X Chromosome Inactivation: The Great Escape

An Insight into the Effects of 'Escapee' Genes on the Sexual Dimorphism of the Human Viral Response

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Upon the entry of a pathogen into the human body, the immune response is our first line of defence. This protection mechanism has multiple ways in which to protect the body depending on the type of pathogen. When viruses enter the body, infected cells can 'warn' neighbouring cells of the viral intruder, allowing them to respond better should they become infected themselves. Although this response would be expected to be consistent between sexes, viruses appear to present differently in men and women. It is believed that a contributing factor to this is the number of X chromosomes present in the individual: females carry two X chromosomes, while males carry only one. For genetic balance to be achieved between sexes, female cells inactivate one X in each of their cells. However, not all the genes on the inactive X are silenced- some manage to escape and are subsequently expressed from both X chromosomes. Some of these 'escapees' contribute to the body's viral response and exhibit a strong female bias. One of these genes, known as Toll-like receptor 7 (*TLR7*), produces a 'viral detector' that induces the body's response upon contact with a virus. This research is of great value during the current COVID-19 pandemic. Due to their greater production of *TLR7*, it has been found that females induce a stronger viral response, allowing them better control of the disease. It has also been suggested that early induction of this response suppresses inflammation of the lungs, thereby reducing the risk of severe breathing difficulties in women. Further research into this phenomenon could help scientists identify a more personalised approach to treating not only COVID-19, but multiple viruses and other infectious diseases.

Introduction

The immune system is an intricate defence mechanism against potential infection consisting of white blood cells and multiple regulatory proteins. Interferon (IFN) responses are essential in controlling the replication of viruses, with mechanisms to 'warn' surrounding cells of the invading pathogen, however, like many of the body's complex processes, this response is regulated by cascades of reactions allowing for the ultimate release of these warning signals.

Despite the tight control of this response, many diseases, including that caused by the SARS-CoV-2 virus (Takahashi *et al.,* 2020), present themselves with clear sex biases, either being more prevalent or inducing greater severity in one sex over the other. In some cases, this may be due to lifestyle, concentrations of sex hormones, or the presence of one or two X chromosomes, as seen in the case of *TLR7*.

It is known that there exist multiple genes along the X chromosome that escape the dosage compensation process of X Chromosome Inactivation (XCI), but it has recently come to light that genes regulating responses like the IFN defence are also affected by this phenomenon. Study into this process could help researchers understand the effects of XCI escape observed within certain diseases, and use this to tailor their treatments.

X Chromosome inactivation and escape

XCI is a mammalian dosage-compensation mechanism whereby gene dosage balance of the X chromosome is achieved between the sexes, via the repression of one of the female X chromosomes.

The master regulator of this process is the long non-coding RNA, *Xist*, which becomes upregulated from the X inactivation centre of one of the X chromosomes in a random fashion (Loda & Heard, 2019). As *Xist* encompasses the DNA, it recruits epigenetic modifiers and chromatin re-modellers to ultimately form a tightly organised constitutive heterochromatic structure, known as a "Barr Body" (Figure 1). This entity is characterised by the depletion of RNA polymerase II, and multiple repressive epigenetic modifications including DNA hypermethylation, and histone modifications associated with gene silencing (Garieri *et al.,* 2018).

Despite the condensation of the inactive X (Xi), biallelic expression has been observed in approximately 15-25% of X-linked genes in females, suggesting that some of these genes escape the silencing process (Katsir & Linial, 2019). It has been shown that while many of the genes on the Xi are relocated to an area devoid of RNA polymerase II and other transcription factors, genetic 'escapees' remain on the periphery of this silenced compartment (Loda & Heard, 2019), continuing to be expressed from the otherwise inactive chromosome.

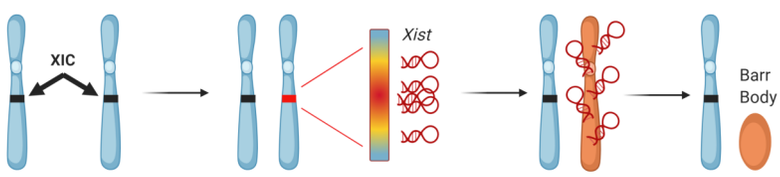

Figure 1. Formation of the Barr Body. The X Inactivation Centre (XIC) is randomly activated (Delaval *et al.,* 2007) on one chromosome. *Xist* is upregulated and gradually spreads over the chromosome, recruiting epigenetic modifiers to condense the DNA into the Barr Body.

Although this pattern of escape would be expected to be consistent, recent studies have shown that a degree of heterogeneity exists not only between individuals, but between tissues and cells of an individual. Perhaps the most surprising phenomenon is illustrated by Garrieri *et al.,* (2018) whereby allele-specific expression was investigated among fibroblasts taken from 5 female individuals. This team identified 60 escape genes, each exhibiting a varying expression profile between cells from the same individual. Different genes presented different levels of escape, with some being predominantly expressed from the active X (Xa) and others showing biallelic expression in >70% of the fibroblast sample. Interindividual inactivation profiles were also explored, revealing that the escape of the *DDX3X* gene (associated with cancer sex-bias) was highly variable among these women, suggesting that this could play a role in varying predispositions to cancer between individuals (Garieri *et al.,* 2018).

