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Review

X marks the spot: Does it matter that *O*-GlcNAc Transferase is an X-linked gene?

Stéphanie Olivier-Van Stichelen, Lara K. Abramowitz, John A. Hanover *

Laboratory of Cellular and Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institute of Health, Bethesda, MD 20892, USA

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ABSTRACT

O-GlcNAcylation has emerged as a critical post-translational modification important for a wide array of cellular processes. This modification has been identified on a large pool of intracellular proteins that have wide-ranging roles, including transcriptional regulation, cell cycle progression, and signaling, among others. Interestingly, in mammals the single gene encoding *O*-GlcNAc Transferase (OGT) is located on the X-chromosome near the *Xist* locus suggesting that tight dosage regulation is necessary for normal development. Herein, we highlight the importance of *OGT* dosage and consider how its genomic location can contribute to a gender-specific increased risk for a number of diseases.

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Contents

1. *O*-GlcNAcylation: OGT as a nutrient sensor

O-GlcNAc cycling links sugar metabolism to post-translational modifications (PTM) that cause changes in protein functions [1]. Two complementary enzymes regulate this modification: *O*-GlcNAc Transferase (OGT), which adds *O*-GlcNAc onto serine and threonine residues of proteins, and *O*-GlcNAcase (OGA), which removes *O*-GlcNAc (Fig. 1). Thus, *O*-GlcNAcylation is a dynamic process, contrary to classical static *N*- and *O*-glycosylation [2,3]. Importantly, *O*-GlcNAcylation is directly modulated by extracellular glucose concentration. Indeed, 2–3% of glucose entering the cell is shuttled through the hexosamine biosynthetic pathway (HBP) whose ultimate product, UDP-GlcNAc, is the substrate of OGT [4]. In addition, because UDP-GlcNAc formation also requires contribution from other metabolic pathways, including those that generate nucleotides, amino acids and fatty acids, *O*-GlcNAcylation is poised to act as a nutrient sensor, regulating proteins involved in major signaling pathways in response to glucose variations [5] (Fig. 1).

Because this glycosylation is added to serine and/or threonine residues on nuclear, cytosolic and mitochondrial proteins, competition exists between *O*-GlcNAcylation and phosphorylation for the same or adjacent sites [6]. Unlike phosphorylation, which is managed by over 1000 kinases and 150 phosphatases [7,8], only two enzymes regulate *O*-GlcNAcylation. Consequently, in response to glucose flux, *O*-GlcNAcylation likely relays a more general signal to the proteome than phosphorylation, which targets particular proteins with specific kinases. Therefore, the interplay between protein *O*-GlcNAcylation and phosphorylation allows the cell to

* Corresponding author. Address: 9000 Rockville pike, Bd8, Room B127, Bethesda, MD 20892, USA.
E-mail address: jah@helix.nih.gov (J.A. Hanover).

