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OGT Glycosylation of Histones in T Cells May Participate in Systemic Lupus Erythematosus

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THE UNIVERSITY OF SOUTH ALABAMA
COLLEGE OF ALLIED HEALTH PROFESSIONS

OGT GLYCOSYLATION OF HISTONES IN T CELLS MAY PARTICIPATE IN SYSTEMIC LUPUS ERYTHEMATOSUS

BY

Jared Luke Ridgeway

A Thesis

Submitted to the Honors College of the
University of South Alabama
in partial fulfillment of the
requirements for the degree of

Bachelor of Science

in

Biomedical Sciences

May 2021

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ABSTRACT

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease that primarily affects women. The etiology is not known yet, but genetics and environmental factors trigger the disease. These epigenetic changes modify gene expression and contribute to the development of the disease. It is known that T cells from patients with lupus overexpress genes that may contribute to the onset and progression of the disease. Dr. Gorelik has shown that OGT (**O**-linked N-acetyl-**G**lucosamine **T**ransferase), which is an X-linked gene, is overexpressed in women with SLE. OGT adds β-N-acetylglucosamine (O-GlcNAc) to serine and threonine residues of proteins. OGT may play a critical role in chromatin structure by O-GlcNAcylation of histones. Furthermore, O-GlcNAc is considered part of the histone code. The effect of OGT in T cells is unknown, but its overexpression may play a role in T cell dysfunction in lupus. This evidence led us to investigate molecular targets of OGT in T cells, particularly its role in the post-translational modification of histones. This work was done with primary T cells isolated form the peripheral blood of healthy female donors. The cells underwent various treatment and protein expression was analyzed via SDS-PAGE electrophoresis. Our results strongly suggest that H2B is a molecular target of OGT in healthy T cells, and the overexpression of OGT in lupus T cells may increase H2B glycosylation. In consequence, it may also modify chromatin structure, causing changes in gene

expression, and may be an epigenetic modulator in lupus, explaining the female preponderance to develop SLE.

CHAPTER I

INTRODUCTION

1.1 Systemic Lupus Erythematosus

Systemic Lupus Erythematosus (SLE), more commonly known as Lupus, is a chronic autoimmune disease that primarily affects women. Some symptoms include swelling and inflammation of joints, lethargy, and a “butterfly” rash spanning across the nose and under the eyes. This disease is characterized by periods of flare ups followed by remission of symptoms and damage to organs such as the skin, liver, kidneys, heart, joints, and lungs. The primary cause is the presence of anti-dsDNA – an antibody that targets genetic material – that accumulates and deposits on healthy tissues in the body, rather than targeting foreign pathogens. This antibody accumulation leads to tissue damage.

Lupus affects women between the ages of 15 and 45 ten times more often than men, and according to the Lupus Foundation of America, it is approximated that 1.5 – 2 million individuals are afflicted with this disease in the United States alone. Lupus pathogenesis has been studied extensively, yet there is still not much understanding about the mechanisms of the disease and why females suffer from it so much more than males. As much as can be elucidated so far is that when genetically predisposed individuals

encounter particular environmental triggers, lupus can develop due to epigenetic modifications.

One modification to DNA that can alter gene expression is methylation. It has been observed that lupus patients present a global T-cell hypomethylation (Jeffries et al, 2011). Because OGT affects many different signaling pathways throughout the cell, it is possible that overexpression of OGT in T cells of female lupus patients alters intracellular signaling and can contribute to lupus pathogenesis.

1.2 O-linked N-acetylglucosamine Transferase

O-linked β-N-acetylglucosamine (O-GlcNAc) was discovered in the 1980s and this discovery eliminated the idea that protein glycosylation is entirely contained to the cell surface and extracellular matrix. **O**-linked N-acetyl**G**lucosamine **T**ransferase (OGT) glycosylates proteins in the nuclear membrane and cytoplasm of cells. Despite only being known so recently due to how difficult to detect it was, O-GlcNAc is ubiquitous, functioning in nearly every cell in the body. Especially high concentrations of O-GlcNAc activity are seen in neurological, endocrine, and immune tissues. O-GlcNAc has been shown to interact with other enzymes such as O-phosphatase, altering its activity on proteins in the cell. Potential pathogenesis resulting from dysregulation of O-GlcNAc is also noted, including but not limited to diabetes and neurological disorders (Hart 2007).

