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Gene-specific and Cell-specific Expression Patterns of Butyrophilins in Mice with Lung Adenocarcinoma

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Gene-specific and Cell-specific Expression Patterns of Butyrophilins in Mice with Lung Adenocarcinoma

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Abstract

Butyrophilins are a part of the immunoglobulin superfamily of transmembrane proteins that bind to undefined receptors on T cells. This binding produces a stimulatory or inhibitory signal. Some butyrophilins have been shown to bind to γδ T cell receptors resulting in γδ T cell activation. γδ T cells exhibit important roles in cancer, but their function is still unclear. We used an inducible murine lung adenocarcinoma model to determine changes in butyrophilin expression relative to healthy murine lungs. A baseline of butyrophilin expression level and pattern was established across different tissues. In the lung cancer model, Btnl2 showed upregulation compared to healthy control lungs. After sorting leukocytes and epithelial cells from the cancerous lung, it appears that the leukocytes may be responsible for this change in gene expression. Butyrophilin gene expression changes in response to another form of inflammation, PR8 influenza, were different than the changes seen in the lung adenocarcinoma model. This finding promotes the idea that butyrophilins differentially bind to specific immune cells. Determining the location and binding affinities of butyrophilins could direct research in new immunotherapies for cancer and other diseases.

Acknowledgements

There were many people that made this project possible. I would like to start by thanking the Seward lab for the mouse model they developed and for letting me hog the qPCR machine whenever I needed. Special shoutout to Sean for his help with sorting and tricky RNA isolations. Thank you to the entire Boyson lab for being so supportive, kind, and patient. Somen, who taught me the tips and tricks behind RNA work and the importance of having your own personal rituals to believe in. Oliver was always available to answer my random questions, even when the answer was right in front of me. The other students of the lab, undergraduates and beyond, that made the lab a fun place to be and laugh at all my silly jokes. Katherine, who helped me plan my experiments and was always there to assist when I inevitably messed something up. Finally, I would like to thank Jon for all the opportunities and guidance he has given me these past two years. Even with all he does, he made time to answer and explain my endless number of questions. The advice and wisdom that he has given me, both in and outside of the lab, has shaped my college career and prepared me for my future endeavors.

Introduction

Butyrophilin Gene Family

Butyrophilins are a family of transmembrane proteins included in the immunoglobulin superfamily, which function in the recognition, binding, and adhesion process of cells. Similar to B7 family proteins, the action of butyrophilins binding to receptors produces a co-stimulatory or co-inhibitory signal[1]. Butyrophilins are composed of two extracellular immunoglobulin proteins, constant IgC and variable IgV. Most also contain a B30.2 domain that acts in protein binding[2]. Butyrophilins are commonly found on dendritic cells, lymphocytes, and macrophages[3]. In the human genome, there are thirteen BTN genes currently identified. There are eleven Btn genes classified in the mouse genome, six of them being orthologs to the respective human genes[2].

Butyrophilin Function

The function of butyrophilins is still unclear. Many studies have shown that butyrophilins negatively affect conventional T cell activation. Btn1a1, Btn2a2, and Btnl2 have been shown to inhibit the proliferation of conventional T cells[4,5]. In addition, Btnl1 negatively regulated T cell activation by interrupting the cell cycle[6]. Btn1a1 and Btn2a2 inhibited the secretion of IFN-γ, an anti-inflammatory and anti-tumoral cytokine[4]. Another cytokine, IL-17A, is known to have a pro-tumor response[7]. Btnl2 interacts with γδ T cells, which are innate like T cells found in mucosal tissues like the lung, gut, and skin. This interaction initiated an increase in IL-17A production, suggesting that Btnl2 may be capable of increasing tumor metastasis[7]. Finally, Btnl1, Btnl4, and Btnl6 expressed in the intestinal epithelia showed regulatory properties in response to colon inflammation. Btnl1 specifically reduces the production of pro-inflammatory mediators after activating TCRγδ+ IELs[8].

However, butyrophilins have also shown positive effects on γδ T cells. For example, Btnl2 promoted the development of Vγ7 TCR+ intraepithelial lymphocytes (IELs). It regulated the frequency of γδ IELs which aid in site-specific homeostatic functions and inflammation responses[9]. Expression of Skint1, another butyrophilin, was determined as a requirement for the development of Vγ5Vδ1+ dendritic epidermal T cells[10]. In the gut epithelium, the Btnl1/Btnl6 complex directs the localization and maturation of Vγ4+ and Vγ7+ T cells[11].

Recently, butyrophilins have been shown to directly activate human γδ T cells. In humans, Vγ9Vδ2+ T cell activation is caused by recognition of phosphoantigens, which are produced by cancer cells[12]. This recognition mechanism is not fully understood but it is known that a butyrophilin complex of BTN2A1 and BTN3A1 is an essential component. BTN2A1 binds directly to the Vγ9+ domain of the TCR in conjunction with BTN3A1, which binds to the Vδ2 and γ-chains[12]. The butyrophilin complex is thought to sense the overproduction of phosphoantigens by tumors, and then send a signal to the γδ T cell. This process results in activating γδ T cell immune response. Evidently, butyrophilins play many regulatory roles in the immune response, but the specifics of how butyrophilins accomplish this influence are unknown.

Locations of Gene Expression

Different butyrophilin genes are expressed in different locations. Although there has not been a lot of general research done on butyrophilin genes in the mouse genome, there are a few types that have been studied. Btnl4 and Btnl6 have shown expression in intestinal epithelial cells[1]. Btn1a1 is commonly expressed in the spleen, thymus, lactating mammary glands, and lymph nodes. Btn2a2 has been identified in the brain, thymus, and lymph nodes[4]. Many members of the butyrophilin family have not been studied. For example, the location

and function of Btnl5, Btnl7, Btnl10, and Skint3-11 are mostly unknown[1]. Taken together, these results suggest that specific butyrophilin genes are differentially regulated in different tissues and contexts. Although these locations and specific functions of butyrophilins have been shown, there is much about them that we do not understand, including what they are binding to and what controls their expression.