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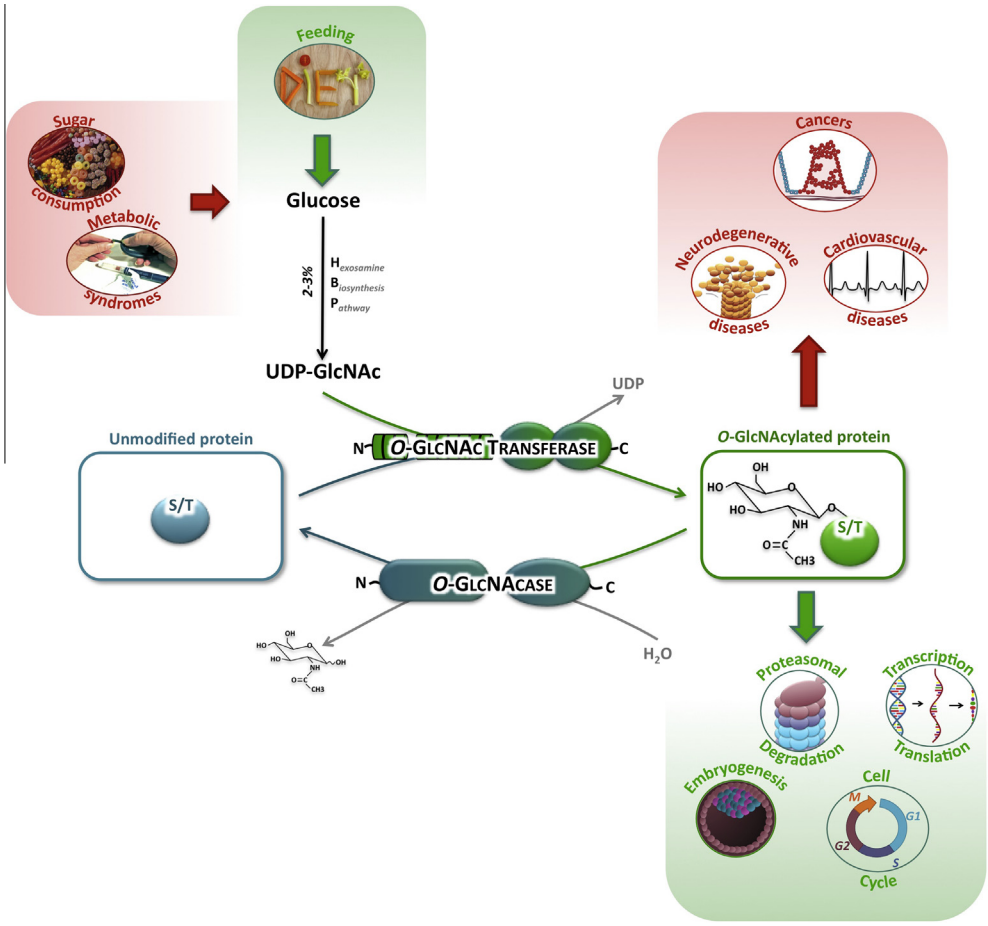

Fig. 1. *O*-GlcNAc cycling: a link between glucose input and physiological/pathological processes. Glucose input is processed partly (2–3%) by the hexosamine biosynthetic pathway, to provide UDP-GlcNAc. This nucleotide sugar is used by *O*-GlcNAc Transferase (OGT) to add a single N-acetylglucosamine onto serine and threonine residues. *O*-GlcNAcase (OGA) removes this modification, yielding a dynamic process. *O*-GlcNAcylated proteins are numerous and varied and impact physiological processes like proteasomal degradation, transcription, translation, cell cycle progression and embryogenesis (outlined in green). Increasing extracellular glucose concentration directly impacts intracellular *O*-GlcNAcylation and may trigger or worsen pathologies such as cancers, cardiovascular and neurodegenerative diseases (outlined in red).

interpret environmental cues and coordinate the appropriate cellular response.

Identified *O*-GlcNAcylated proteins (~4000) have a wide array of functions, from structural proteins to transcription factors to OGT and OGA themselves [9–11]. Hence, the consequences of *O*-GlcNAcylation are likely broad, ranging from conformational changes and altered partner interactions, to changes in protein half-life, sub-cellular localization or activity. By modification of a large number of proteins, *O*-GlcNAcylation is able to alter transcription, translation or proteasomal degradation, each of which are known to regulate complex processes such as the cell signaling and embryonic development [12]. Importantly, aberrant *O*-GlcNAcylation is involved in pathologies including neurodegeneration, cardiovascular disease, type II diabetes and cancer development [13–16]. In these cases, deregulation of nutrition could impact protein modification and exacerbate disease development [17,18] (Fig. 1).

Overall, *O*-GlcNAcylation is a good candidate linking environmental factors, like nutrition, to signaling pathway regulation or deregulation. Modifying intracellular OGT levels impacts *O*-GlcNAc cycling in cells [19]. Accordingly, it is imperative to understand the mechanisms underlying normal OGT regulation in order to gain insights into the consequences of *O*-GlcNAc deregulation. Due to its location on the X-chromosome the gene encoding OGT is subject to complex mechanisms of dosage compensation in females [20], implicating that tight control of OGT dosage is necessary for normal health.

2. Mammalian sex-chromosome dosage compensation

In mammals, sex is determined by the presence of a Y chromosome. Whereas females have two X chromosomes, males have an X and Y chromosome. Thus, Dosage compensation mechanisms are necessary in order to balance transcription from the X-chromosome between males and females. This process for dosage compensation in mammals is called X-inactivation, and is defined by silencing of one of the two X-chromosomes in females [21]. X-chromosome inactivation occurs during early embryogenesis, however, this process occurs differently for extraembryonic *vs* embryonic tissues. In the trophectoderm, which gives rise to extraembryonic tissues, the paternally inherited X-chromosome (Xp) is silenced (imprinted X-inactivation, iXCI) [22]. Within the inner cell mass (ICM), from which the embryo is derived, cells undergo random X-inactivation (rXCI), where about half the cells silence the Xp and the other half silence the maternally-inherited X-chromosome (Xm) [23].