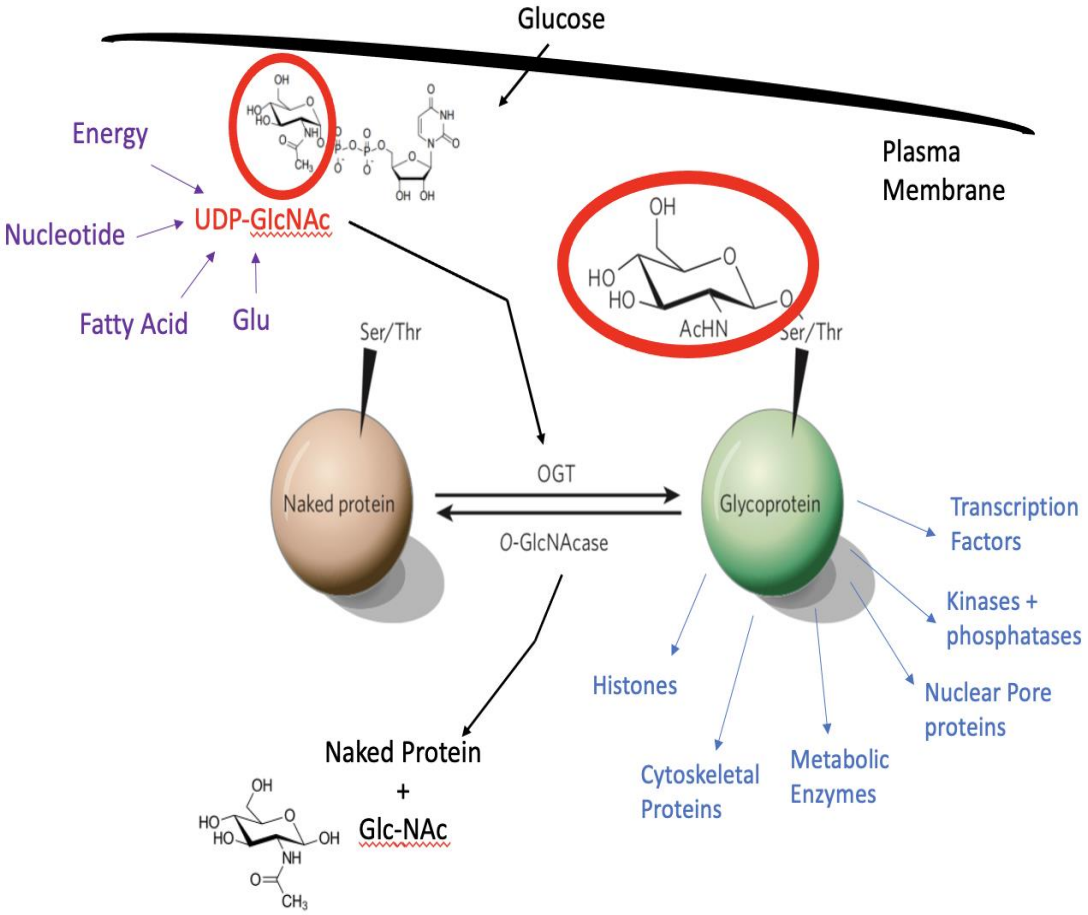


Figure 1. *Cycling of O-GlcNAc on serine or threonine residues of nuclear and cytoplasmic proteins is controlled by two highly conserved enzymes, OGT and O-GlcNAcase (OGA). O-GlcNAc cycles at rates similar to that of O-phosphate in response to various cellular stimuli and does not require a consensus sequence.* (figure modified from Hart 2007, doi:10.1038/nature05815)

OGT transfers GlcNAc from UDP-GlcNAc serine and threonine residues of proteins. This post-translational modification occurs in the cytosol and nucleus, modifying signal transduction. The cycling of O-GlcNAc onto proteins is regulated by the highly conserved balance of OGT and O-GlcNAcase (OGA), which removes O-GlcNAc from proteins (Hart 2007).

O-GlcNAc is described as a major modifier of chromatin in cells, suggesting that O-GlcNAcylation plays a critical role in transcriptional regulation of gene expression. OGT also has been shown to directly modify histones due to a similar HAT (Histone acetyltransferase) domain to that of the GCN5 gene, which has also been associated with transcriptional regulation (Sakabe 2010). This is believed to facilitate OGT's association with histone complexes. Dr. Gorelik's team has shown that OGT, which is an X-linked gene, is overexpressed in women suffering from SLE (Hewagama 2013), shown in Figure 2.

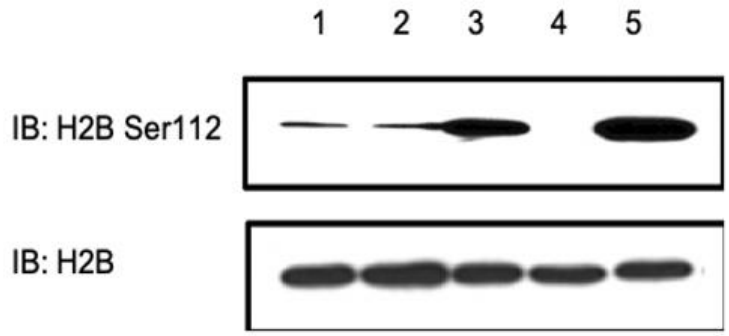


Figure 2. Glycosylation of H2B in T cells. *T cells were isolated form two healthy female donors (lane 1-2); female with active lupus (lane 3); healthy female donor and transfected with OGT siRNA (lane 4); or incubated with 5 mM glucosamine (lane 5). Cells were stimulated with PMA/Ionomycin for 4 h and the chromatin-bound proteins were isolated from the insoluble nuclear fraction. Proteins were subjected to SDS-PAGE gel electrophoresis followed by immunoblot with anti Glc-H2B antibody. The blot is representative of three experiments. H2B was used as a loading control.*

