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The X Factor: Skewing X Inactivation towards Cancer

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Increased expression of the epidermal growth factor receptor HER-2/ErbB2 is frequently observed in breast cancer and is targeted by the anticancer drug Herceptin. Now, Zuo et al. (2007) reveal that an X-linked gene encoding the transcription factor FOXP3 is a breast cancer tumor suppressor that represses expression of HER2/ErbB2.

One of the first lessons taught in tumor genetics courses is that activation of a proto-oncogene requires mutation of a single allele, whereas inactivating a tumor suppressor gene requires biallelic inactivation. This difference was recapitulated in Knudson's two-hit theory to explain the genetics of retinoblastomas (Knudson, 1971) and helped identify the first tumor suppressor genes. Following this easy rule of thumb, the tumor suppressor protein p53 was originally classified as an oncogene, because introduction of a single mutant allele provides transforming activity. But once it was realized that p53 is a tumor suppressor that can acquire dominant-negative mutations, it became clear that tumor suppressor genes do not always require biallelic inactivation (Levine et al., 2004). In another variation on this theme, two new studies describe identification of the first X-linked tumor suppressor genes. One study describes involvement of the X-linked *WTX* gene in Wilms tumor (Rivera et al., 2007), and in this issue of *Cell*, Zuo et al. (2007) report that *FoxP3* is an X-linked tumor suppressor gene involved in breast cancer.

What makes an X-linked tumor suppressor gene special? First, as males only carry one copy of the X chromosome, this allows for "one-hit" inactivation. Second, X chromosome inactivation results in transcriptional silencing of one of the two X chromosomes in female somatic cells to equalize the dosage imbalance of X-linked genes between males and females. Which of the two X chromosomes is inactivated is completely random, but once established, this pattern of inactivation is stably propagated to the daughter cells. As a result, females normally display a mosaic pattern of X inactivation: 50% of the cells inactivate the maternally inherited X chromosome, whereas the other 50% inactivate the paternal X chromosome. Consequently, X chromosome inactivation in combination with a single mutation in the active X chromosome can inactivate an X-linked tumor suppressor gene. Indeed, *WTX* is inactivated by monoallelic mutation targeting the active X chromosome in female patients (Rivera et al., 2007). Similarly, mammary tumors in *FoxP3* mutant heterozygous mice have invariably inactivated the X chromosome carrying wild-type *FoxP3*. Thus, inactivation of X-linked tumor suppressor genes can be achieved by a "single-hit" event, both in males and in females.

Why have tumor suppressor genes on the X chromosome not been selected against during evolution? This may reflect a lack of sufficient selective pressure against these genes, especially considering that reproductive age generally precedes the onset of cancer. In fact, the risk that comes with an X-linked tumor suppressor gene is comparable to that associated with a haploinsufficient tumor suppressor gene, of which several examples exist (Santarosa and Ashworth, 2004). Nonetheless, the genetics of X-linked tumor suppressors might be more complex. First, X inactivation does not silence all genes on the X chromosome. Although there is clear evidence that *FoxP3* is subject to X inactivation, silencing at the *FoxP3* locus might be incomplete (Wildin and Freitas, 2005). Indeed, in 38% of the analyzed breast cancer tissue samples carrying somatic *FoxP3* mutations, the wild-type *FoxP3* allele is lost, suggesting leaky X inactivation at the *FoxP3* locus. Secondly, in females carrying mutations in critical X-linked genes, selective X inactivation is observed due to negative selection of cells expressing the mutant allele. This selection, known as skewed X inactivation, allows for phenotypic suppression of X-linked dominant disorders in females by selecting for expression of the wild-type allele in relevant tissues (Figure 1). In fact, although inactivation of *FoxP3* causes an autoimmune disorder (IPEX syndrome) in males, females with a single mutant *FoxP3* allele are not affected by the disease, as skewing can select against expression of the mutant allele. Now, Zuo et al. (2007) show that FoxP3-heterozygous female mice develop breast cancer at an enhanced rate as they age. This leads to the peculiar notion that in female carriers of a mutant

FoxP3 allele, skewed X inactivation selects against expression of the mutant allele in certain tissues to prevent autoimmune disease, whereas skewing selects for expression of the mutant allele during tumorigenesis in breast epithelial cells to promote breast cancer.

