Jupe and R. J. Santen (2012). "Estrogen receptor-dependent and independent mechanisms of breast cancer carcinogenesis." Steroids.

Zang, E. A. and E. L. Wynder (1996). "Differences in lung cancer risk between men and women: examination of the evidence." Journal of the National Cancer Institute **88**(3-4): 183-192.

Zhang, F., J. L. Ram, P. R. Standley and J. R. Sowers (1994). "17 beta-Estradiol attenuates voltage-dependent Ca²⁺ currents in A7r5 vascular smooth muscle cell line." American Journal of Physiology-Cell Physiology **266**(4): C975-C980.

Zhang, H., J. S. Thomsen, L. Johansson, J.-Å. Gustafsson and E. Treuter (2000). "DAX-1 functions as an LXXLL-containing corepressor for activated estrogen receptors." Journal of Biological Chemistry **275**(51): 39855-39859.

Zhang, W., S. Andersson, G. Cheng, E. R. Simpson, M. Warner and J.-Å. Gustafsson (2003). "Update on estrogen signaling." FEBS Letters **546**(1): 17-24.

Zhang, W., S. Saji, S. Mäkinen, G. Cheng, E. V. Jensen, M. Warner and J.-Å. Gustafsson (2000). "Estrogen receptor (ER) β , a modulator of ER α in the uterus." Proceedings of the National Academy of Sciences USA **97**(11): 5936-5941.

Zhao, C., K. Dahlman-Wright and J. A. Gustafsson (2008). "Estrogen receptor beta: an overview and update." Nuclear Receptor Signaling **6**: e003.

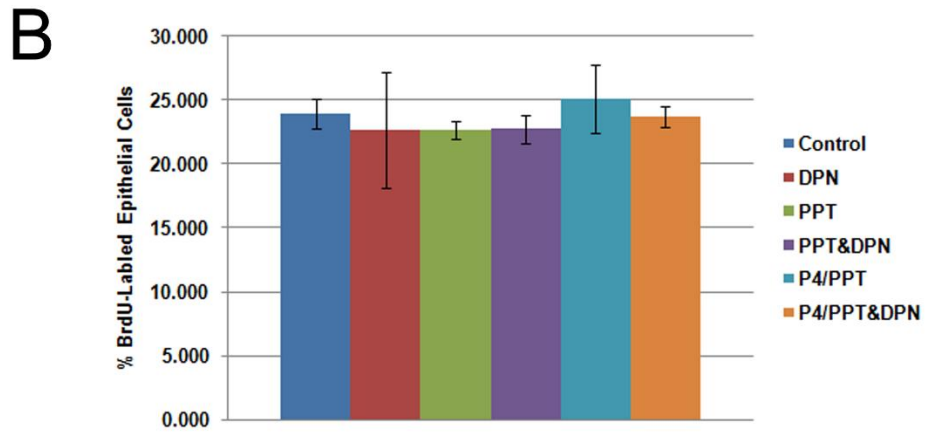
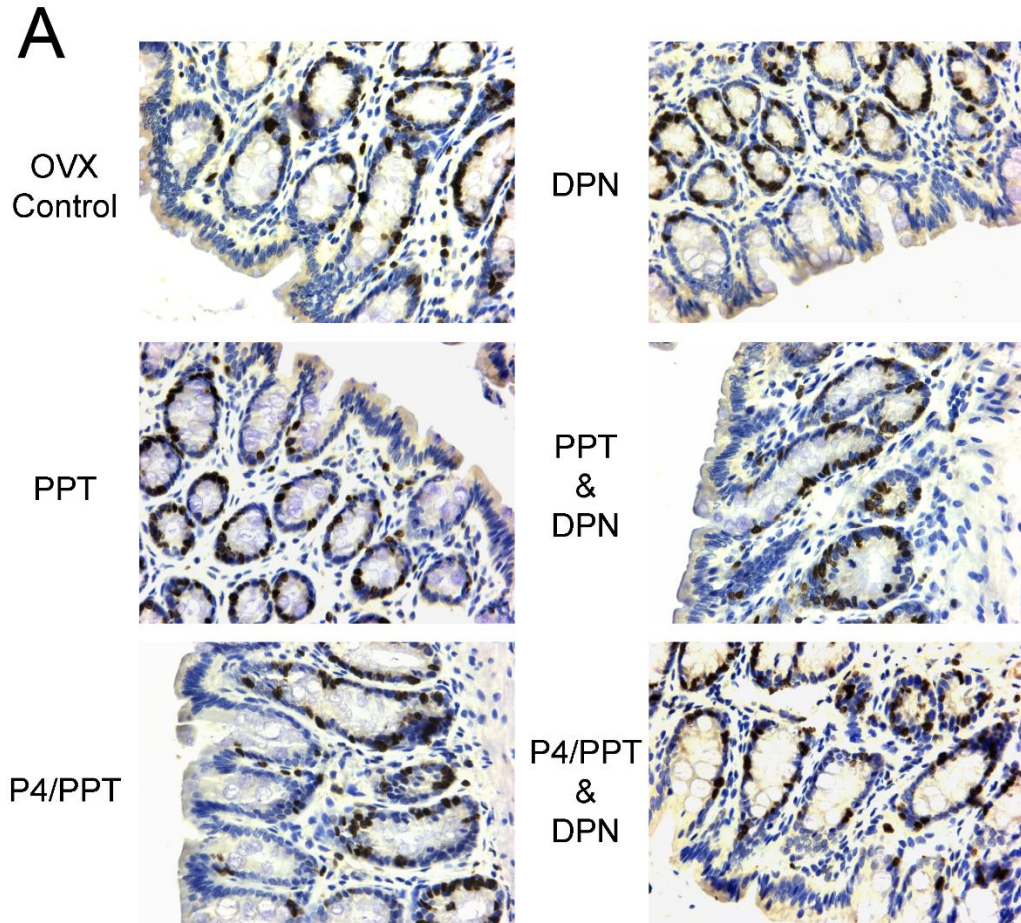
Zhao, C., E. W. Lam, A. Sunter, E. Enmark, M. T. De Bella, R. C. Coombes, J. A. Gustafsson and K. Dahlman-Wright (2003). "Expression of estrogen receptor beta isoforms in normal breast epithelial cells and breast cancer: regulation by methylation." Oncogene **22**(48): 7600-7606.