1.3 OGT and Histones

Histone O-GlcNAcylation may be the response to serum glucose levels and/or cellular energy states within certain cell types (Fujiki 2011). The effects of histone modifications by OGT are observed to be especially pronounced in the cells of the

nervous and immune tissues. The histone complex H2B is shown to serve as a substrate for O-GlcNAcylation by OGT. This modified H2B protein was frequently located near transcribed genes, suggesting that histone O-GlcNAcylation plays a critical role in facilitating transcription of genes within the cell (Dehennaut 2014). However, the significance of O-GlcNAcylation in chromatin reorganization remains elusive. Relating specifically to my project, experiments on HeLa cells have shown that O-GlcNAc modifies all four of the core histone proteins and is believed to compete with or promote modifications which are associated with activation or repression of transcription in gene expression (Bond 2015).

OGT and its cycling of O-GlcNAc, along with OGA, is considered to be part of the histone code (Sakabe 2010). It serves as an epigenetic controller which regulates gene expression. Evidence presented showed that overexpression of the OGT protein affects the structure of chromatin condensation around the histone. Modifications such as methylation, acetylation, and glycosylation of histones affect how DNA associates with them as part of the nucleosome. Chromatin structure will adopt a more or less tight configuration that will prevent or facilitate, respectively, the access to gene promoters by the transcription machinery. Thus, OGT – through the post-translational glycosylation of histones – may regulate gene expression in T cells from patients with lupus.

1.4 Epigenetics

Observations like this indicate that O-GlcNAcylation could have an important role in epigenetic expression, affecting development and gene differentiation. As OGT is an X-linked gene, its overexpression could be due to an incomplete X inactivation in

females. Males only have one X chromosome, whereas females have two. Therefore, one of these X chromosomes must be silenced in order to balance transcription of X-linked genes. Due to numerous variables such as maternal nutrition, diet, and differentiation of this gene silencing, female placentas will occasionally have higher levels of OGT and O-GlcNAcylated proteins than male placentas (Olivier-Van Stichelen 2015).

Interestingly, the levels of these glycoproteins are also elevated in the T cells of women with lupus (Hewagama 2013). It had been previously noticed that estrogen was linked to SLE flare-ups in women of child-bearing age, including those who received therapeutic estrogen treatment (Cutolo 2006). However, pre-pubertal girls and post-menopausal women also have an increased prevalence of SLE when compared to men. This suggests an alternative reason for the female preponderance of SLE. It has been noted that men affected by Klinefelter's Syndrome (XXY) suffer from SLE at approximately the same rate as women (Dillon 2011 and Scofield 2008). This indicates that the presence of a second X chromosome contributes to the development of SLE. The function of OGT within immune cells has emerged as a prime candidate for a pleiotropic effector in X-linked diseases (Abramowitz 2014). We hypothesize that OGT escapes X-inactivation in the tissues of the nervous system and immune system and contributes to X-linked diseases, including ones like systemic lupus erythematosus, by overexpression of the *OGT* gene.

1.5 Overview

OGT being overexpressed in the T cells of females suffering from SLE due to gene demethylation has been reported (Hewagama 2013). However, very little is known

about the role of OGT in T cells. This project focuses on the signaling of normal T cells in order to characterize the role that OGT plays. This knowledge can then help us to better understand the significance of abnormal OGT expression in patients who are suffering from SLE. Due to the role that histone modifications have in the availability of transcription machinery to access DNA, we are interested in studying how the activity of OGT on histones could act as a regulator of immune response and contribute to pathogenesis of SLE. The findings could contribute to a better understanding of how abnormal signaling in T cells contributes to SLE, as well as the female preponderance to develop it.

CHAPTER II

HYPOTHESIS & AIMS

The aim of this project was to characterize and identify molecular targets of OGT, which are key proteins in T cell signaling and may participate in the pathogenesis of lupus.

We hypothesized that the increase in OGT may alter intracellular signaling in T cells of women with lupus and may contribute to the female preponderance to develop the disease. T lymphocyte cells have a critical role in the immune system and T cell signaling impairment may lead to autoimmune diseases. We studied the targets of OGT in normal T cells from healthy donors to analyze the contribution of OGT overexpression to the disease. To test this hypothesis, we identified the molecular targets of OGT in T cell signaling in order to explain the biological relevance of its overexpression in lupus.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

The following reagents were used: density gradient media/Ficoll-Paque (GE health sciences), EasySep cell separation kit (Stem Cell Technologies), nitrocellulose membrane (Bio-Rad), BSA protein standard (Pierce), nitrocellulose membrane (Bio-Rad), PUGNAc (100 μM; Tocris Bioscience), OSMI (50 μM), Glucosamine (5 mM), PMA (10 ng/mL), and Ionomycin (250 ng/mL; Sigma-Aldrich). All other reagents were from Sigma-Aldrich unless otherwise specified.