The X-inactivation process is divided into three steps: (1) counting and selection of inactive X (Xi)/active X (Xa) chromosome(s); (2) inactivation, including coating of the Xi by *XIST* RNA and transcriptional repressors; and (3) maintenance of Xi status through long-term modifications of DNA and histones (Fig. 2). The initiation of X-inactivation is achieved by the counting of X-chromosome(s) present in the cell. Only one X has to be active and, as a consequence, each extra X-chromosome is subjected to X-inactivation [24,25]. During this step, the cell also selects the

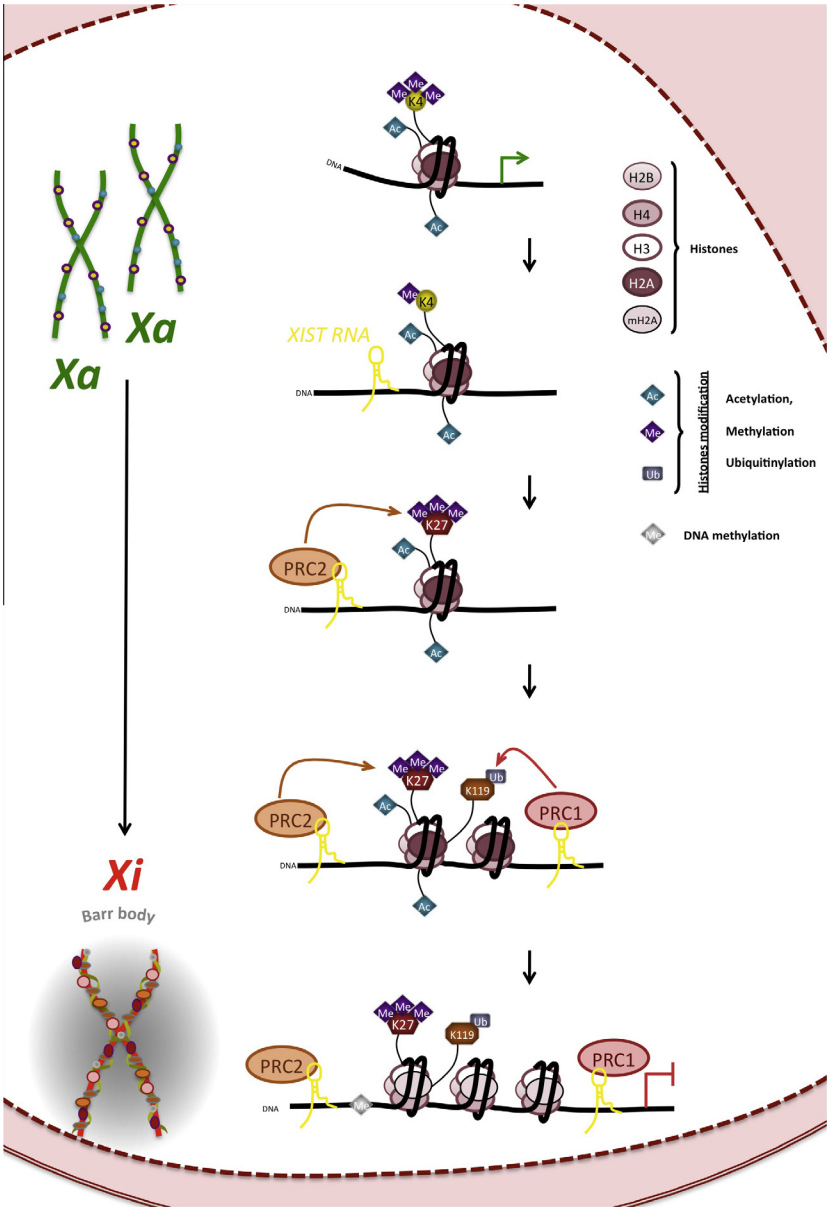

Fig. 2. Mechanism of X-inactivation. In the early blastocyst both active X-chromosomes (Xa) have active histone modifications, like H3K4me3 and acetylated H4 and H2B. *XIST* lncRNA is synthesized by the future inactivate X-chromosome (Xi) and coats the entire chromosome in cis, creating a docking platform for Polycomb group-Related Complexes (PRC1 and PRC2). PRC2 and PRC1 modify the histone core by adding repressive histone modifications H3K27me3 and H2Ak119Ub, respectively. Finally, promoters of X-linked genes are methylated to maintain X-inactivation and the Xi is relocated in a peri-nuclear structure called the Barr body.