How does FoxP3 act to suppress the development of breast cancer? FoxP3 belongs to the Forkhead family of transcription factors, of which several members (FoxO, FoxM, FoxG1) have been implicated in tumorigenesis. Initially, it was shown that FoxP3 is essential for the commitment of thymocytes to become regulatory $CD4^+$ T cells (Tregs) with dedicated immunosuppressive functions (reviewed in Zheng and Rudensky, 2007). Indeed, FoxP3 is predominantly expressed in Tregs, where it can act as a transactivator and repressor of a large variety of genes. Zuo et al. present evidence for FoxP3 expression in breast epithilium and indicate that the *HER-2/ErbB2* oncogene is a relevant target of FoxP3. Most tantalizing is their observation that ectopic expression of ErbB2 completely reverses the antitumorigenic effect of FoxP3, at least as measured in colony-formation assays in vitro. This outcome suggests that *ErbB2* is the single most important target of FoxP3 in preventing tumorigenesis. Interestingly, Liu and colleagues previously proposed that *ErbB2* is a pivotal target of FoxP3 in thymic epithelium where FoxP3 regulates differentiation of Tregs (Chang et al., 2005). This suggests that deregulated *ErbB2* expression is responsible for both the autoimmunity in males and the increased tumor formation seen in females. However, a role for FoxP3 in thymic epithelium was recently disputed (Zheng and Rudensky, 2007), and *ErbB2* was not identified as a major FoxP3 target by expression profiling in Tregs. Possibly, FoxP3 requires specific cofactors to repress *ErbB2* expression, similar to the cooperation between NFAT and FoxP3 to repress IL-2 expression (Wu et al., 2006). In addition, several FoxP3-negative breast cancers express low levels of ErbB2, and FoxP3 can also suppress growth of ErbB2-negative breast cancer cell lines. Based on expression profiling of FoxP3-regulated genes in ErbB2-negative cells, the authors propose multiple other players in the ErbB2 signaling pathway. However, this seems to contradict the observation that ErbB2 expression alone can overcome the tumor-suppressive effect of FoxP3. Clearly, the situation downstream of FoxP3 is more complex than mere regulation of ErbB2 signaling.

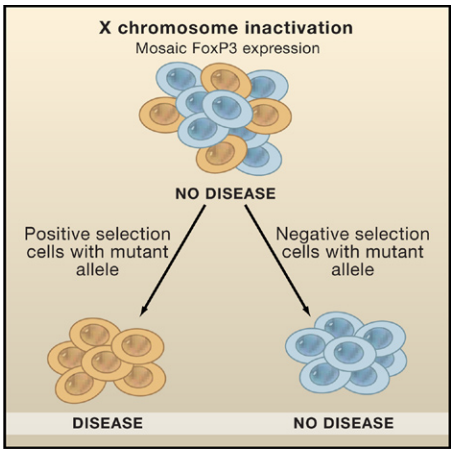

Figure 1. Inactivation of X-Linked Tumor Suppressor Genes and Tumor Formation

Mosaic expression of a mutant X-linked gene, such as *FoxP3*, in females depends on X chromosome inactivation. Growth of cells carrying a mutant allele can be actively suppressed by negative selection resulting in skewed X inactivation (right). Mechanism(s) for selection can be diverse, such as increased intrinsic cell death of cells carrying the mutant allele. However, clonal outgrowth of cells carrying a mutant allele can also occur, and this results in disease (left). Clonal outgrowth can be caused by secondary genetic changes (such as Ras activation), resulting in hyperproliferation of cells carrying a mutant allele or silencing of the wild-type allele through loss of heterozygosity or, in the case of *FoxP3* and other X-linked tumor suppressor genes, through X chromosome inactivation.

Although the data at hand indicate that FoxP3 acts in breast epithelium to suppress breast cancer, it remains possible that Treg function also contributes. Mosaic FoxP3 expression in Tregs may result in partial impaired Treg function without disease and elevated expression of inflammatory cytokines. Given that inflammation is increasingly recognized as a contributing factor in tumorigenesis (reviewed in Karin and Greten, 2005), increased and sustained inflammation may provide a mechanism for a more general involvement of FoxP3 in cancer development. Clearly, X-linked tumor suppressor genes are the new kids on the block, and it will be of interest to find out why and how nature tolerates these genetic booby traps.

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