The following primary antibodies were used: anti-OGT (1.2 μg/mL; 1:1000; Cell Signaling), anti-H2B (1 mg/ml; 1:5000; Abcam), anti-GlcH2B Ser/Thr (1 mg/mL; 1:2000; Abcam), anti-RL2 (1 mg/mL; 1:1000; Abcam). β-actin (1 mg/mL; 1:2500; Sigma-Aldrich) for loading control.

The following secondary antibodies were used: goat anti-rabbit (1:5000; Cell Signaling) and goat anti-mouse (1:2000; Sigma-Aldrich).

3.2 Subjects

Healthy controls aging from18-58 were recruited via advertising, with an average age of 41. These studies were reviewed and approved by the University of South Alabama Institutional Review Board for Human Subject Research.

3.3 T Cell Extraction

Peripheral blood mononuclear cells (PBMCs) were acquired from healthy donors by drawing blood in a 60 mL syringe with 5 mL of Heparin in them to prevent clotting. 10 mL of PBS was then added to 50 mL tubes to dilute the blood and 20 mL of blood was added to it. The blood and PBS were mixed to homogeneity. Approximately 20 mL of blood was then pipetted carefully into tubes containing Ficoll-Paque solution. The tubes were turned at a 45^{o} angle while pipetting so that the blood would sit on top of the Ficoll and not mix with it. The tubes were then centrifuged in a *Centrifuge 5810R* at 22^{o}C and 1250 rpm for 30 minutes. The top layer of plasma was removed via aspiration and pipette, being careful not to disturb the second layer. The buffy coat containing the PBMCs were then removed via pipette and transferred to a new tube. PBMCs from all tubes were then combined and homogenized, and then 10-12 mL aliquots were transferred to new tubes.

The cells were washed by adding PBS to the top of the tubes, and then centrifuged at 18^{o}C and 1250 rpm for 10 minutes. The supernatant was discarded and the pellet was then loosened and washed with 10 mL of PBS. The cells were then transferred to half as many new tubes (if there were 8 tubes, then the cells were consolidated into 4 tubes). The tubes were then centrifuged again at 18^{o}C and 1250 rpm for 10 minutes and the

supernatant was discarded. This was repeated until there were only two tubes. Then, ~3 mL of PBS was added to the tubes to loosen the pellet and mixed, and the tubes were combined into one. PBS was then added to the top of the tube and a small aliquot was taken for cell counting.

The cells were centrifuged for 10 minutes at 18^{o}C and 1250 rpm and the supernatant discarded. The T cells were then isolated from the PBMCs using the *EasySep Human CD4+T Cell Isolation Kit* following the manufacturer instructions. The resulting enriched CD4+ T cell fraction was then diluted to 20 mL and a small aliquot was removed for cell counting.

The cells were then centrifuged again at 18^{o}C and 1250 rpm for 10 minutes at maximum acceleration and maximum brake. The supernatant was then discarded; the cells were counted and resuspended in 10% FBS 1% penicillin/streptomycin RPMI-1640. Then they were transferred to a 6-well plate in a concentration of $2x10^{6}$/mL. The corresponding drugs for each sample treatment were then added, and the cells were incubated at 37^{o}C in a 5% CO_2 incubator for 60 minutes. Following the 1h incubation, cells were stimulated with 10 ng/mL PMA and 250 ng/mL Ionomycin for 4 hours. Each cell sample underwent one of the following treatments: 100 μM PUGNAC (O-GlcNAcase inhibitor), 5 mM D-(+)-Glucosamine hydrochloride (glycosyl donor), and 50 μM OSMI (OGT inhibitor). Afterwards, the cells were harvested and placed into 15 mL tubes and washed by centrifugation at 4^{o}C and 1250 rpm for 10 minutes. Supernatants were then discarded and cell pellets were stored at -80^{o}C.

3.4 Nuclear Fraction Isolation

Isolated, treated T cells were washed with PBS buffer and centrifuged at 1,250 rpm and 18^{o}C for 10 minutes with maximum brake and maximum acceleration. The supernatant was then removed via aspiration. The pellet was then resuspended in 5 pellets volumes of cytoplasmic extract (CE) buffer containing NP-40 detergent and transferred to a microfuge tube. It was then incubated on ice for 5 minutes, mixing intermittently. The sample was then centrifuged again at 3,000 rpm and 4^{o}C for 4 minutes. The supernatant containing the cytoplasmic extract was then removed and placed into a separate tube labeled CE. The pellet containing the nuclei was then washed with 5 pellet volumes of CE buffer without the NP-40. It was then centrifuged at 3,000 rpm, 4^{o}C, for 4 minutes. The supernatant containing the cytoplasmic wash was then removed and transferred to a new labeled tube. One pellet volume of nuclear extract (NE) buffer was then added to the nuclei pellet and vortexed to resuspend. The suspension was then incubated on ice for 10 minutes, mixing intermittently. Both the CE and NE tubes were centrifuged at 14,000 rpm for 10 minutes at 4^{o}C. The supernatant of each was then transferred to a new tube and labeled. All samples were then stored in the freezer at -80^{o}C until need for the next experiment.

