Clinical Interpretation of Skewed X Inactivation

To the Editor:

The recent study by Fey et al[1] presents a number of troubling issues regarding the use of the M27β (DXS255) probe for analysis of tumor clonality. First, they propose a derived value called allele cleavage ratio (ACR) for comparison of DNA samples. The definition of ACR is ambiguous because it arbitrarily specifies the less represented allele as the denominator. But, if the fully inactive allele gives no hybridization signal, then the ACR should be ∞. The effect is to distort the ACR value toward very large numbers as the allele contribution gets more skewed. This presumably explains the nonnormal distribution of ACR values in their data set. I suggest that a better way to describe skewing is to calculate the ratio of each allele to the sum of both alleles. This ratio gives a linear relationship between allele contribution and cell mixture and avoids the distortion of the distribution curve.

Of greater concern is that the data presented by Fey et al tends to undermine the link between X inactivation and methylation status at M27β. In some samples, the inactive X is partially methylated; in several samples, there was a mismatch between PGK analysis and M27β; and in two lymphomas proven to be clonal by immunogenotype, the M27β profile was not skewed. We have also observed several cases of mismatch between human androgen receptor (HAR) analysis[2] of X inactivation and M27β results.[3] These data call into question whether M27β should be used at all in quantitative X-inactivation analysis. The conclusion that the M27β data "are more diverse than predicted by Lyon's hypothesis" may reflect the inaccuracy of the M27β assay rather than unusual patterns of X inactivation in the blood. Thus, Fey et al observed nearly 20% of women with extreme skewing of X inactivation—a figure that is approximately

twofold higher than observed in other control populations. They speculated that there may be X-linked genes that cause skewing or that clonal succession events contribute. Because of the number of women that have skewed X inactivation (7% to 20%), it is unlikely that deleterious mutations account for most skewing. An X-linked mutation mechanism would predict a greater distortion in birth sex ratio than actually observed. Puck et al[4] analyzed human T lymphocytes using an unambiguous interspecific hybrid assay. Those data show a unimodal distribution, a result that is most consistent with binomial sampling of a limited number of coherent clones (~10). We obtained a similar estimate in both B and T cells.[5] Because X inactivation is probably determined well before the beginnings of embryonic hematopoiesis, the coherent clones probably arise from mesodermal cells fated to be precursors for the hematopoietic system. However, an age effect, if confirmed, might suggest clonal dominance or an age-related restriction in the size of the hematopoietic stem cell pool.

A final mechanism may be operative as an explanation for constitutional skewing. There may be allelic variation in the X-inactivation process. In mice, the Xce locus (X-inactivation control element) exhibits strain variation.[6] Allelic variation in human X-chromosome inactivation might cause skewing if one chromosome tended to inactivate before the other. This scenario makes the explicit prediction that skewing would be inherited as a Mendelian trait, a speculation for which there is no evidence. Regardless of the explanation, to derive a clinically useful interpretation of skewed X inactivation in an individual, it is important to identify and analyze an appropriate control tissue or cell type. The application of quantitative polymerase chain reaction assay at the androgen receptor locus solves many of the associated technical problems.[5]

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