The pattern of X-inactivation in Human X-linked Disorders and their Model Organisms: A literature review

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Abstract. X-inactivation is a strategy in female mammals aiming at maximizing gene inactivation of one single X chromosome in order to balance X dosage between males and females. Various human X-linked disorders have been reported related to one or more X-inactivation patterns. It is necessary to understand their relationship to study X-linked disorders. Current researches largely rely on clinical phenotype research and model organism. Considering the differences between human and model organisms, it is vital to find a suitable model of a specific disorder. The paper reviews different patterns and specific disorders linked with X-inactivation. Also, the advantages and disadvantages of applying specific model organisms in different disorders will be discussed.

1 Introduction

X-inactivation is a dosage-compensation strategy in female mammals, aiming at eliminating differences in phenotypic dosages between female and male. This strategy could randomly inactivate one of the parental X chromosomes to ensure the same amount of X chromosome expression among male and female mammals. Long non-coding (lnc) RNA is required to inactivate X-chromosome, which is encoded by a gene called X-inactive specific transcript (XIST) gene. Inactivated X chromosomes are coated with lnc RNAs and are condensed to form Barr Bodies. X-inactivation takes place in the early phase of embryonic development, a process that will be inherited to all daughter cells. Random strategy and inheritability during mitosis are combined to form the mosaic pattern in heterozygous, which is a phenomenon that is evident in female calico cats coated in tortoiseshell color [16].

Various X-inactivation patterns have been found related to multiple human X-linked disorders. The first pattern to be reviewed in this article is pseudoautosomal gene, which is a group of genes that are able to escape from X-inactivation because of their presence on X and Y chromosomes without the need for dosage compensation. Either short of or overexpression of pseudoautosomal genes could lead to pathogenic phenotypes, a major cause of symptoms in X chromosomes aneuploidy[3, 24]. Next, the paper reviews the cellular hypothesis, a term coined by Wieland et al. in 2004[34]. It plays a vital role in certain genetic disorders due to its heterogenic pathogenesis. The paper then reviews X-linked recessive disorders for its mosaic pattern of X-inactivation. Interestingly, variants of symptoms can take place in carriers of X-linked recessive disorders with the same genome sequences regardless of the lifespan of an individual. Skewed X-inactivation could

eventually lead to imbalance of active paternal and maternal X chromosomes. Thus, various patterns of inactivation contribute to different phenotypes, including pathogenesis. Mosaic pattern in heterozygous could be problems of some disorders, whereas homozygous or hemizygous with paternal or maternal X chromosome would be asymptomatic.

Just like various X-inactivation patterns in human disorders, inactivation patterns can take place in mammals. The paper focused on two model organisms, namely marsupial and mouse. Marsupial is considered as a model organism with a X-inactivation pattern drastically different from human while mouse is regarded as the most commonly used model organism. The advantages and disadvantages of using them as model organisms to study X-inactivation patterns will be further discussed.

In this paper, various X-inactivation patterns and how they are linked with human disorders will be reviewed. Also, the differences of X-inactivation patterns between human and other mammals as well as the possible disadvantages of using other mammals (mice, marsupials, etc.) as model organisms will be discussed.

2 Different X-inactivation Patterns Are Related with Human Disorders

2.1 Pseudoautosomal genes and Aneuploidies

By applying X-inactivation, there should be only one active X chromosome in female mammals. However, about 20%-30% of genes in human are believed to be able to escape from X-inactivation[1]. Among these genes, a group of genes are present on both X and Y chromosomes as homologous, thus no dosage compensation for these genes is needed (see figure 1).

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Figure 1. Chromosome pair 23 with escaping genes and pseudoautosomal genes in normal female, male, female with Turner Syndrome and Klinefelter Syndrome.

These genes are termed as pseudoautosomal genes. Most of them have no Y homologous, leading to higher female than male expression [31]. Most of the pseudoautosomal genes are believed to be involved in the establishment and maintenance of sex-specific differences and dosage balance of chromatin [10]. They behave like autosomal genes and are recombined during meiosis. There are two pseudoautosomal regions (PAR1 and PAR2), PAR1 is thought to be important in fertility, whereas only four genes are found in PAR2 have known functions [14]. Still, there are some isolated pseudoautosomal genes, such as some promoters are reported to have various X-inactivation status, too [12]. Expression of both copies are vital for normal phenotypes, and the lack of one X could possibly contribute to aneuploidy X-linked symptoms, such as Turner syndrome (TS) and Klinefelter syndrome (KS).