X-chromosome to silence. This choice is skewed toward the paternal chromosome in iXCI but is random in rXCI.

Silencing of the chromosome is dependent upon a cis-acting regulatory element known as the X-inactivation center (XIC), which in humans has been mapped to Xq13 [26]. The XIC synthesizes noncoding RNAs (ncRNAs), such as *XIST*, which coordinates silencing of most genes on the Xi. At the onset of the X-inactivation process, the XIC of each X-chromosome expresses *XIST* ncRNA at low levels [27]. During the counting/choice step, there are increases in both Xi *XIST* RNA expression and DNA methylation of the *XIST* ncRNA promoter on Xa [28]. This ncRNA coats the entire Xi by interacting with Long Interspersed Elements (LINEs) [29,30]. Next, *XIST* ncRNA recruits the polycomb group protein (PcG) complexes, PRC1 and PRC2, which deposit repressive histone modifications that act to silence transcription. First, the PRC2 complex tri-methylates lysine 27 of histone 3 (H3K27me3) [31], followed by ubiquitination of lysine 119 of histone 2A (H2AK119Ub) by PRC1 [32].

To maintain the X-inactivated state, histones 3 and 4 of the Xi are hypoacetylated [33] and histone 2A is replaced by macroH2A [34], which is known to inhibit both the binding of transcription factors and histone acetylation [35,36]. Furthermore, DNA methylation is required to durably silence these X-linked genes [37].

3. X-Chromosome inactivation: imprinted (iXCI) *vs* random (rXCI)

At zygotic gene activation repetitive elements on the Xp are already silenced, similar to their state within the paternal germline [38,39]. Although genes on the Xp are initially active, at around the 4-cell stage transcription from the Xp is progressively inactivated and remains repressed in extraembryonic tissues (iXCI) [22,40,41]. Thus, within the tissues that interact with the maternal