Significance of Butyrophilins

Butyrophilins are a gene/protein family that are not completely understood. We know that they regulate immune responses and play an inhibitory role in conventional T cell responses. Recent studies have shown a connection between butyrophilins and γδ T cell immune response, which inspires potential new mechanisms to change or regulate immune responses, such as tumor response. This poses the question of what the function of butyrophilins is in the presence of cancer. If butyrophilins play a role in tumor progression, understanding how they function can potentially influence the study and treatment of cancer.

γδ T cells have responsibility in anti-tumor immunity. Some butyrophilins are expressed on γδ T cells and have been shown to activate their anti-tumor immunity. Recent data implies that γδ T cells preferentially recognize specific butyrophilin proteins for this reason. In the human genome, BTN3A1 and BTN2A1 were suggested as possible ligands for γδ T cells and as potential prevention and treatment of breast cancer[13]. It has been proposed that low expression of intestinal butyrophilins could lead to increased chronic inflammation, suggesting that these butyrophilins usually activate γδ T cells with anti-inflammitory secretion. The downregulation of these proteins may contribute to tumor progression and colon cancer[14]. In addition, since some γδ T cells secrete IL-17, which is thought to promote tumor metastasis[15], it's possible that butyrophilins may induce a stimulatory or inhibitory effect on

the secretion of IL-17. This could potentially impact tumor progression[16]. Understanding the interactions between butyrophilins and γδ T cells and the resulting impact on the immune response in the setting of cancer could lead to the development of therapeutics to block those interactions for cancer treatment.

Immunotherapy is a newly developed form of cancer treatment. One type of immunotherapy focuses on the inhibition of immune checkpoints. Normally, when T cells become activated, they begin to express a number of cell surface receptors that act to inhibit T cell function. The expression of these inhibitory receptors, known as immune checkpoints, is to prevent runaway T cell activation which may cause autoimmunity[17]. In the setting of cancer, however, the binding of these proteins sends an inhibition signal to the T cells, which prevents the T cells from attacking the tumor cells. Programmed cell death protein 1 (PD-1) is a member of the B7 family of immunoglobulin proteins, like butyrophilins. PD-1 is found on the surface T cells and can inhibit their activity[18]. This interaction has become a target area for therapeutics. Immunotherapy consists of drugs that inhibit the inhibition signal, allowing the T cells to attack the cancer cells[19]. In addition to PD-1, butyrophilins could potentially be involved in the recognition and binding of cancer cells and may play a role in the inhibition of the immune checkpoints. Determining how butyrophilins are inhibiting these immune checkpoints can influence the engineering of immunotherapy drugs for the treatment of cancer.

Lung Adenocarcinoma

The majority of cancer deaths in the United States are caused by lung cancer[20]. Lung adenocarcinomas account for almost 50% of lung cancer diagnoses, making it one of the deadliest forms of cancer. This non-small cell lung cancer develops from mucosal glands in the

periphery of the lung[21]. Smoking is a leading cause of lung adenocarcinoma, but cases can also occur from mutations not linked to smoking. One mutation is in the oncogene KRAS which is known to influence infiltration of leukocytes and produce tumors in the epithelial cells of lung airways, also known as club cells. Understanding which cells are being affected and what response is being produced could be useful in preventing lung adenocarcinoma. Currently, treatment options for lung adenocarcinoma include chemotherapy, radiation therapy, immunotherapy, targeted therapy, and varying levels of surgical removal[21].

Objectives

The goal of this project was to identify changes in butyrophilin expression in response to inflammation using a lung adenocarcinoma cancer model. We aimed to identify if potential changes were due to the genes being upregulated or if specific cell-types were being recruited to the location of inflammation.

Methods

Mice

Male and female C57BL/6 mice were used to collect cells from the spleen, thymus, liver, lungs, and gut. The lungs of C57BL/6J mice with PR8 influenza were also used. B6.Scgb1a1$^{CreER/+}$.Rosa26$^{LSL/+}$.Kras$^{LSL\text{-}G12D/+}$ and B6.Scgb1a1$^{CreER/+}$.Rosa26$^{LSL/+}$.Kras$^{+/+}$ mice were used in these experiments as a KRAS-driven lung adenocarcinoma model and control. Both strains of mice were given an intraperitoneal injection of 2 mg of tamoxifen in corn oil for three consecutive days. After at least thirty days, the mice were euthanized using carbon dioxide and the lungs were obtained. All the experimental procedures are approved by the

University of Vermont Institutional Animal Care and Use Committee, PROTO202000154 and PROTO2022000333.

Lung Digests & Antibody Staining

Lungs were harvested and digested using either 1mg/ml of elastase or collagenase, and 200 µg/ml DNase in DMEM. All lungs were minced in digest media and incubated in a shaker. The elastase protocol had one 45-minute incubation in the shaker at 37°C and 200rpm, followed by one trituration with a blunted needle. The collagenase protocol had two 20-minute incubations in the shaker at 37°C and 200rpm with a trituration using a blunted needle after each incubation period. Samples were resuspended in phosphate-buffered saline (PBS) and treated with red cell lysis buffer. The reaction was stopped, and the samples were resuspended in PBS with 2% fetal bovine serum, also known as sterile staining buffer (SSB). The cells were filtered, resuspended in SSB, and counted. Cells were stained with a live/dead viability dye and washed with SSB. Cells were then stained with Alexa488-conjugated CD326 as a marker for lung epithelial cells and eFlour506-CD45 for lung leukocytes. The lung epithelial cells and the lung leukocytes were FACS-sorted based on CD326, CD45, and tdTomato expression in the Larner College of Medicine Flow Cytometry and Cell Sorting Facility. The $CD326^{+}$ $tdTomato^{+}$ induced epithelial cells and $CD45^{+}CD326tdTomato^{-}$ leukocytes were sorted from each strain. The sorted cells were washed, pelleted, and immediately used for RNA extraction.