TS is caused by a lack of one copy of X chromosomes (45, XO) in the females. Female embryos carried with only one copy of X chromosome would usually die early during development, but survived ones have symptoms of webbed neck, short stature and infertility. Multiple genes in these two regions are found to be correlated with X-linked aneuploidy disorders. Researchers have suggested that the pseudoautosomal deletions of one gene among homeobox named as SHOX (short stature homeobox-containing) gene, which resides in PAR1, would cause the growth failure in TS [24]. Despite growth failure, this deletion also causes skeletal abnormalities and symptoms of Leri-Weill dyschondrosteosis [18]. Many pseudoautosomal genes are transcriptional or chromatin regulators [2], thus improper expression number of these genes can cause a huge effect. Histone H3 lysine 27 (H3K27) demethylase has been found downregulated in TS, whose encoding gene is located on PAR of X chromosome, which is called UTX (ubiquitously transcribed tetratricopeptide repeat). In both mice and human, haploinsufficient of UTX leads

to the deficiency of T follicular helper cell [29]. Many other genes on PAR that have been reported are possibly correlated with TS symptoms. For instance, KDM6A has been suggested that it could have correlations with hyperinsulinism in TS, because the prevalence of hyperinsulinism is quite high among TS children. Inhibition of KDM6A, a gene which is associated with hyperinsulinism in Kabuki syndrome, has been reported that it can reproduce TS-like hyperinsulinism in mice [19]. These facts could be evidence for that KDM6A is probably responsible for hyperinsulinism in TS infants [13]. However, a conflicting result has shown by comparing with genome-wide RNA expression, that KDM6A has the same expression levels in TS patients and normal females [30]. Although, interestingly, this researcher has also demonstrated different methylation status of KDM6A compared with TS (45, XO) and 46, XX [30]. Based on these research, abnormal methylation status of KDM6A could be the reason of hyperinsulinism in TS patients. Revealing the mechanism of how genes specifically escape from X-inactivation is vital for understanding how pseudoautosomal genes contribute to TS symptoms, however the mechanism is not clear yet. RPS4X is a gene encoding for S4 ribosomal protein, which resides in premature ovarian failure region II (POF2) on long arm of X chromosome (Xq). Whether Xq is involved in TS symptoms or not is still conflicting, since no excess symptoms were found in isochromosome Xq compared with 45,X [5]. Still it could be a model of escaping, which provides escaping principle of other pseudoautosomal genes. X-inactivation of human RPS4X can happen in knock-in mouse, which should normally be silenced in mice. A previous research has suggested that RPS4X gene must contain a recognizable region to be specifically inactivated [23].

KS is caused by improper nondisjunction during maternal or paternal meiosis 1, which induces one extra X chromosome in males eventually (47, XXY). Most of the symptoms could be termed as feminization, including breast development, small testes, poor beard growth and so on. Also, patients can have low blood level of testosterone (TR), whereas the blood level of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are higher. Unlike TS, as the majority of its symptoms are suggested to be caused by hypoeexpression of pseudoautosomal genes, correlation of X-inactivation and symptoms of KS is more complicated. Usually, mammals with more than one X chromosome inactivate all but one X chromosome, so only one copy of X chromosomes is expressed. This strategy is thought to happen in KS patients as well [7], except for pseudoautosomal genes, which continually express from both X chromosomes. However, it has been suggested that the morbidity of some cancers is higher in KS than normal males, such as breast cancer [28]. Researchers indicated that the overexpression of double X chromosomes in KS-linked cancers is not achieved by reactivation of an inactive X chromosome, but by multiplication of the active one following loss of the inactive one [17]. Despite KS-linked cancers, a recent study suggested that the differentially expressed non-coding RNA (ncRNA) which is located close to X-inactivation center (XIC) and escape genes, and indicated the possible regulation of X-inactivation status in KS [27]. Despite possibly altered X-

inactivation status in KS, overexpression of genes escaping from X-inactivation, especially those who reside in PAR, are hypothesized to be involved in KS symptoms. For instance, SHOX is not only involved in the short stature and growth abnormalities of TS, but also in the tall stature in KS[20].