3.5 Histone Extraction:

Isolated, treated T cells were flash-frozen using a dry ice/methanol bath. The frozen pellet was resuspended in ice-cold T-Cell Buffer (HEPES) supplemented with DTT and a Protease inhibitor cocktail (1 mL buffer/10x10^{6} cells). The cells were then incubated on ice for 30 minutes. The cells were then sonicated to disrupt the membrane

and resuspend the pellet. We then added 0.6% NP-40 and vortexed for 10 seconds. The suspension was then centrifuged at 14,000 rpm and 4^{o}C for 5 minutes. The supernatant was then removed, but not discarded, and placed into a separate sample tube. The pellet was then resuspended in 0.2 N HCl (100 μL/10x10^{6} cells) and mixed via pipette, then transferred to a 1.5 mL microfuge tube. The sample was then incubated overnight in a cold room (4^{o}C) with continuous inversion (orbital set: 24 rpm/2 min, reciprocal: 14^{o}/1 min, vibro/pause: 5^{o}/5min, time: 22 hrs.). Sample was then removed and centrifuged at 14,000 rpm and 4^{o}C for 10 minutes. The supernatant containing the histones was then transferred to a new tube. We then added 2M NaOH at 1/10 of the volume of the supernatant (10 μL/10x10^{6} cells). Protein concentration was then determined, and an Amicon Ultra 10K column was used to concentrate the sample, if needed. This method of histone extraction is based on the solubility of histones in acids, in this case HCl, conditions where most other nuclear proteins and nucleic acids will precipitate (Schechter 2007).

3.6 Quantification of Proteins

Amounts of protein in the samples were quantified in a Varioskan LUX microplate reader. Samples were loaded into wells at ¼ dilution. A standard protein curve was then created using bovine standard albumin (BSA) dilution. The plate was incubated in a 37^{o}C incubator for 30 minutes before being quantified in the Varioskan using 526 nm wavelength.

3.7 Electrophoresis and Western Blotting

Once proteins were quantified, an amount of each sample containing ~10 μg of proteins was added to 1.5 mL tubes. A volume of SDS Sample Buffer (6x) was added to each sample in a 1:5 ratio of buffer to sample. Once the sample buffer was added, the tubes were capped immediately and placed in a dry bath at 100^{o}C for 5 minutes. The samples were then removed, vortexed, and momentarily centrifuged to get any condensate off the sides of the tube. The samples were then inserted into their lanes of a *Bio-Rad 4–20% Mini-PROTEAN TGX* gel, along with a standard marker (Bio-Rad). The gel was run at 30 mA until completion, using a *Bio-Rad Power Pac 300*. The electrophoresis was then transferred overnight at 70 V onto a 0.2 nitrocellulose membrane. Once transferred, the blot was stained with *ChemCruz Ponceau S* to visualize the proteins that had successfully transferred. Once visualized, the Ponceau was removed using 0.1 M NaOH before being rinsed with DI water. The gel was also stained with *Thermo Scientific Imperial^{TM} Protein Stain* to visualize any histones that had not transferred.

3.8 Imaging

Once the blotted proteins had been incubated with various antibodies to target different aspects of the samples, the blots were visualized via chemiluminescence. This was done by developing the blots for 30-40 seconds in the Bio-Rad Clarity^{TM} Western ECL Substrate mixed in a 1:1 ratio, and minimizing exposure to light. The blots were then imaged using a *LI-COR Odyssey Fc* exposed for 2 minutes.

CHAPTER IV

RESULTS

4.1 Successful Purification of Histones

The focus of many of our experiments were on the effect that OGT has on histones. In order to conduct these experiments, an effective method of isolating histones from a whole cell lysate was needed. The purpose of the experiment shown in Figure 3 was to show that methodology to isolate histones was appropriate. As described above, T cell lysates were first isolated into a nuclear fraction using a detergent. From this nuclear fraction the solubility of histones in acid was utilized. The pellet containing the nuclear fraction was dissolved in HCl, which allowed the histones to be retrieved in the supernatant while most other nuclear proteins were insoluble and remained in the nuclear pellet once centrifuged. After concentrating and quantifying, the histones were loaded into an SDS-PAGE. The membrane was stained with a protein stain and the results are shown in Figure 3. Bands can be seen corresponding to histones H2A, H2B, H3, and H4 without very much other background protein confirmed the successful purification of histones. The histone that we are particularly interested in, H2B, has a molecular weight of 17 kDa. Being able to isolate histones was important to our experiments because it allowed us to load a known amount of histone proteins into each well for electrophoresis.