Whole Tissue Homogenization

Parts of whole tissues intended only for qPCR were homogenized using a bead beater. Tubes were filled 1/3 full of 2.33mm diameter zirconia/silica beads and lysis buffer with beta-

mercaptoethanol. The lysis buffer used was RLT Buffer from the Qiagen RNeasy Mini Kit. Lungs were thoroughly minced and mixed before measurement to control for sampling bias. Approximately 20mg/sample of tissue was homogenized in the bead beater for 1.5 minutes and immediately used for RNA extraction.

RNA Extraction & cDNA Synthesis

RNA was extracted from whole tissues using the Qiagen RNeasy Mini Kit. RNA was extracted from sorted lung epithelial cells and lung leukocytes using a Thermo Scientific GeneJET RNA Purification Kit. All procedures were done according to the manufacturer's specifications. Cells were lysed with lysis buffer. If not already homogenized, the cells were spun down in a homogenizing spin column or vortexed. Ethanol was added to the lysate and transferred to a spin column. The column was washed, and RNA was eluted with RNAse-free water. The yield and purity of RNA was measured by the NanoDrop spectrophotometer. RNA was stored at -80°C.

cDNA was synthesized according to the Invitrogen SuperScript IV first-strand cDNA synthesis reaction procedure. RNA was combined with oligo$(dT)_{20}$ as a primer, dNTP mix, and nuclease-free water. The RNA-primer mix was heated at 65°C for five minutes and then incubated on ice. A reaction mixture of 5x SSIV buffer, DTT, RNaseOUT recombinant RNase inhibitor, and SuperScript IV reverse transcriptase was added to the RNA-primer mixture. The combined reaction was incubated at 50-55°C for ten minutes and then inactivated at 80°C for ten minutes. The resulting cDNA was stored at -20°C.

Quantitative Real-Time PCR

qPCR was conducted according to the Applied Biosystems Taqman Fast Advanced Master Mix protocol. Primer/probe sets specific for Btn1a1, Btn2a2, Btnl1, Btnl2, Btnl4, Btnl6, Btnl9, Btnl10, Skint1, and Skint3 were used (Assay on Demand; Applied Biosystems). Primer/probe sets for endogenous control, genes that have high and constant expression levels in a variety of cell types, were used as a reference comparison to the tested butyrophilin gene expression. The endogenous controls used were beta-2m and actin. A PCR reaction mix was made for each primer, using the Taqman Fast Advanced Master Mix, and transferred to an optical 96-well plate. The cDNA template was added, the plate was sealed with an optical adhesive film, and run in the QuantStudio 3 Flex Real-Time PCR system.

Data Analysis & Interpretation of Results

Sorted lung epithelial cells and lung leukocytes were assessed for purity after sorting using flow cytometry. qPCR data was analyzed using the comparative C_t method. The C_t is the number of cycles it takes for the fluorescence signal of a sample to be accurately measured by the machine. The ΔC_t is the difference between the C_t of the gene of interest and the C_t of the endogenous control genes. The $-\Delta C_t$ was used to show the differences between data sets in a more intuitive way. The $\Delta\Delta C_t$ was determined by calculating the difference between the ΔC_t of the sample and the ΔC_t of the chosen reference control. Relative quantity was calculated according to the following formula: $2^{-\Delta\Delta Ct}$. To test for significant differences in butyrophilin gene expression, Welch's T-test was used. Differences with a p-value < 0.05 were considered significant.

Results

Baseline Butyrophilin Expression in Various Tissues

To begin researching butyrophilin expression changes in response to inflammation, we needed to determine a baseline of expression throughout various tissues in the mouse. We harvested thymus, liver, spleen, gut, and lungs from C57BL/6 mice. The RNA was isolated from all samples and converted to cDNA for qPCR to assess the expression pattern of butyrophilin genes (Figure 1A). The thymus expressed all the butyrophilins tested and was chosen as a reference sample to compare expression across the different tissues. We found that butyrophilin expression levels and patterns varied among the tissues tested (Figure 1B). The gut showed highly increased expression of Btnl1, Btnl2, Btnl4, and Btnl6 compared to the thymus. Most of the tested butyrophilins had lower levels of expression in the liver and lungs compared to the thymus. Btn1a1 was not detected in liver but was expressed in the other tissues tested. Skint1 and Skint3 expression levels had limited expression, if any, in tissues other than the thymus. The differing levels of butyrophilins in different tissues showed that expression pattern varied based on location.

Effect of KRAS-Driven Lung Adenocarcinoma on Butyrophilin Gene Expression in Whole Lung

With a baseline of butyrophilin expression pattern determined in healthy B6 mice, we wanted to investigate if the presence of cancer initiated any changes. A murine lung adenocarcinoma model was used in which a KRAS mutation induced tumors in lung epithelial cells after administration of tamoxifen. The controls for these mice were $Scgb1a1^{+/CreER}.Rosa26^{+/LSLtdTomato}$ mice that were also injected with tamoxifen but did not develop cancer. Mice were injected once daily for three consecutive days. The lungs of mice

with the KRAS mutation and the lungs of control mice were harvested around thirty days post-injection. Tumors were evident in the lungs of cancer model. Both sets of lungs were minced and homogenized in the bead beater. Immediately after, RNA was isolated and used for cDNA synthesis. Butyrophilin expression in lungs with induced lung adenocarcinoma and the control lungs was assessed using qPCR. The control lungs were chosen as the reference sample. Btnl2 showed a significantly different $-\Delta C_t$ value compared to the control ($p=0.033$), with a mean relative quantity value of 2.96 compared to the control (Figure 2A, 2B). Other butyrophilin genes, such as Btn2a2, Btnl1, and Btnl6 appeared to be downregulated in lungs with induced adenocarcinoma when compared to the control (Figure 2B). Btnl10 specifically was nearly undetected in the lung cancer model. Based on these data, the presence of lung adenocarcinoma changed the butyrophilin expression pattern in the lung with increased Btnl2 expression.