Zhou, S., Y. Zilberman, K. Wassermann, S. D. Bain, Y. Sadosky and D. Gazit (2001). "Estrogen modulates estrogen receptor α and β expression, osteogenic activity, and apoptosis in mesenchymal stem cells (MSCs) of osteoporotic mice." Journal of Cellular Biochemistry **81**(S36): 144-155.

Zhu, B. T. and A. H. Conney (1998). "Functional role of estrogen metabolism in target cells: review and perspectives." Carcinogenesis **19**(1): 1-27.

Appendix A

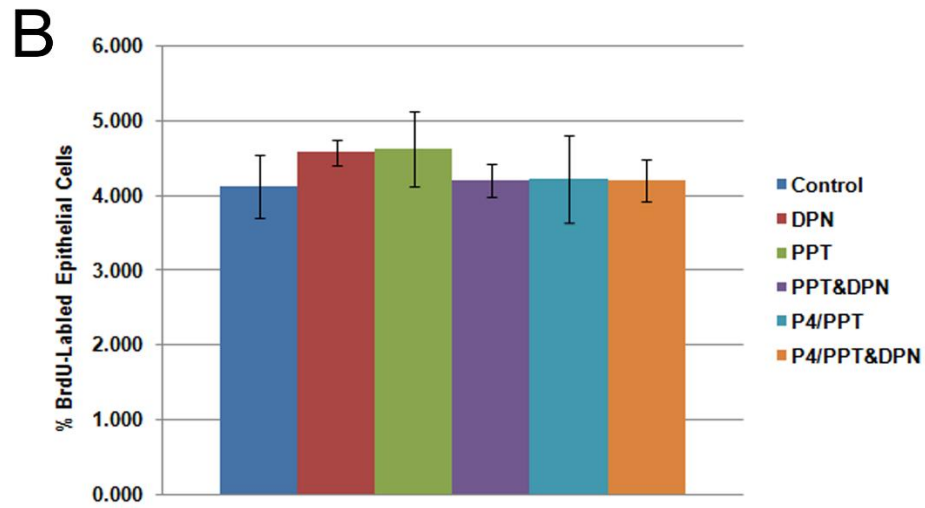
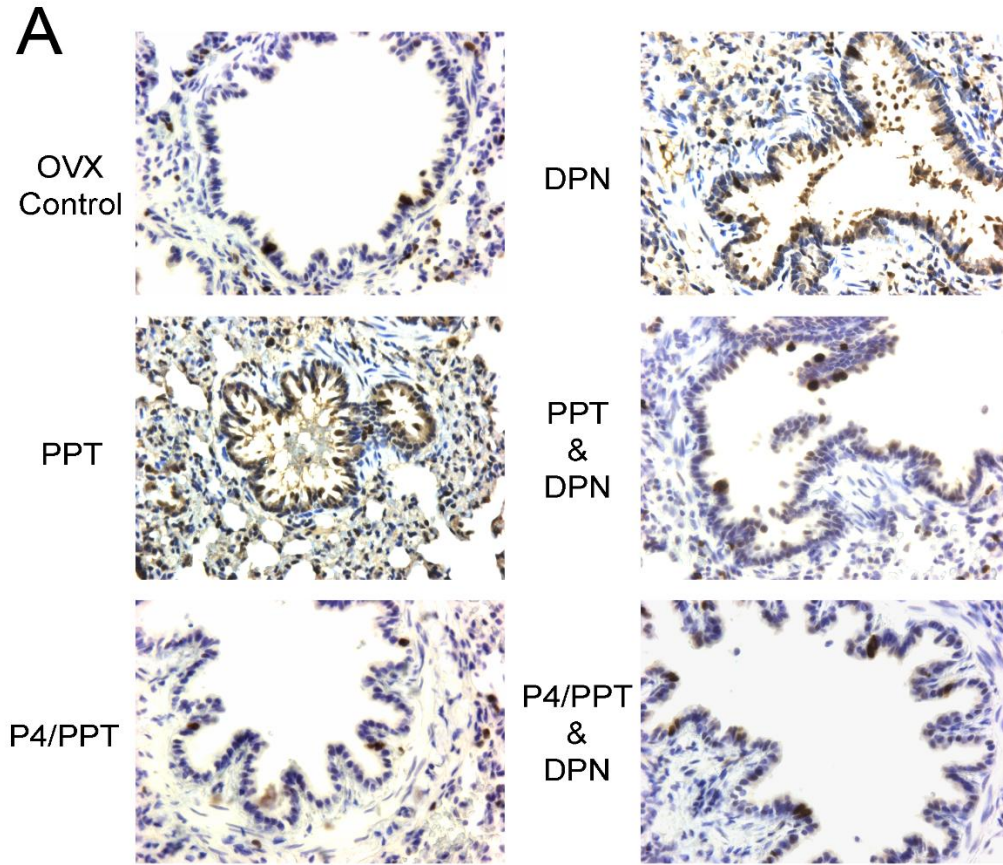
The effects of ER agonists on colon cell proliferation



OVX rats were administered by i.p. injection once a day for three consecutive days with DMSO (control), DPN, PPT, PPT and DPN (PPT/DPN), respectively. BrdU was injected concurrently with each drug administration to label proliferating cells. (A) Representative microimages showing BrdU-labeled proliferation cells in the colon from different groups of rats. BrdU labeled cells were detected by IHC staining with DAB as substrate to give brown color. Magnification, $400\times$. (B) Percentage of BrdU-labeled proliferation cells in the colon of different groups of rats. The proliferation rate is similar among different treatment groups.

Appendix B

The effects of ER agonists on lung cell proliferation



OVX rats were administered by i.p. injection once a day for three consecutive days with DMSO (control), DPN, PPT, PPT and DPN (PPT/DPN), respectively. BrdU was injected concurrently with each drug administration to label proliferating cells. (A) Representative microimages showing BrdU-labeled proliferation cells in the lung from different groups of rats. BrdU labeled cells were detected by IHC staining with DAB as substrate to give brown color. Magnification, $400\times$. (B) Percentage of BrdU-labeled proliferation cells in the lung of different groups of rats. The proliferation rate is similar among different treatment groups.