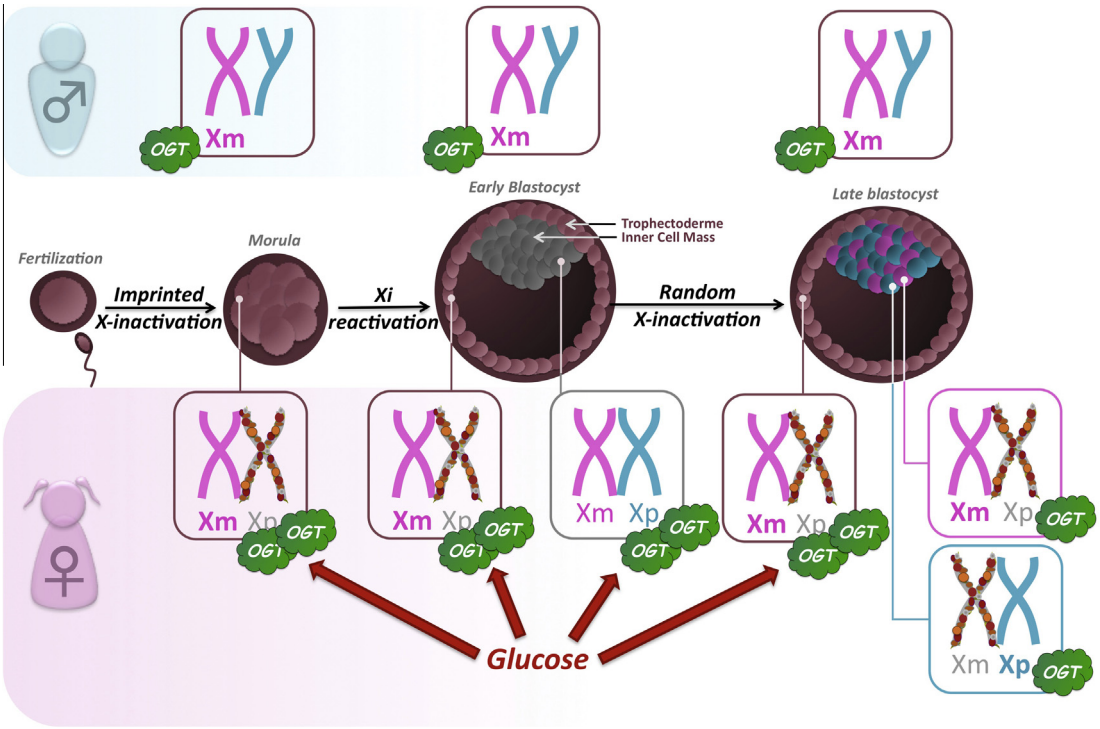

Fig. 3. Male *vs* female embryogenesis and X-inactivation. From the 4-cells stage, female embryos undergo imprinted X-inactivation (iXCI), preferentially silencing the paternal-X (Xp), which is maintained in extraembryonic tissues. Cells derived from the inner cell mass (ICM) reactivate their Xp and trigger random X-inactivation (rXCI), silencing either the paternal or maternal X-chromosome (Xm). The dosage of OGT (green cloud) is different in males *vs* females as well as between extra-embryonic tissue *vs* inner cell mass in females. Differences in OGT dosage could explain embryonic sex-specific sensitivity to maternal glucose level.

environment, like the placenta, transcripts come predominantly from the Xm. Nevertheless, a re-emergence of Xp transcripts appears in the ICM [42]. At the blastocyst stage, the ICM possesses two active X-chromosomes that later undergo random X-chromosome inactivation (rXCI) [23] (Fig. 3).

Despite the fact that iXCI and rXCI may be initiated differently [43], both rely on the Xi [26], expression of *XIST* and heterochromatization of the Xi [44]. For rXCI the choice of Xi is made randomly, between maternal and paternal X-chromosomes. Thus, after reactivation of the two X-chromosomes in the ICM, *XIST* will coat either the paternal or the maternal X, causing silencing [23] (Fig. 3). Each daughter cell maintains the same pattern of Xi (paternal or maternal), which causes females to be mosaic in their X-inactivation patterns [21]. Nevertheless, a skewed choice may appear when mutations are present on one of the X-chromosomes, which confers advantages to females *vs* males by allowing silencing of deleterious mutations [45,46].

Despite X-inactivation, in humans around 15% of genes on the Xi remain active [47]. Therefore, some genes are biallelically expressed in females [48], highlighting the importance of a second X-chromosome. Indeed, females with only one X-chromosome have Turner syndrome and exhibit a number of defects [49,50]. These observations showcase the requirement of a second X-chromosome despite being mostly inactivated in females. The fragile balance between single or double copies of X-linked genes is crucial for female biology, but is not yet fully understood. Knowing that OGT is an X-linked nutrient sensor whose dosage is important for human health and disease, the question arises as to *OGT's* X-inactivation status in extraembryonic and embryonically-derived adult tissues, and how this can have effects on human disease in a sex-dependent manner.

4. OGT dosage in extraembryonic tissues

As demonstrated by Shafi et al., OGT is essential for embryonic development. Knock-out of *Ogt* causes lethality, with mouse embryos dying around 4.5 days post coitus (blastocyst) [20], suggesting that OGT is required during pre-implantation development. Intriguingly, heterozygous *Ogt* knock-out mice are viable when the mutant allele is paternally inherited, whereas maternal inheritance of the mutant allele is embryonic lethal [19]. Based on these observations, it has been proposed that OGT is required in preimplantation development and in extraembryonic tissues, where the Xp is silenced. Thus, in mice that have paternally inherited the truncated *Ogt* allele, normal *Ogt* expression can occur from the Xm throughout early embryogenesis and in extraembryonic tissues. Surprising though, more recent studies have shown that *Ogt* expression does not undergo normal iXCI in extraembryonic tissues. Indeed, a number of recent studies have identified *Ogt* as an iXCI-escaping gene in mouse trophoblast stem cells [51–54], suggesting that *Ogt* is never subjected to iXCI in extraembryonic tissues. Accordingly, biallelic expression of *Ogt* is detected in female mouse trophobast stem cells [51,53]. Thus, female placentas have higher levels of OGT and *O*-GlcNAcylated proteins than male placentas [55] (Fig. 3). Although the transcriptional status of *Ogt* remains unknown in early development, these recent studies indicate that the lethality observed in mice that inherited a mutant Xm [19] resulted from decreased *Ogt* expression during preimplantation development rather than decreased expression in extraembryonic tissues.