Finding a good loading standard is difficult in nuclear proteins, as many of them are affected by the activities of OGT, which we are manipulating in these experiments.

4.2 Levels of O-GlcNAcylated H2B in T Cells from Healthy Women

Cells were subjected to the various treatments previously described in order to either increase or decrease the total O-GlcNAcylation level of the histone H2B. These changes in the levels of O-GlcNAcylation were measured via immunoblotting (Figures 4 and 5).

In Figure 4, histones were purified from CD4+ T cells. The first lane is our control cells with no treatment. The second lane were cells treated with 5mM $GlucosNH_2$, and the third lane cells treated with 100 μM PUGNAc, which both increase O-GlcNAcylated protein levels in the CD4+ T cells. PUGNAc does this by inhibiting OGA, which prevents O-GlcNAcylated proteins that have already been added by OGT from being hydrolyzed. $GlucosNH_2$ increases O-GlcNAcylation by increasing the available amount of sugar donor for OGT, UDP-GlcNAc. The fourth lane was treated with 50 μM OSMI, which directly inhibits OGT activity. The blot was stained with Ponceau protein stain in order to visualize and confirm that similar amounts of protein were loaded into each lane. The blot was incubated with anti-glycosylated H2B primary antibody and anti-rabbit secondary to elicit chemiluminescence. From this, it can be seen in Figure 4 that the lanes treated with PUGNAc and $GlucosNH_2$ had increased levels of H2B glycosylation relative to the control. Also, it can be seen that the lane treated with OSMI had decreased levels of H2B glycosylation relative to the control.

In Figure 5, the first lane is our control. In the second lane, CD4+ T cells were stimulated with PMA/Ionomycin, which is an unspecific stimulator and calcium ionophore. In lane 3, the T cells were incubated with PUGNAc. Lane 4 was incubated with OSMI. The blot was incubated with anti-glycosylated H2B primary antibody. Total H2B protein was used as a loading control. This is done by calculating the optical density relative to total H2B. It should be noted that less total protein was loaded into the 3rd lane due to a sample shortage. This can be seen in the reduced level of total H2B. Despite this, these results showed that the T cells treated with PUGNAc had increased levels of H2B glycosylation relative to the control, and the T cells treated with OSMI had decreased levels of H2B glycosylation relative to the control.

FIGURES:

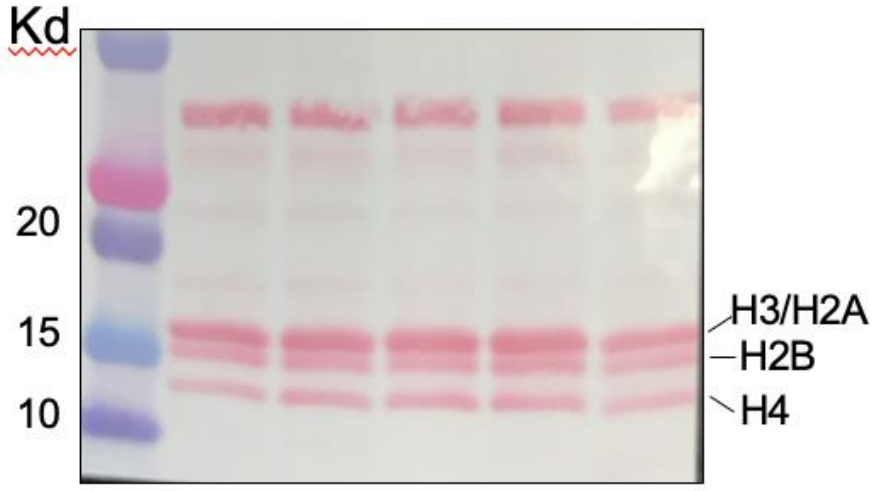
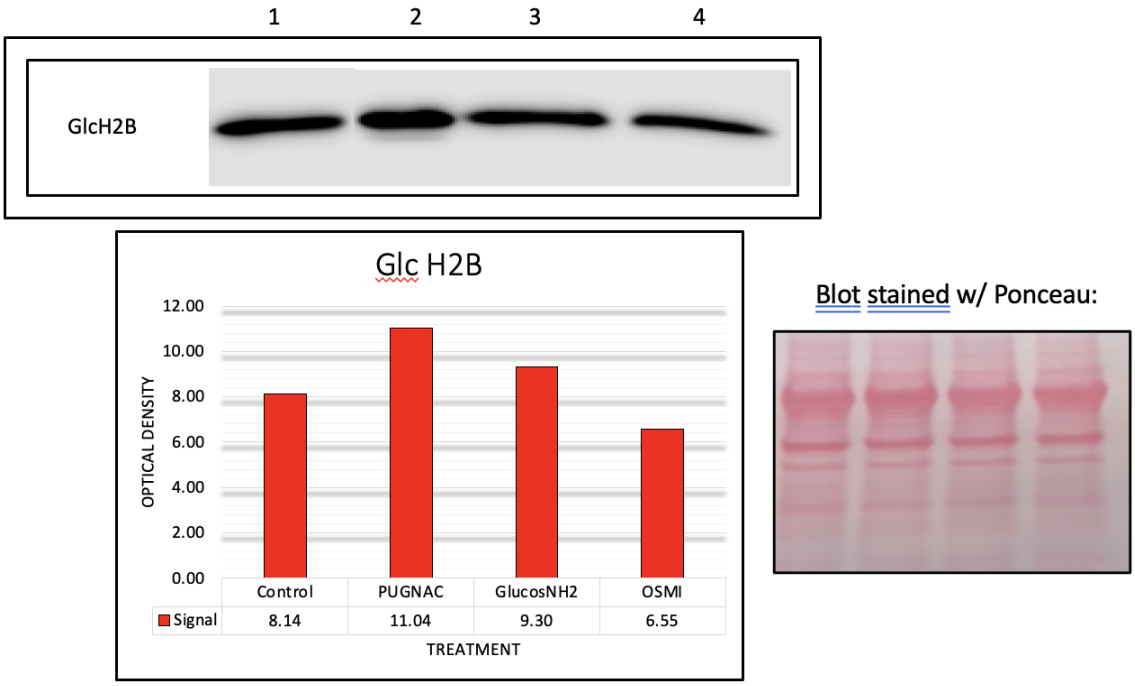