Cell-Specific Butyrophilin Expression in Lungs with KRAS-Driven Lung Adenocarcinoma

Butyrophilin gene expression pattern changed within the whole lung in response to the lung cancer model. We wanted to investigate if this change was a result of specific cell types, such as tumor cells or leukocytes inhabiting the area. To answer this, we needed to determine the butyrophilin expression in different cell types. We aimed to develop a lung digest protocol that yielded viable epithelial cells as well as leukocytes. The lungs of normal B6 mice were digested using collagenase or elastase in order to compare viability outcomes. The lungs processed in collagenase had a higher number of cells overall, but a low number of viable epithelial cells. The lungs digested with elastase lost total cell quantity when filtered but showed a significant increase in the number of viable epithelial cells (Figure 3). Leukocytes and club cells were sorted from the lungs of mice with the $KRAS^{G12D}$ mutation around ninety days post-injection.

The controls were leukocytes and epithelial cells from B6.Scgb1a1$^{CreER/+}$·Rosa26$^{LSL/+}$ mice. In leukocytes, neither Btn1a1 nor Btnl10 was detected in any of the samples tested. Expression of Btn2a2 was seen in the control group, but not in the leukocytes of the cancer model. The -ΔC_t values showed that Btnl4 was not detected in the control group but did show expression in the leukocytes from the cancer model (Figure 4A). There appeared to be slight upregulation of Btnl2 and possibly Btnl9 in the leukocytes of the cancer model compared to the control leukocytes (Figure 4B). The lung club cells of the cancer model were compared to the lung club cells of the control mice. Out of the butyrophilins tested, Btn1a1, Btnl1, Btnl4, Btnl6, and Btnl10 were not detected in either group. Btnl2, the only gene detected in both the control group and the cancer model group, showed no difference in -ΔCt values. Btn2a2 and Btnl9 had low expression in the club cells of the mice with induced adenocarcinoma, but no expression in the control club cells (Figure 4C). An increase in Btnl2 expression was seen in lung leukocytes from the cancer model when compared to the controls, but this increase was not determined in the cancerous lung epithelial cells. This suggests that the previously recorded change in Btnl2 expression in the whole lung cancer model is due to the increased frequency of leukocytes.

Butyrophilin Gene Expression Changes in Influenza-Infected Lungs

Butyrophilin expression pattern changes are seen in the lung adenocarcinoma model. Lastly, we wanted to examine if butyrophilin expression pattern was specific to the type of inflammation. To test this, influenza-infected lungs were used to identify if changes in butyrophilin expression pattern were the same across different types of inflammation. The two influenza-infected lungs were compared to the healthy lungs from a B6 mouse. All butyrophilin genes tested exhibited upregulation of expression when compared to normal lungs. Btnl4, Btnl6,

and Btnl10 specifically showed highly increased expression in the presence of influenza infection compared to the control (Figure 5). These data combined contribute to the hypothesis hat butyrophilin expression pattern changes in response to specific types of inflammation.

Discussion

Butyrophilins bind and activate γδ T cells, playing a role in the regulation of immune response. Cancer studies have shown that γδ T cells may contribute to the metastasis of tumors depending on the cytokine they secrete[12]. Therefore, butyrophilins are a potential target for the treating and preventing of inflammatory conditions, such as lung adenocarcinoma. In order to know which butyrophilins to target, the upregulated or downregulated responses to the specific inflammation need to be determined.

Butyrophilin expression patterns vary across different tissues. It is difficult to determine exactly how much a butyrophilin gene is being expressed, as the data is relative to the values measured in the thymus. However, even with this relative quantification it is evident that different tissues express butyrophilins in varying amounts. We can see that a select number of butyrophilins are very highly expressed in the gut. γδ T cells are very populated in gut as well. There could be a connection between the specific upregulated butyrophilin genes seen here and the large number of γδ T cells in the area. If butyrophilins are acting as a ligand to γδ T cells, there could be select butyrophilin genes recognizing them and possibly affecting the development of specific γδ T cells subsets.

With their regulatory roles in immune responses, we wanted to see how inflammation would change butyrophilin expression pattern or level. γδ T cells are found in the lungs, and some subsets have a pro-tumor cytokine response when activated. Butyrophilin levels in lungs

with induced lung adenocarcinoma showed a slight upregulation of Btnl2 when compared to the control. Previously, Btnl2 has been shown to assist with tumor metastasis. When Btnl2 was blocked using an antibody, the number of infiltrating IL-17A producing γδ T cells and tumor progression was reduced.[7]. By sorting the cells in the cancerous lungs, we saw that this upregulation appears to be due to leukocytes. The club cells, where the tumors are produced, did not show this upregulation. We evaluated butyrophilin expression via qPCR due to the lack of butyrophilin antibodies. With the data gathered on Btnl2, the next step would be to find a Btnl2 antibody to accurately identify butyrophilin expression on different cell types and compare results to RNA sequencing data.

Club cells sorted from the cancer model showed low expression of Btn2a2 and Btnl9. These genes were not expressed in the control club cells. It is possible that these butyrophilins were undetected due to the amount of cDNA input from the control club cells. We collected a limited amount of the control club cells after sorting, which yielded a small amount of RNA. These results can be confirmed by looking at RNA sequencing data. If Btn2a2 and Btnl9 are only being expressed in the club cells cancer model, it would be beneficial to see if expression is upregulated in more severe stages of lung adenocarcinoma.