Because the placenta is the interface between mothers and embryos, it is the tissue that directly interacts with the maternal environment to protect the developing fetus from outside

influences. As a nutrient sensor, the differential dosage of OGT between male and female placentas can cause an *O*-GlcNAcylation-dependent sex-specific sensitivity to the intrauterine environment, having consequences later in life.

5. Interaction of the intrauterine environment and OGT

Maternal stress and nutrition are risk factors for diseases including neurodevelopmental and metabolic disorders [56–58]. Interestingly, aberrant *O*-GlcNAcylation and sex-specific risks have been identified for these disorders [13,15,59]. Therefore, sex differences in expression of *Ogt* in the placenta could be a key component contributing to these adult diseases. In a study investigating changes in gene expression in a mouse model of early prenatal stress (where mothers are subjected to stress during early gestation), *Ogt* was identified as being significantly downregulated in the placenta [55]. Importantly, decreased expression of *Ogt* specifically in the placenta resulted in robust gene expression changes in the hypothalamus. These data suggest that placental *Ogt* expression could have consequences in the presentation of neurodevelopmental diseases [55].

Diet is a key component of pregnancy affecting embryonic development and susceptibility to disease. Indeed, maternal malnutrition is a key influence in some adult-onset diseases such as metabolic disorders like obesity and type II diabetes (T2D) [57,58]. T2D has been linked to *O*-GlcNAcylation, as proteins involved in insulin signaling are largely modified by this PTM [15]. Subsequently, *O*-GlcNAcylation is a good candidate to link maternal diet to affects on offspring. Indeed, as a consequence of either maternal low fat diet that is high in carbohydrates or a very high fat maternal diet, a greater increase in *Ogt* expression was detected in female *vs* male placentas in mice [60]. This study further implicates the contribution of sex-dependent differences in placental *Ogt* expression for a sex-biased risk in disease. Similarly, as *O*-GlcNAcylation is involved in type-2 diabetes, it is not surprising that female rodents who had experienced malnutrition, or defects in glucose homeostasis during perinatal development, had increased incidences of metabolic syndrome [61,62]. Moreover, maternal exposure to high sucrose during pregnancy caused glucose homeostasis defects in female but not male offspring in mice [63]. While it should be noted that diet during pregnancy does have effects on offspring of both genders [64], female and male mouse preimplantation embryos still have around 600 differentially expressed transcripts that could potentially explain the sex-specific sensitivity to maternal diet [65]. Therefore, further investigation into the role that placental OGT dosage plays in the increased risk for metabolic syndrome in females exposed to intrauterine malnutrition is warranted (Fig. 3).

6. rXCI and OGT dosage

Although *Ogt* escapes iXCI, it is silenced on the Xi during rXCI. Due to its location near *Xist, Ogt* appears to be under tight transcriptional control in mice. Prior to widespread X-inactivation and upregulation of *Xist,* most X-linked genes are biallelically expressed in mouse embryonic stem cells. However, allelic analysis in these cells indicated that *Ogt* expression was monoallelic, suggesting that due to its close proximity to *Xist*, *Ogt* can be repressed prior to *Xist* upregulation [66]. Furthermore, a comparison between X-inked gene expression in male and female adult human tissues demonstrated that *OGT* is expressed equally between males and females, except in the adrenals where *OGT* expression was higher for females [67]. Taken together, these studies indicate that the gene encoding OGT in mammals undergoes random X-inactivation in most tissues.