2.2 Cellular interference hypothesis

Some human disorders, for example, the Epilepsy, only appears in X chromosome heterozygous, whereas homozygous of neither allele would be asymptomatic. The mechanism is not clear yet, but a hypothesis suggests it could be the interference between two different cell populations which leads to a gain-of-function at tissue level. This hypothesis is termed as cellular interference hypothesis [39]. Epilepsy and mental retardation (EFMR) is a disorder limited to female, which leads to symptoms of epileptic seizure with physical pains [21]. The causes of most EFMR cases are not clear, only a small proportion of them are caused by the mutation on protocadherin 19 (PCDH19) gene, which is located on X chromosome [8]. PCDH19 is a protein for cell-cell adhesion involved in neuronal development. Only heterozygous of mutation in females can cause symptoms, whereas hemizygous of mutation in males or homozygous of mutation in females is asymptomatic, which indicates that the constitutive loss of function of PCDH19 is not the pathogenesis. Possible guess of heterozygotic pathogenesis in EFMR is the alteration of cell-cell interaction between mutated and wild-type cells, which causes gain-of function at the cell level, termed as cellular interference hypothesis. The discovery of mosaic pattern in fibroblasts of male carriers is also a solid support of this hypothesis [6].

2.3 X-linked recessive disorders variants

X-inactivation of parental allele is randomly happened during eight-cell stage and can be inherited to all daughter cells during mitosis, which will lead to mosaic pattern in female mammals (see figure 2). Mosaic pattern can cause various symptoms of X-linked recessive disorder. So far, both distinct phenotypes of monozygotic twins of Menkes Disease and recurrence of Epilepsy in carriers have been reported [4, 9].

Figure 2. Random X inactivation results in different expressions of X chromosome.

Research has shown that some X-linked recessive disorders, such as epilepsy and mental retardation, cause more symptoms as the male carriers grow older, instead of staying asymptomatic [9], which indicates the dynamic expression of mutated genes. EFMR has a unique reverse pattern in which male hemizygous of mutated PCDH19 carriers develop symptoms from seizures to cognitive impairment; however, based on cellular hypothesis of epilepsy[39], only heterozygous of mutation has symptoms. According to literature, parents with gonadal mosaicism pattern of PCDH19, who already have one affected daughter, undergo the risk of having another affected daughter or transmitting son [9]. Several other X-linked disorders have been found to have correlations with mosaic pattern of X-inactivation. X-linked adrenoleukodystrophy (ALD), the most common peroxisomal disease caused by mutated gene ABCD1, encodes for the peroxisomal transporter of very long-chain fatty acids. According to a study, X-linked ALD carriers develop neurological symptoms of myelopathy and/or peripheral neuropathy [11]. Furthermore, the incidence of female carriers with symptoms increases significantly with age, and the levels of very long-chain fatty acids have been found elevated in fibroblasts [11]. However, although this study has suggested the X-inactivation status is correlated with pathogenesis of fibroblasts, it did not find any correlations between X-inactivation and symptoms [11]. But this situation could be attributed to the limitations of the study, for instance, it had insufficient samples of ALD carriers. As other X-linked recurrent disorders suggest, dynamic X-inactivation status in this disorder could also be induced by gonadal mosaicism. The incidence of mosaicism is relatively higher in disorders, including a large proportion of mutated genes, such as Duchenne Muscular dystrophy (DMD). DMD is an X-linked recessive genetic disorder caused by the fragile protein called dystrophin and characterized by weakened and degenerated muscles. As an X-linked recessive disorder,

DMD carriers of heterozygous mutation usually have no symptoms. However, in some rare cases, they can also develop symptoms. Disorders have the same reason of recurrence risk, including neurofibrosis type 1 [15], Dravet syndrome [7] and so on.

2.4 Skewed X-inactivation

Although X-inactivation is expected to be a random case, it has been reported that preferential inactivation commonly occurs in female population (nearly 50%). More than 80% of cells show a preferential inactivation of X chromosome, which is termed as skewed X-inactivation. Mechanism behind the skewed X-inactivation is unclear, but two possibilities have been raised to explain this so-called skewed X-inactivation, either by chance or based on the physical characteristics of each allele. Since X-inactivation starts early during the embryonic development when there is only a limited number of cells and the status of X-inactivation would be inherited to all daughter cells, it could be likely to cause imbalanced prevalence of parental alleles eventually. Negative selection of cells carried with X chromosomes with lethal or growth-limited mutations can lead to apoptosis or a slow growth, which leads to the different ratio between paternal and maternal alleles.