Butyrophilin expression was examined in different types of inflammation. Induction of lung adenocarcinoma showed an increase in Btnl2 expression and downregulation of other butyrophilins. When influenza was introduced to the lungs, all the butyrophilins tested showed increased expression compared to healthy B6 lung. Btnl4, Btnl6, and Btnl10 all showed very high levels of expression in influenza-infected lung. Based on cell-specificity of the lung adenocarcinoma model, we would expect that this change is due to leukocytes expressing butyrophilins are infiltrating the area in response to influenza. All the butyrophilin genes had low

detection in the cancerous lungs. These results suggest that different infections or sources of inflammation have differing effects on butyrophilin expression and pattern.

In this work, a baseline of butyrophilin expression level across various tissues was established. The changes in gene expression were examined in response to different types of inflammation. It was shown that the patterns of butyrophilin expression changes were exclusive to the type of inflammation present. When focusing on a model of lung adenocarcinoma, Btnl2 showed increased expression and could potentially promote tumor metastasis via γδ T cell activation. Most of the butyrophilin detection was found on the leukocytes of both the cancerous lungs and controls. The changes in butyrophilin expression in response to lung adenocarcinoma also seem to be due to expression on leukocytes, not the lung epithelial cells. By knowing which butyrophilins are experiencing gene expression changes in response to inflammation, we can then identify what they are binding to and how this binding occurs. Determining whether changes in expression are cell-specific or gene-specific will allow for precise testing of potential immunotherapies, which could have a profound effect on the treatment of cancer and other diseases.

List of Figures

Figure 4. Cell-Specific Butyrophilin Expression in Lungs with KRAS-Driven Lung Adenocarcinoma. Controls were lung leukocytes (n=4) and lung epithelial cells (n=4) from B6.Scgb1a1$^{CreER/+}$.Rosa26$^{LSL/+}$ tamoxifen injected mice. KRAS values were lung leukocytes (n=4) and lung epithelial cells (n=4) from B6.Scgb1a1$^{CreER/+}$.Rosa26$^{LSL/+}$.Kras$^{LSL-G12D/+}$ tamoxifen injected mice. Butyrophilin genes tested were Btn1a1, Btn2a2, Btnl1, Btnl2, Btnl4, Btnl6, Btnl9, and Btnl10. (A) The $-\Delta C_t$ values of butyrophilins genes testes in lung leukocytes. The limit of detection is represented at y=-18. (B) Expression levels of butyrophilins in cancerous leukocytes relative to control leukocytes. Butyrophilin gene expression of the control leukocytes is represented at y=1. (C) The $-\Delta C_t$ values of butyrophilins genes tested in lung epithelial tdTomato+ cells. The limit of detection is represented at y=-17. Any genes not detected were labeled “nd”.

Figure 5. Butyrophilin Gene Expression Changes in Influenza-Infected Lung. Butyrophilin expression of PR8 influenza-infected lungs (n=2) relative to normal B6 lungs (n=1). The butyrophilin gene expression of the healthy B6 lungs is represented at y=1. Butyrophilin genes tested were Btn1a1, Btn2a2, Btnl1, Btnl2, Btnl4, Btnl6, Btnl9, and Btnl10.

Figure 1.