Sex biases arising from XCI escape

In line with having two X chromosomes rather than one, genes that escape XCI demonstrate a strong female bias. A 2017 study investigated the male-female differences brought about by XCI escape using RNA-seq data. Tukiainen *et al.,* (2017) found that, of 186 X chromosomal genes investigated, ~23% indicated incomplete XCI with 57% of these demonstrating variability of Xi expression between tissues, including a subset of genes appearing biallelic in just one of the 29 tissues assayed. Both *KAL1* and *CLIC2* illustrated biallelic expression specifically in the lungs and skin respectively, suggesting male-female biases in expression can arise directly from tissue-specific XCI escape, potentially influencing varying disease phenotypes between the sexes.

Conversely, genes within pseudo-autosomal region 1 (PAR1) were shown to have a consistent male bias (Tukiainen *et al.,* 2017). PARs are homologous regions along both the X and Y chromosomes, allowing for recombination between them during meiosis. It was previously believed that genes within PARs were not subject to XCI, however, Tukiainen *et al.,* (2017) demonstrated that, on average, PAR1 expression from Xi reached ~80% of Xa expression, indicating a spread of XCI beyond nonPARs, and presenting an incomplete balance of this region leading to subsequent male bias of these genes. The team ensured that these effects were from the direct influences of XCI, and not from any further regulation of the Y chromosome; there were no detections of up- or down- regulation of the PAR1 region of the Y.

Figure 2. Male-Female bias of genes on the X chromosome. The blue region represents male bias, while red represents female bias.

The rigorous investigation of 82 reported 'escapee' genes across 29 tissues illustrates an overwhelming female bias of multiple genes along the Xp arm, as well as a significant male bias of the PAR1 domain (Tukiainen *et al.,* 2017) (Figure 2).

The *TLR7* gene

Multiple genes linked with the immune response are located on the X chromosome. From the information presented above, it is hardly surprising that a number of infectious diseases present with different phenotypes between men and women.

Following the systematic analysis performed by Tukiainen *et al.,* (2017) a study was released describing the XCI escape of the *TLR7* gene, located along the Xp arm (Souyris *et al.,* 2018). *TLR7* forms part of the innate immune system capable of detecting single-stranded RNA, thus playing an essential role in antiviral responses via the induction of the type I interferon (IFN I) response and production of other inflammatory cytokines (Petes *et al.,* 2017). It was established that *TLR7* escapes XCI in primary immune cells including B cells, monocytes, and plasmacytoid dendritic cells (pDCs), presenting biallelic expression in a mosaic fashion (Souyris *et al.,* 2018). Further studies were performed to analyse the effect this had on immune responses between the sexes: it was found that the number of X chromosomes present was directly associated with pDC activation and the *TLR7*-mediated IFN response, with females indicating a significantly higher IFNα response from pDCs than males (Webb *et al.,* 2019). This higher production of interferons along with an enhanced ability to detect viral loads could potentially provide a critical immune advantage for women in the event of infection.

What could this mean for COVID-19?

Throughout the SARS-CoV-2 pandemic, it has become increasingly apparent that COVID-19 induces greater disease severity among men than women (Takahashi *et al.,* 2020). Although this is a novel virus, its sexual dimorphism is being studied continuously to enhance our understanding of how this disease behaves within the population.

Recent papers have described sex differences in the IFN response amongst pDCs, specifically analysing the effects of *TLR7* XCI escape. Cells exhibiting biallelic expression of *TLR7* have been shown to induce significantly higher levels of IFNα and IFNβ (Hagen *et al.,* 2020). This cascade of events has proved to be essential in the control of SARS-CoV-2: loss of function mutations within *TLR7* were reported in four young men with no pre-existing or underlying health conditions, and yet the progression of the disease was reported as severe, with three requiring mechanical ventilation and one succumbing to the disease (Van Der Made *et al.,* 2020). Studies investigating the related virus, MERS-CoV, identified *TLR7* as a critical player in the production of IFNs after the infection of lung epithelial cells, as well as finding that early IFN induction suppresses proinflammatory cytokine and chemokine

production in the lungs (Channappanavar *et al.,* 2019) thereby reducing the severe inflammatory phenotype observed in coronavirus patients. Following this premise, XX pDCs exhibiting biallelic *TLR7* expression may partially explain the seemingly better control of COVID-19 infection observed in women.

Further enhancing this evidence, a paper analysing the sexual dimorphism of COVID-19 found that, although viral load was equal between sexes, men exhibited higher levels of the proinflammatory cytokines, IL-8 and IL-18 (Takahashi *et al.,* 2020). The evidence from Channappanavar *et al.,* (2019) is therefore consistent with the data presented here: the higher concentration of IL-8/18 renders males more susceptible to severe inflammatory responses in the lungs than females.

The future of COVID-19 treatment

The extent of immune effects caused by XCI escape remains to be explored, but expanding on the knowledge of particular genes like *TLR7* may help scientists to further understand the clear sex bias presented by the SARS-CoV-2 virus, potentially providing a different approach to how this virus should be treated. With female genes escaping XCI in a heterogenous fashion between individuals, there may be an advantage in using a more personalised approach to treatment by examining the expression levels of particular genes. The study of sexual dimorphism observed in disease has the potential to enhance treatment not only for COVID-19, but for multiple infectious diseases, and any future pandemics yet to come.

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