7. Implications for OGT and *O*-GlcNAcylation's involvement in X-inactivation

Given that OGT is an epigenetic regulator [68], the question arises as to whether OGT has a role in X-inactivation, and thus is involved in a self-regulatory loop. Most interesting is the relationship between OGT and the PcG proteins, which, as previously mentioned, are necessary for proper X-inactivation [31,32]. Intriguingly, OGT is encoded by the PcG gene super-sex comb (sxc) in *Drosophila* [69]. Furthermore, the PRC1-equivalent in *Drosophila*, Ph, is *O*-GlcNAcylated [70]. However, *Drosophila* dosage compensation is radically different than mammals. Compensation in *Drosophila* is accomplished though upregulation of genes on the male's single X-chromosome to equal that of the female's two X-chromosomes [71]. Nevertheless, a relationship between PcG and OGT has also been found in mammals [72,73]. Indeed, OGT interaction with, and *O*-GlcNAcylation of, EZH2, a component of PRC2, stabilizes the protein. Additionally, depletion of OGT led to a decrease of H3K27me3 levels [73]. More generally, the histone core can also be *O*-GlcNAcylated (H2A, H2B, H3 and H4) [74], potentially contributing to heterochromatization of the DNA. Further studies are necessary to elucidate the potential role of *O*-GlcNAcylation in mammalian X-inactivation.

8. Consequences of X-linked gene biallelic expression

Because X-linked gene dosage is tightly regulated, females are at an increased risk for disease if the Xi is reactivated. In fact, a number of diseases, including autoimmune disease and cancers, have been associated with reactivation of the inactive X [75–79]. As a regulator of a diverse panel of cellular processes, OGT overexpression is a candidate causative factor for the progression of these diseases.

Systemic lupus erythematosus (SLE) is an autoimmune disease that predominantly affects woman. In fact, 90% of SLE patients are women [80]. It has been hypothesized that the second X chromosome is a factor for the increased risk of SLE in woman. This hypothesis is supported by the fact that in mice 2 X-chromosomes were required for disease development in a model of SLE [81], as well as the fact that an increased incidence of SLE has been reported in Klinefelter's syndrome men (who have an XXY karyotype) [82]. Altered DNA methylation patterns have been reported in SLE patients [83,84], including hypomethylation of X-linked genes [85]. It has been reported that *OGT* is reactivated from the Xi during SLE progression due in large part to DNA demethylation [78]. This reactivation causes increased levels of *O*-GlcNAcylation that may worsen or even trigger SLE development [78].

Two active X-chromosomes are often observed in female cancers [75–77]. Accordingly, deletion of *Xist* in mouse adult hematopoietic stem cells led to reactivation of the Xi in females. This reactivation resulted in aggressive and lethal blood cancer [79]. These observations suggest that *Xist* acts as a tumor suppressor by downregulating oncogenes on the X-chromosome in females. Similarly, Klinefelter's syndrome men have 20- to 50-fold increased risk to develop breast cancer [86,87]. Although reactivation of *OGT* from the Xi has not yet been identified in cancers, increased *O*-GlcNAcylation has recently been reported as a mechanism of breast cancer development, linking glucose metabolism and tumorigenesis [88]. Further studies investigating reactivation if *OGT* in cancer progression in females is necessary to provide insights into the mechanism by which reactivation of the Xi contributes to cancer development.

Interestingly, observations from patients with sex-chromosome abnormalities also implicate the potential that an increased dosage of OGT yields an increased risk for metabolic diseases. Indeed,

subsets of Turner syndrome patients have a heightened risk for type 2 diabetes depending on their genetic alteration. Compared to classic Turner syndrome XO females (who have a T2D rate of 17.5%), females who have an iso-Xq profile, meaning that they have two Xq arms and no Xp arm, have a much greater incidence of type 2 diabetes (40%) [49]. These Iso-Xq females have two *OGT* alleles on the same X-chromosome, and thus potentially overexpress *OGT*. However, further investigations are needed to determine if OGT levels are increased in these females and if this is the cause for the heightened incidences of type 2 diabetes.

9. Conclusions

As a nutrient sensor, OGT is uniquely poised to interpret environmental influences and coordinate the proper cellular response. Thus, regulation of OGT dosage is critically important to maintain cellular homeostasis. Due to its location on the X-chromosome, the gene encoding OGT in mammals is subject to complex mechanisms of dosage compensation in females, which potentially allows for differential regulation between the sexes. Having two *OGT* alleles puts females at an increased risk for a number of diseases. This increased risk could be a consequence of OGT's escape from iXCI in the placenta as well as the potential reactivation of the silenced allele in adult tissues. Therefore, further research into the sex-specific differences in normal OGT dosage in various tissues will help to elucidate mechanisms underlying sex biases in numerous diseases. Additionally, identification of factors involved in regulating OGT dosage could be potential therapeutic targets. Overall, it is important to consider *OGT* as an X-linked gene for future *O*-GlcNAcylation studies as female and male systems could interact differently to external factors.

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