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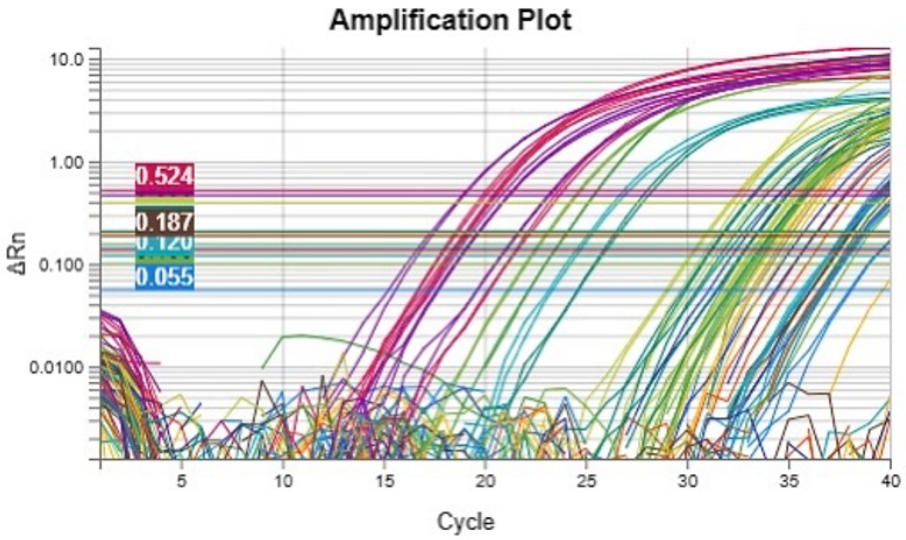
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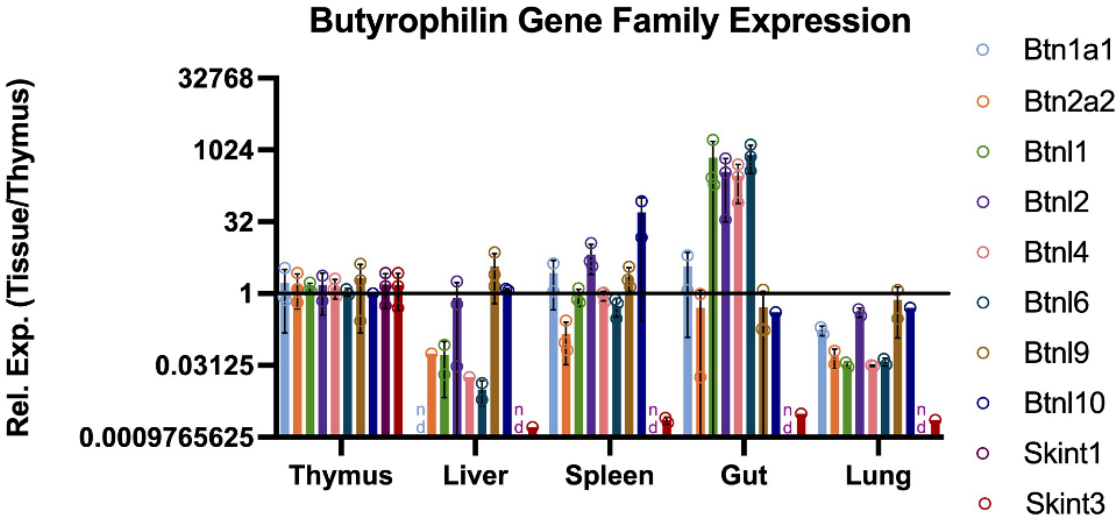
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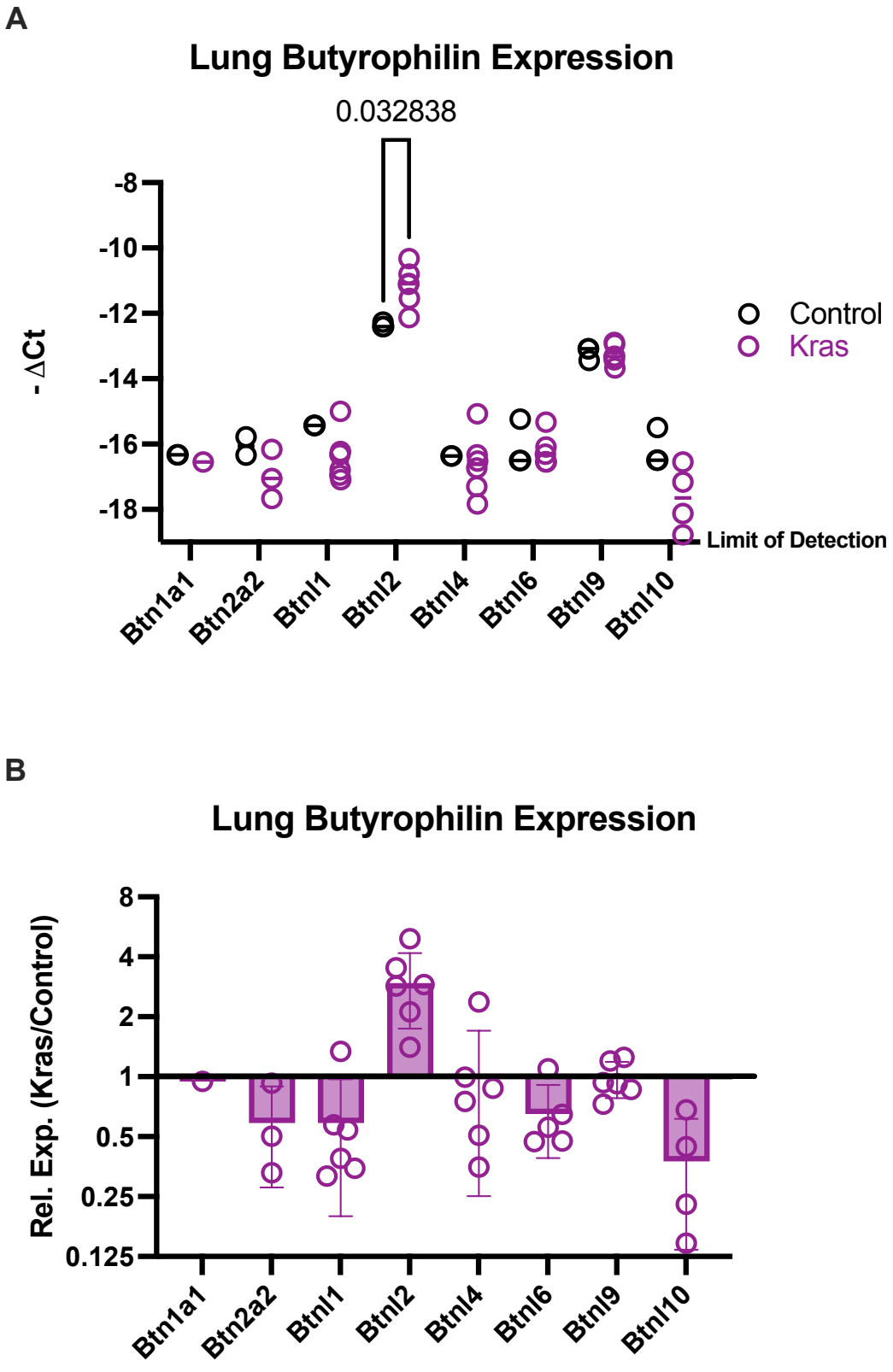
Figure 2.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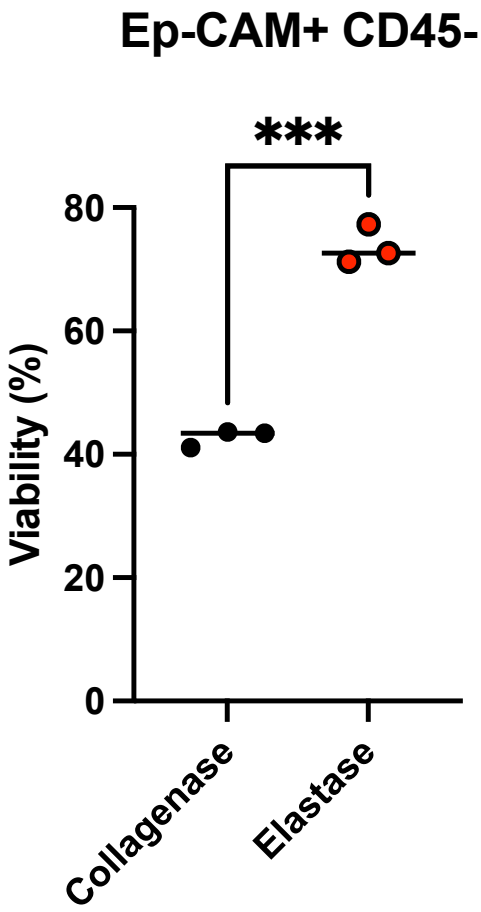

