

# Skewed X-Chromosome Inactivation: Cause or Consequence?

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X-chromosome inactivation occurs early during female mammalian development to transcriptionally silence one of the two X chromosomes, thereby achieving dosage compensation with males who have only a single X chromosome and the sex-determining Y chromosome (1). The choice of which X chromosome to inactivate is generally random in somatic tissue; however, once chosen, the inactivation is stably maintained, and the same chromosome is inactivated in all progeny cells. Therefore, females are mosaics of two populations of cells that differ in the X chromosome that is active. For more than three decades, researchers have used this mosaicism as a tool to examine the potential clonal origin of neoplasias in females (2), since, if the tumor(s) arose from a single cell after the time of X-chromosome inactivation, then it will have the same X chromosome active in all cells. This skewed (nonrandom) pattern of inactivation has been observed in a wide range of neoplastic tissues [see (3) for recent review] and can be considered a consequence of the monoclonal origin of the neoplasia. However in this issue of the Journal, Buller et al. (4) report the finding of an elevated incidence of nonrandom X-chromosome inactivation in the somatic tissue of females with invasive ovarian cancer and BRCA1 mutations.

Buller et al. (4) make the interesting suggestion that the observed nonrandom inactivation may be indicative of a putative tumor suppressor gene on the X chromosome and that the combination of a germline mutation of this gene as well as nonrandom X-chromosome inactivation has eliminated wild-type activity of the gene and thus results in an elevated risk of developing cancer in these females. As males who inherit the mutation would also lack expression, either the gene functions in a female-specific tissue (such as the ovary) or the male carriers would also have an elevated predisposition to cancer. In this model, skewed X-chromosome inactivation would arise independently but would cause enhanced tumor susceptibility. The difficulty with such a model is that females with the germline mutation and random X-chromosome inactivation would still have approximately 50% of their cells that would be predisposed to tumor development, suggesting that they too would have significantly elevated predisposition to cancer. There are some demonstrated X-linked cancer predispositions [e.g., (5)], and loss of heterozygosity for regions of the X chromosome is seen in a number of cancers, including ovarian cancer [references in (4); (6,7)]; however, there are numerous causes of skewed X-chromosome inactivation (Table 1). Therefore, in addition to the skewed inactivation being a cause of tumor susceptibility, it may be that skewed inactivation and elevated cancer risk are both consequences of one event or are independent events.

A large proportion of skewed inactivation patterns results from selection for or against alleles on the active X chromosome. Such selection can be variable among different tissues [e.g., see (8)], dependent on the expression of the gene, whether or not the gene product is cell diffusible (9), and interactions with other genes [reviewed in (10)]. Furthermore, depending on

**Table 1.** Examples of different known causes of skewed (nonrandom) X-chromosome inactivation

Selection
Growth disadvantage
X-chromosome rearrangements (20)
Mutations in X-linked genes (21)
Growth advantage
Possibly much skewing observed in human population (10) and seen in blood with aging [reviewed in (3)]
Decrease in precursor pool size
Twinning (22)
Confined placental mosaicism (14)
Tissue-specific, e.g., cancers; severe anemia (3,21); and possibly the skewing seen in blood with aging [reviewed in (12)]
Primary nonrandom X-chromosome inactivation
Imprinting
Marsupials (23)
Mouse extraembryonic tissues (24)
X inactivation centre variants
Mouse Xce alleles (25)
XIST mutations [mouse-(18); human-(15)]

the fitness conferred by the variant, selection can be slow or fast, resulting in different extents of skewing. Because hematopoiesis continues throughout life, the greatest amount of skewing is often observed in blood (10). The proportion of individuals showing skewing of X-chromosome inactivation in blood [skewing defined, as in the study by Buller et al. (4) as one allele being on the active X chromosome in >75% of cells and demonstrated by a modified allelic cleavage ratio of <0.33 or >3.0] increases dramatically with age (11,12), rising from under 10% of neonates to more than 35% of women older than 60 years of age (11) and up to 45% of women older than 75 years of age (13). Therefore, in assessments of skewed inactivation (particularly those that examine blood; however, extensive surveys of other tissues have not been reported), the age of the individuals examined must be considered. While Buller et al. did some age comparisons (and also found similar patterns of skewing in some other tissues), it is anticipated that females presenting with cancer may include a high proportion of older individuals; therefore, increased age-related skewing of X-chromosome inactivation is a concern. In addition, relatively small selective advantages may lead to extensive skewing over time; therefore, it is possible that an X-linked gene is providing a growth advantage, causing skewing of inactivation as well as resulting in an increased predisposition to certain cancers.

Skewed inactivation can result from a decrease in the size of

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the pool of cells undergoing X-chromosome inactivation or contributing to the tissue being examined. Such reductions increase the chance that stochastic variation alone will result in skewed inactivation. Confined placental mosaicism (CPM), which may account for a substantial proportion of the skewed X-chromosome inactivation seen in neonates (14), likely results in skewed inactivation by reducing the number of cells functionally contributing to the embryo. The disomic rescue of a trisomic conceptus will result in CPM, and the subsequent loss of the trisomic cells will substantially reduce the pool of cells in the embryo, substantially increasing the chance of observing skewed X-chromosome inactivation. An interesting possibility is that some of the trisomic cells may survive, and the continued existence of such cells may be the predisposing factor for ovarian cancer. In this case, the skewed inactivation would be a consequence of the CPM that also predisposes to neoplastic progression. Factors from the trisomic chromosome(s) may interact with the BRCA1 mutation to explain the preferential association of skewed X-chromosome inactivation with BRCA1 mutations.

Primary nonrandom inactivation has been demonstrated in other mammals, although there is only limited evidence for its occurrence in humans (15), and it is extremely difficult to distinguish it from very early cell selection. The known changes altering the choice of chromosome to inactivate involve the X inactivation centre (XIC), a region of the X chromosome that is necessary in *cis* for inactivation to occur. Expression of the XIST gene from the XIC of the inactive X chromosome appears to initiate the inactivation process, and the XIST RNA coats the inactive X chromosome [reviewed in (16)]. Mutation and imprinting of the XIST gene have been described in mice and humans as resulting in skewed inactivation (15,17,18); however, such changes are rare in humans, and it is not obvious how they would be associated with an increased risk of neoplasia.

Skewed inactivation can result in heterozygous females manifesting X-linked diseases that are usually seen only in males [see (19)], and a similar mechanism may result in the increased skewing seen in the ovarian cancer patients reported by Buller et al. (4). However, it is also possible that the skewed X-chromosome inactivation is the result of age-related changes or is a consequence of a process that also increases the probability of tumor development or progression. Although this study serves as yet another cautionary note that somatic tissue needs to be examined before declaring a sample "clonal" on the basis of skewed inactivation patterns, it also emphasizes the utility of studying inactivation patterns. If the results of Buller et al. are substantiated, the analysis of X-chromosome inactivation patterns, whether cause or consequence, could be used to predict which females might have an elevated risk of carrying BRCA1 mutations.

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## NOTES

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