X chromosome abnormal inactivation: a unique factor for women's diseases?

“In addition to women's unique diseases in reproductive organs, many other diseases also have clear gender preferences. Whether this can be explained by the unique process of Xi in women is an interesting question.”

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X chromosome inactivation (XCI) is unique gene regulation mechanism to counter the potential dosage effect of two X chromosomes in women's cells instead of one in men's. The inactivated X chromosome (Xi) is highly condensed and viewed as a Barr body in microscope. Most genes (>85%) on this Xi are in a state of transcriptional silence and leave the remaining escaping the inactivation and likely performing needed functions. The XCI process is a typical example of epigenetic control where DNA coding sequence is not changed but gene transcription is modified through DNA and histone modifications and 3D wrapping. The XCI is regulated by an X-inactivation center, a key locus that is required for the initiation of X inactivation with a long noncoding RNA *XIST* [1]. On the X chromosome to be inactivated, a large number of the *XIST* RNA molecules are expressed, deposited, and spread along [2]. The afterward recruitment of polycomb proteins, occurrence of DNA methylation, and histone modifications lead to the condensation and inactivation of the X chromosome [3]. New advances for the complex epigenetic changes have been made in recent years [3,4]. Once established, the XCI can be stably inherited in the differentiated cells [5].

The XCI is a random process, in other words, the two X chromosomes from mother (Xm) and father (Xp) have an equal chance to be inactivated and in the end there are about

50% women's cells with Xm inactivated and other 50% with Xp inactivated, although it is more theoretical. Any significant deviation from this mosaic state is termed as skewed X-chromosome inactivation (SXCI), which is clinically relevant and is a hot topic of research. There are more than 1100 genes located on human X chromosome, which take about 5% of total human genes; however, the chromosome has accumulated a disproportionately higher number of genes concerned with neural development and mental functions [6].

The tightly controlled inactivation process can go awry and cause diseases or it can be a subsequent event of another disease. Different types and degrees of abnormal Xi exist. The commonly studied one is SXCI where one of the X chromosomes (Xp or Xm) is significantly favored for inactivation over another. The SXCI may not always be bad and it actually can have three outcomes. If both X chromosomes are healthy (i.e., without a mutation), there would be not much impact. If one of the X chromosomes has a defect but the skewed inactivation happens to be on this chromosome, the defect might be silenced and the inactivation may have a protective effect. The worse and more clinically relevant scenario might be the case in which skewed inactivation occurs in the healthy chromosome but leaves the defective one active. An array of studies has shown

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SXCI is associated with disease development, clinical phenotypes or outcomes. For example, extremely SXCI (95% or above) is associated with a very higher incidence (>four-times) of idiopathic recurrent spontaneous abortion from a meta-analysis of 12 datasets [7]. PHACE syndrome is a developmental disorder with unknown cause. Studies show consistent SXCI in the unaffected mother but consistent random X-inactivation in the affected proband, suggesting that the mother is protected through favorable skewed X-inactivation but the proband is not [8]. X-linked dominant Rett syndrome is another example of modifying effect of SXCI on manifestation of clinical phenotype [9]. SXCI has been extensively studied for women's cancer risk such as breast or ovarian cancer. These studies are mostly epidemiologic using blood leukocytes. Early data shows that SXCI occurs more in individuals with *BRCA1* mutation compared with control subjects, and among the patients with breast or ovarian cancer, the SXCI is found to be significantly associated with age at diagnosis [10]. The latter is interesting as studies also find that SXCI increases with aging in normal population [11]. Contrary to the notion, SXCI is reported in young females with breast cancer but no difference is found for those in regular breast occurring ages [12–14]. Ovarian cancer patients with invasive tumors have high skewed X inactivation compared with those with borderline cancer and healthy control subjects [15]. The significant association is also observed in other cancer such as early development of lung cancer in females [16] and esophageal cancer [17]. Certainly not all studies are positive. A study assessing SXCI between cases with *BRCA1* mutation with those without did not see any difference [18]. SXCI appears not different between patients with cervical cancer and age-matched controls [19].