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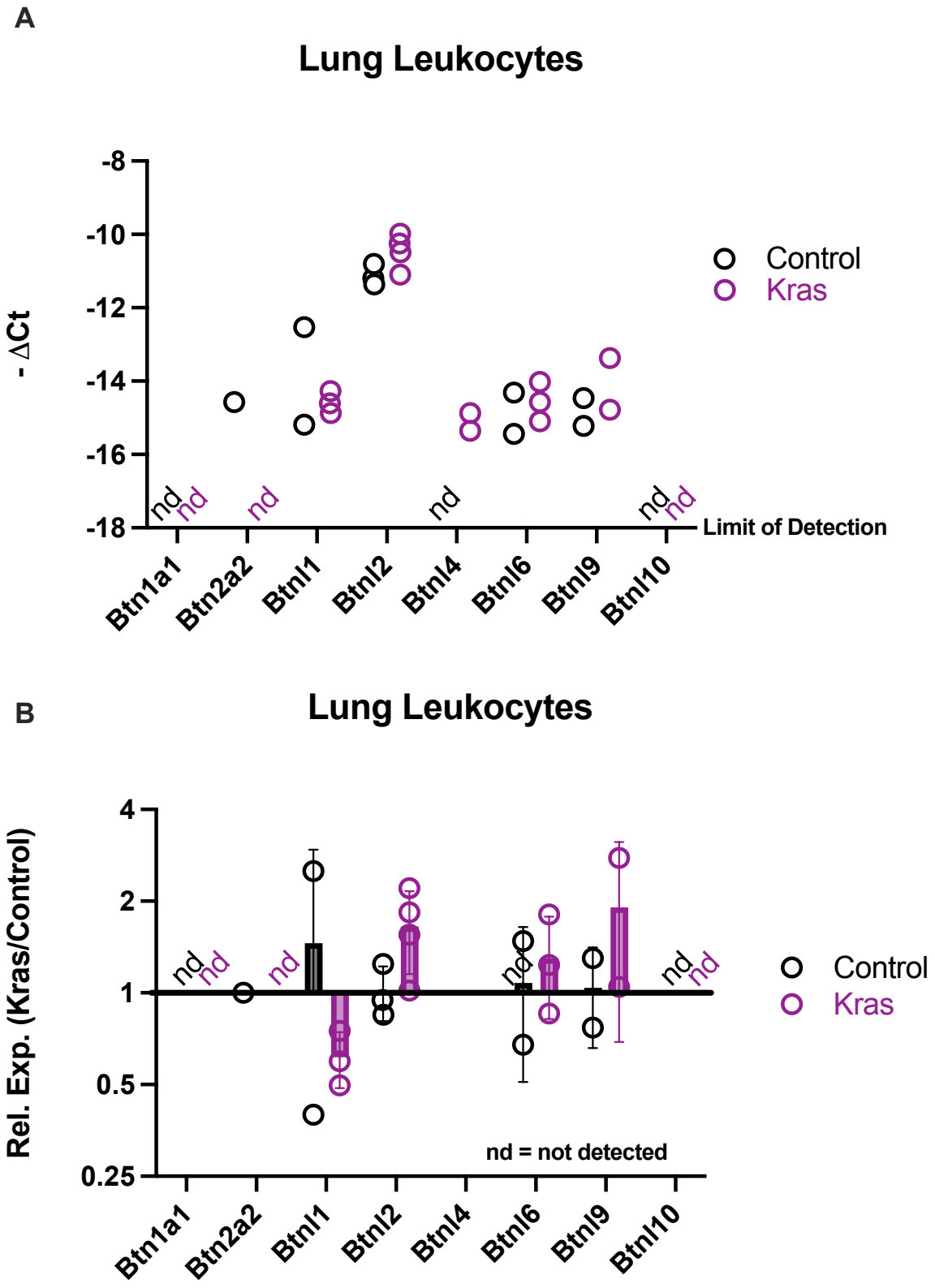
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nd = not detected
Control
Kras