To support the random nature of X-inactivation, a study of mother and daughter X-inactivation status has suggested that there is no correlation of X-inactivation between two generations [26]. Furthermore, X-linked recessive carriers with same genome sequences (monozygotic twins) can have distinct phenotypes due to distinct choices of X-inactivation. Menkes Disease (MD) is an X-linked neurodegenerative disorder induced by a mutated copper transporter and encoded by ATP7A gene on X chromosome. A study of a couple of monozygotic twins who are carriers of MD has shown the distinct phenotypes of these two sisters [4]. According to this study, one of the twin sisters has no symptoms at all, whereas the other one has significant symptoms of MD. The causal reason of the variant is the different X-inactivation status in this pair of monozygotic twins, as one of them preferentially inactivated the mutated X chromosome, the other one preferentially inactivated the normal X chromosome. However, the mechanism of distinct selections of X-inactivation in this disorder is not yet clear, so more similar monozygotic experiments are needed for further investigation. Skewed X-inactivation has also been reported to be related to phenotypic differences in ALD [32]. This study reported a high frequency of skewed X-inactivation of preferentially inactivating mutant ABCD1 in a family of inherited ALD, which is also related to symptoms in heterozygous. However, according to the authors of this paper, current suppositions of skewed X-inactivation, including negative selection against deleterious mutations, are not consistent with their results. How the skewed X-inactivation works still remains unrevealed, thus further investigations are needed. Interestingly, a study failed to find any correlations between skewed X-inactivation and neurological pathogenesis of ALD, which disagreed with the results provided by Wang et.al[11]. Whether skewed X-inactivation is respon-

sible for ALD phenotypes or not appeared in various studies and further research are required.

2.5 Model organisms have various X-inactivation patterns

X-inactivation patterns in mammals are various and differences between human, marsupials and mice will be discussed. The following paragraph will focus on the advantages and disadvantages of using mouse as a model organism in different disorders.

Marsupials are not suitable to be used as model organisms as they have enormous differences of X-inactivation to human. Despite the imprinted X-inactivation, marsupials have three homologues of human PAR1 genes in autosomes. But the investigations of them totally make out that species of X-inactivation could be meaningful in evolution. Mouse is the most common model organism used in epigenetic research. However, there are still many differences of X-inactivation patterns between human and mouse. Compared to marsupials, mice have a PAR with only one active gene. Take skewed X-inactivation as an example, despite the unclear mechanism of human skewed X-inactivation, it has been found that a genetic single polymorphism at Xce locus in mice has the ability to affect X-inactivation preferentially [25]. In the pseudoautosomal gene pattern, we can take mice as model organisms for KS, because mice also have XXY chromosome. These models have suggested the extra X chromosome in KS patient eliminates the spermatogenesis and reduces testicle size as well as lowers levels of testosterone [33], which make mouse an ideal model of human KS. But for TS, because of the different pseudoautosomal genes in human and mouse (ZFX and RPS4X in human but not in mouse), symptoms in XO mice are much milder than in XO human. XO human are usually infertile, whereas XO mice are fertile. A study has also shown that a mice model of loss-of-function mutated Pcdh19 does not play a vital role in neuronal development as human PCDH19 does, although it is also widespread in mice brain [22]. This result indicated that for investigating correlations of X-inactivation pattern and phenotypes, mouse is not a suitable model.

3 Conclusion and Perspectives

X-inactivation is a popular theory because it has correlations with genetic disorders. To understand the patterns of X-inactivation, we need to get an insight into X-linked recessive disorders to find further treatments. For future studies, more researches on X-linked disorders and their correlation with X-inactivation patterns are required. So far, the mechanisms of X-inactivation patterns has still remained unknown to researchers, such as the mechanism that enables pseudoautosomal genes to escape from X-inactivation. The next stage of X-inactivation research should concentrate on the mechanisms of different patterns in specific X-linked disorder as lots of disorders have similar X-inactivation patterns. Efforts are needed to identify these mechanisms. Also, since most of the mechanism studies are based on model organisms, it is vital to choose model organism that has similar X-inactivation mechanism as

human. Mouse is the most widely used organism in epigenetic research for its similarity with human with its economical feature. However, as an animal model, mouse is not suitable for some X-linked disorders such as TS. Therefore, finding another model organism that shares characteristics with human in terms of X-inactivation patterns to facilitate future epigenetic researches on these disorders is needed.

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