Figure 3. *Isolation of Histones from primary human T-cells. Histones were isolated and proteins were subjected to electrophoresis. After transfer, blot was stained with Ponceau. The bands correspond to the molecular weight of the different histones, H2B, H3/H2A, and H4, confirming the successful purification of histones.*



	PUGNAc	GlucNH$_2$	OSMI
% of Control	136	114	80

Figure 4. *Glycosylation of H2B after treatment. Histones were isolated from T cells from healthy donors. Cells were treated to increase the levels of glycosylated proteins with PUGNAC (lane 2), GlucosNH$_2$ (lane 3), or to decrease OGT activity with OSMI (lane 4). All cells were stimulated after 1-hour treatment with PMA/Ionomycin for 4 hours. The chromatin-bound histones were purified from the nuclear fraction. Histones were subjected to SDS-PAGE gel electrophoresis followed by immunoblot with anti Glc-H2B antibody. The blot was stained with Ponceau protein stain to visualize proteins and confirm that similar amounts of sample were loaded.*

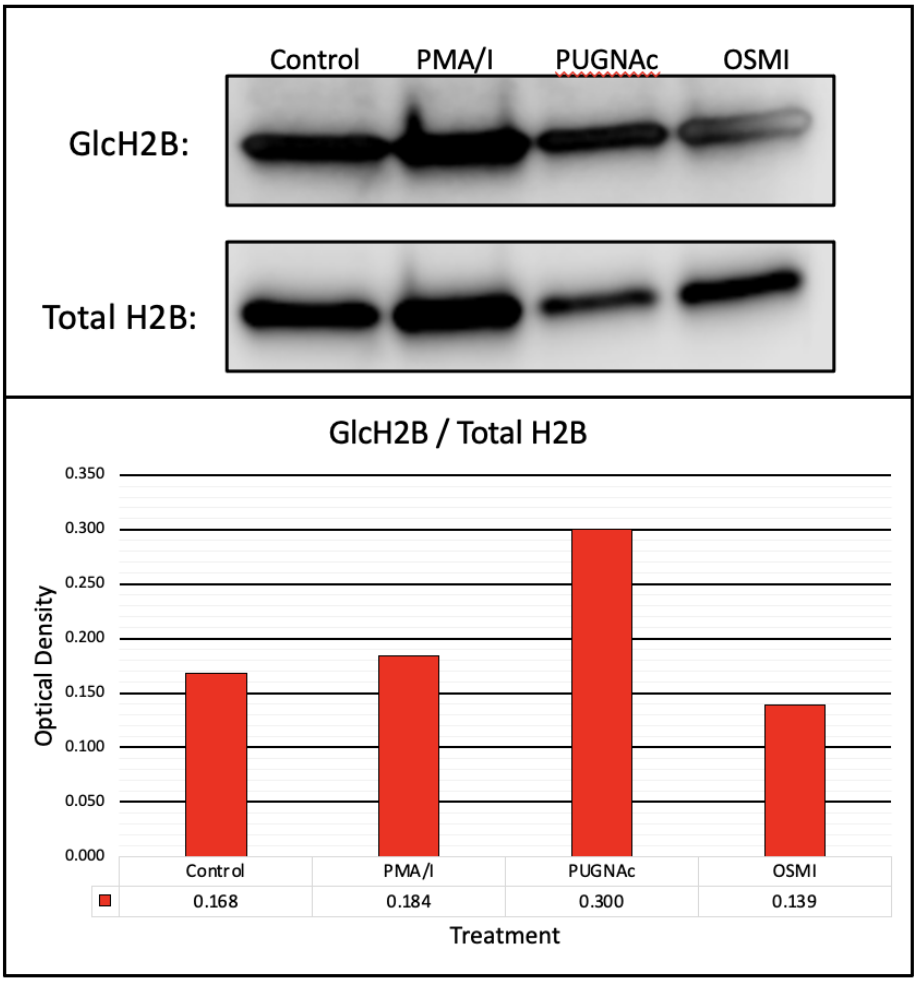


Figure 5. *<u>Glycosylation of H2B from nuclear extract.</u> Nuclear proteins were isolated from T cells of healthy donor. Cells were treated to increase O-GlcNAcylation of H2B with PUGNAc (lane 3), or to decrease OGT activity with OSMI (lane 4). Cells in Lanes 2 through 4 were stimulated after 1h treatment with PMA/Iono for 4h. Nuclear proteins were subjected to SDS-PAGE gel electrophoresis followed by immunoblot with anti Glc-H2B antibody. Total H2B protein was used as a loading control.*

***Lane 3 had less sample loaded than the other lanes.*

CHAPTER V

DISCUSSION

Current evidence suggests that the presence of two X chromosomes increases the risk of developing lupus in both humans and mice, relative to individuals with just one X chromosome. This is indicated by work showing that 90% of people who have lupus are women (Cooper 2003), and that men suffering from Klinefelter's (XXY) have a 14-fold increased risk of developing lupus compared to healthy men (Scofield 2008).

Previous work in my lab has shown that OGT is overexpressed in women with lupus. OGT was overexpressed in CD4+ T cells following induced DNA demethylation, as well as in CD4+ T cells from lupus patients. In lupus patients, the degree of overexpression was directly related to disease activity, and OGT mRNA and protein levels were significantly higher in women with lupus compared to men with lupus, suggesting a contribution from the inactive X (Hewagama 2013). This suggests that OGT overexpression could potentially contribute to immune dysregulation and autoimmunity in women.

Our results suggest that changes in OGT activity alter the O-GlcNAcylation level of histone H2B. When OGT activity was increased using PUGNAc or glucosamine, the proportion of O-GlcNAcylated H2B could be quantified as having increased, and when OGT activity was inhibited by OSMI, the relative proportion of O-GlcNAcylated H2B