C

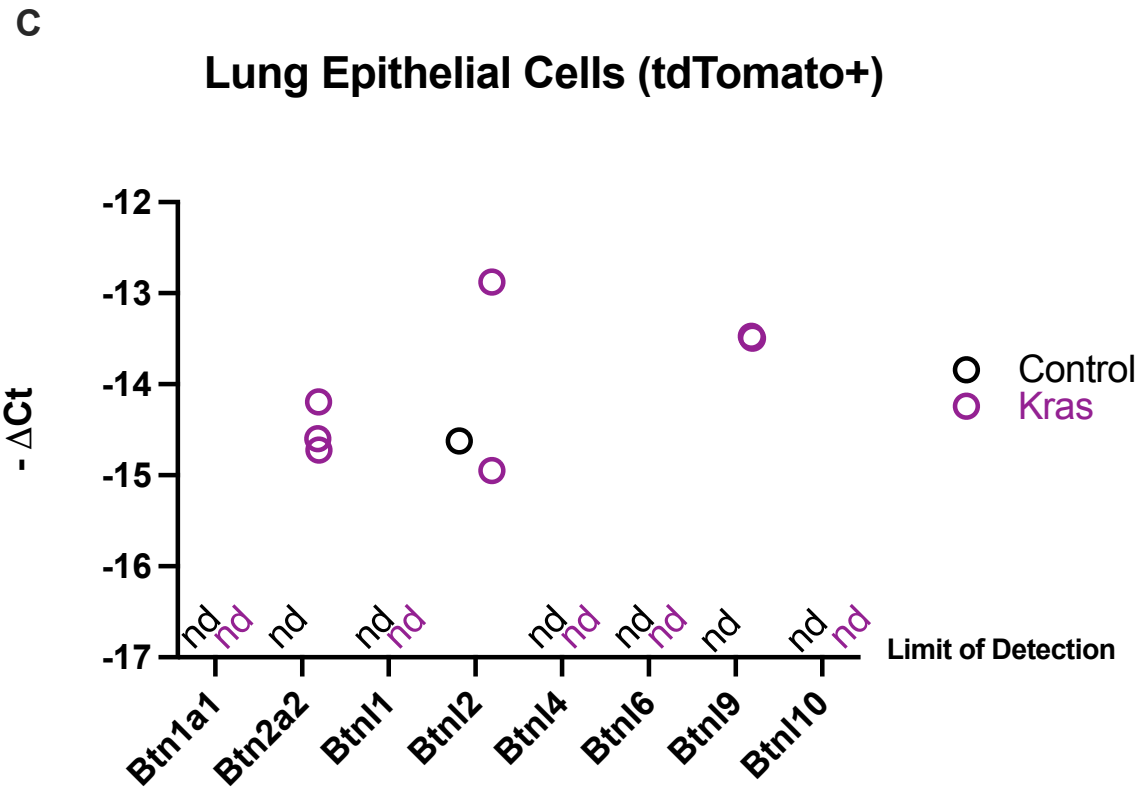
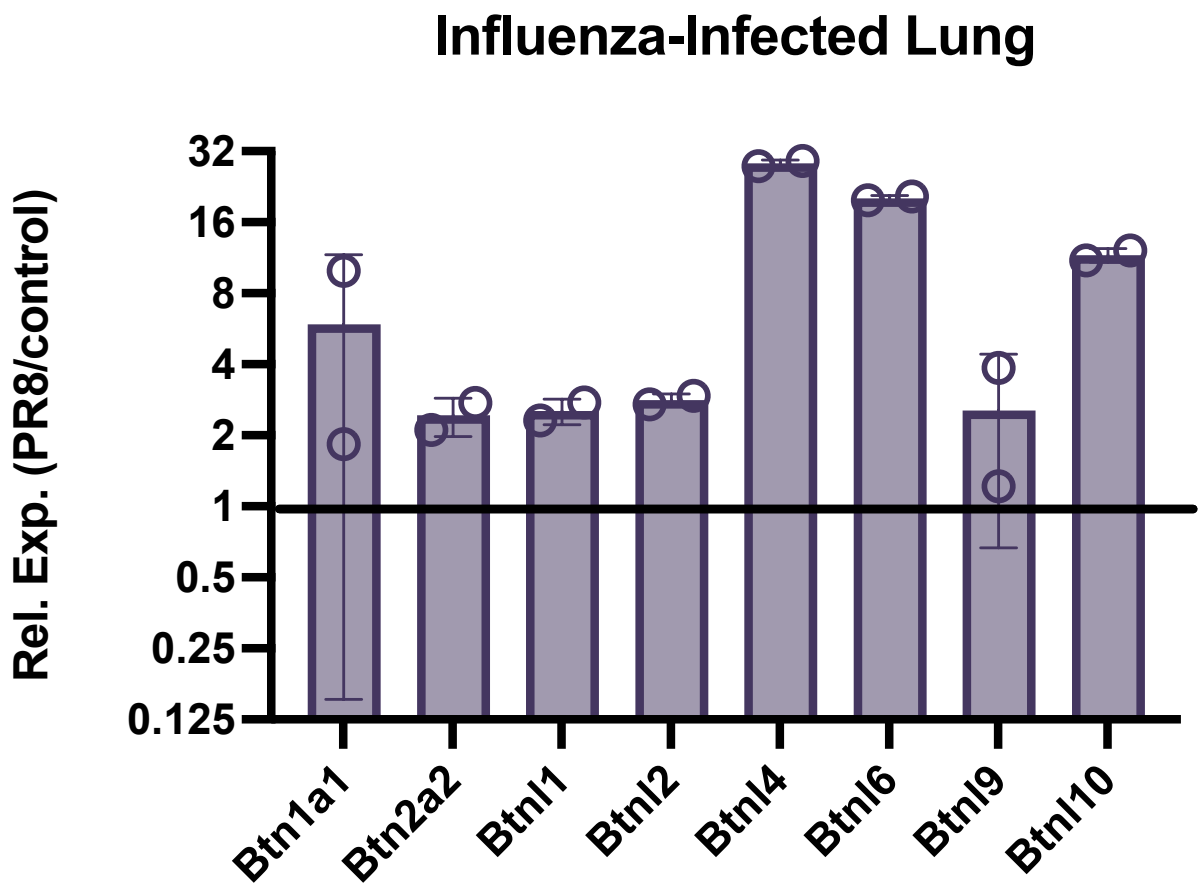

Lung Epithelial Cells (tdTomato+)
-12
-13
-14
-15
-16
-17
- ΔCt
nd
nd
nd
nd
nd
nd
nd
nd
nd
nd
nd
nd
nd
nd
nd
nd
Limit of Detection
Control
Kras
Btn1a1
Btn2a2
Btnl1
Btnl2
Btnl4
Btnl6
Btnl9
Btnl10

Figure 5.


Influenza-Infected Lung
Rel. Exp. (PR8/control)
32
16
8
4
2
1
0.5
0.25
0.125
Btn1a1
Btn2a2
Btnl1
Btnl2
Btnl4
Btnl6
Btnl9
Btnl10

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