The inconsistencies can be the result of multiple factors. Cancer initiation and development in different organs may differ. SXCI may play a role in one organ but may not in another. Second, many studies may not have a sufficient sample size or power to detect the association or it may be confounded by other factors such as age. Most studies use blood lymphocytes as a surrogate; however, they are highly regenerative with a short life span and it is questionable if they are representative of the tissue origin of women's cancer like breast and ovarian. Last and most importantly, SXCI has either a damaging or protective effect depending on the location of a defective allele and the X chromosome skewed. As our knowledge for risk alleles is limited, it is impossible to conduct allele-specific inactivation analysis. Without that information, the indiscriminating SXCI analysis may turn out to be null association as the protective and damaging effects cancel each other out. With tech-

nology and knowledge advance, we expect this is going to be changed. Combined genotype and SXCI analysis indeed suggests there is an additive interaction between the two and the risk allele along SXCI significantly increases breast cancer risk [20]. This will also help us to understand why some X-linked genetic disorders do not have clinical presentation and SXCI might be one of the underlying mechanisms.

Loss or gain of additional X chromosome is a severe genetic disorder that is well characterized clinically such as 46, XX-male, 47, XXX, Klinefelter syndrome (47, XXY) and Turner syndrome (45, X). Patients with these karyotypes generally have developmental abnormalities but often are not lethal, with many living a relatively normal life. For example, patients with triplicate X syndrome (47, XXX) may have low IQ and higher stature but can be fertile with normal children [21]. One of the reasons may be the normal X inactivation. If there is more than one X chromosome, the extra ones should be all inactivated randomly. However, cells with multiple X tend to be compromised for the inactivation process and can leave more than one active X chromosomes (Xa). In analysis of a 49, XXXXX girl, the 4 Xs are found to be maternal and preferentially inactivated but incomplete, causing the patient's severe physical phenotype and mental retardation [22].

Abnormal Xi has been long recognized in cancer; however, how it happens and what are the implications are little known. Loss of Xi with two co-existing active ones was described in breast cancer cell lines long time ago [23]. The original Xi likely got lost and duplication of Xa made both active. The loss appeared cancer subtype specific. By observing the disappearance of the Barr body in cancer cells, it was found that it occurred in more than half of the sporadic basal-like breast cancer, the subtype without expressing ER, PR and Her2 and in high grade (i.e., more aggressive). The Barr body disappearance was accompanied with two Xa, either from duplication of the Xa or reactivation of Xi. Both may lead to a small subset of X chromosomal genes overexpressed [24]. Genetic instability may cause Xi loss physically [25], which is not surprising considering chromosome gain or loss is common in cancer cells. The interesting question is why there is accompanying Xa duplication. A more plausible mechanism may be that Xi process dysfunctions and then leads to reactivation of the Xi [24,26]. Through high-resolution sequence techniques, a recent study provides strong evidence of epigenetic instability of the inactive X chromosome [27]. Although the underlying mechanisms differ, the overall result from either tends to be similar, in other words, increased expression of some or all X-linked genes. The overdosing could potentially provide a selective advantage and promote can-

cer development or progress. From both breast cancer cell lines and primary tumors, a core set of commonly changed genes likely associated with abnormal X inactivation was characterized [26]. More importantly, survival analysis of the primary breast tumors found important genes associated with tumor progression and aggressiveness [26]. Loss of XCI is more common in endometrial serous adenocarcinoma and associated with increased expression of cancer–testis antigens. Tumors with two Xa render worse clinical outcome to patients [28]. High-grade serous ovarian adenocarcinomas with loss of XCI showed more aggressive behavior and led to shorter patient survival [29].

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Recent evidence shows that reprogramming of somatic cells to naive pluripotency is coupled to X chromosome reactivation and at molecular level DNA demethylation and removal of *XIST* silencing [29,30]. One of unique features in cancer cells is the loss or

dysfunction of its ability to differentiate into intended mature cells. Loss of Xi in cancer may represent unexpected retro-differentiation.

The single X/Y separates male from female. In addition to women's unique diseases in reproductive organs, many other diseases also have clear gender preferences. Whether this can be explained by the unique process of Xi in women is an interesting question. Although we have learned much over the past few decades, much more needs to be learned and more complex and well-thought studies are essential.

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