increased. These changes in O-GlcNAc levels on H2B may alter intracellular in T cells of women with lupus, and could contribute to the increased rates of lupus seen in women when compared to men.

Future experiments would first focus on confirming these observed results. The primary weakness of this study is a simple lack of sufficient results. COVID-19 had a pretty significant impact on our ability to perform all of the experiments that we would have liked to. The lab was not accessible to students for the last half of the spring 2020 semester, and for the entirety of summer 2020. It was impossible to work with donors during a pandemic due to COVID restrictions, and without donor samples, our work is impossible.

Beyond this, additional future experiments would focus on comparing these results found in healthy individuals to those seen in the T cells of lupus patients in order to investigate whether these increased levels of O-GlcNAc on the histone H2B alter gene expression and intracellular signaling – if so, then we could investigate what kind of changes in expression that these modifications elicit. It would also be beneficial to analyze other pathways that could be affected by OGT activity, and their possible contribution to lupus pathogenesis. This work could help lead to development of new treatments for lupus that therapeutically target OGT activity.

CHAPTER VI

CONCLUSIONS

In conclusion, this project suggests that H2B is a molecular target of OGT in normal, healthy CD4+ T cells. In addition to this, these results suggest that the increase in OGT expression in lupus T cells causes increases in H2B glycosylation. OGT may modify chromatin structure, causing changes in gene expression, and may be an epigenetic modulator in lupus. OGT could also modify signal transduction by glycosylating other proteins, and may contribute to the female predisposition to develop lupus, as it is an X-linked gene. Further experiments are needed to confirm our hypothesis.

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REFERENCES

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APPENDICES

Appendix A

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INSTITUTIONAL REVIEW BOARD
June 16, 2020

IRB Number and Title:	[1036746-8] T cell signaling in health and disease
Reference Number:	17-103
Principal Investigator:	Gabriela Gorelik, Ph.D.
Next Report Due Date:	June 15, 2021

This project has transitioned to the HHS Final Rule dated July 19, 2018, provision 2) The allowance for no annual continuing review of certain categories of research

The annual Check-In has been completed to notify the IRB that the research remains ongoing. Stamped study related materials have been updated to remove the date of expiration

An annual notification will be automatically generated through IRBNet 60 and 30 days prior to the next Check-In. Failure to submit the annual Check-In form will result in the project being closed.

BIOGRAPHICAL SKETCH

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