Good news for the aging population?

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Studies of XCI in elderly females have previously suggested that the pool of blood stem cells normally diminishes with age, resulting in oligoclonal or even monoclonal hematopoiesis. In this issue of Blood, Swierczek and colleagues provide convincing evidence against this hypothesis by analyzing the pattern of allele-specific gene expression rather than DNA methylation of X-encoded genes.

Hematopoiesis is normally polyclonal with the progenitors and mature blood cells being derived from an unknown number of hematopoietic stem cells. In contrast, in premalignant and malignant disorders (eg, myelodysplastic syndromes and leukemia), hematopoiesis may be derived from a small number or even just one abnormal, mutated stem cell (or self-renewing progenitor) that has acquired a selective advantage, giving rise to oligoclonal or monoclonal hematopoiesis. Assessing clonality in peripheral blood cells provides an important way to distinguish premalignant and malignant disorders from reactive conditions and has also been of great value in tracing the cellular origins of many malignant blood diseases.

In the absence of either a cytogenetic abnormality or an acquired, disease-specific mutation, clonality has most frequently been assessed by studying the pattern of X-chromosome inactivation (XCI), although, of course, this can only be carried out in females (XX) and not males (XY). Most normal females are mosaics in whom individual cells contain either an inactivated paternal or maternal chromosome (usually 50:50). However, the pattern of XCI is established randomly in a small number of cells early in development before hematopoietic stem cell specification. Therefore, by chance, up to approximately 5% of hematologically normal young females have a skewed (90:10 or 10:90) pattern of XCI. Similarly skewed XCI may also be acquired as a result of clonal hematopoiesis in individuals who started life with random (50:50) XCI. This results from predominance of cells in which either the maternal or paternal chromosome was inactivated.

The process of XCI results in most of the genes on the inactivated chromosome being silenced, and many of the CpG dinucleotides on these chromosomes also become methylated. This, in turn, results in heritable mononucleotide (50:50) XCI assay into the assay (HUMARA assay) distinguishes methylation (inactivation) of the maternal and paternal alleles. Such studies have consistently suggested that skewed XCI occurs in the peripheral blood cells (particularly granulocytes) of approximately 23% to 39% of hematologically normal women over the age of 60 years, 1-4 much more frequently than in younger women. This trend was confirmed by looking at methylation of the phosphoglycerate kinase gene in a small number of women. 5 These observations have been widely interpreted as showing an increase in clonal hematopoiesis resulting from a reduction in functional stem cell numbers with increasing age (by implication both in females and males). It also meant that these X-inactivation assays could not be used to reliably distinguish between normal (polyclonal) and clonal hematopoiesis in the elderly population.

Taking an alternative approach, Swierczek et al have evaluated XCI by measuring allele-specific expression rather than allele-specific methylation. They used a modified Real Time polymerase chain reaction assay 6 to quantitate expression from 5 X-chromosome loci. At each locus, expression of the 2 alleles could be distinguished in this assay by exonic single nucleotide polymorphisms (SNPs). The combined rates of heterozygosity at these loci allow most ethnic populations to be assayed, and in this study, about 50% of the subjects were informative for more than one SNP. Importantly, in taking this different approach to assaying XCI in females over 65 years of age, the authors found no evidence of extreme skewing for any marker, in any subject, in either granulocytes or platelets. By contrast, when they used the HUMARA assay in the same samples, they replicated previous findings which implied that approximately 30% of this group have skewed X inactivation. This study shows that methylation at the androgen receptor is not an accurate indicator of XCI in elderly females. The reasons for age-dependent skewing of methylation at this locus are still not clear, although several possibilities (technical and biological) are discussed by Swierczek et al. The authors conclude that the previously reported skewing of XCI in elderly females is apparent rather than real and certainly does not support the suggestion of a tendency to clonal hematopoiesis in the elderly. This conclusion is consistent with data from mice, showing that hematopoietic stem cell numbers are not depleted with aging, although there may be functional changes that alter cell fate choices made by aged hematopoietic stem cells. 6-10

In conclusion, these new findings from Swierczek et al may revise our view of hematopoiesis in the elderly and make a convincing case for using nonmethylation-based and allele-specific expression assays to assess clonality.

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REFERENCES

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