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Genome instability: cancer or aging?

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Mutations are essential as a substrate for evolution by natural selection. However, since mutations occur at random, they are mostly deleterious and would be expected to have adverse effects in somatic tissues of multicellular organisms. While in theory somatic genomes could be stabilized to a much greater extent than germline genomes, evolutionary theory dictates that such maintenance would not stretch much further than until shortly after the reproductive period. Hence, mutations would be expected to accumulate in somatic tissues, a concept that has been proposed as a universal explanation of the aging process (Failla, 1958; Szilard, 1959).

The somatic mutation hypothesis of aging was immediately challenged, for example, by John Maynard Smith, who suspected that the natural rate of such mutations would be too low to cause an effect (Maynard Smith, 1959). This is probably very similar to some of the arguments made by Aubrey de Grey in this Opinion Paper. The idea to apply different thresholds for adverse effects of somatic mutation loads to cause cancer or non-cancer aging-related phenotypes is original and very difficult to rule out at this stage. However, there are a few recent developments to suggest that alterations of the genome or epigenome can cause both cancer and lead to functional decline of highly differentiated tissues in aging.

First, there is abundant evidence by now that somatic mutation loads are far higher than previously imagined. Our own data obtained with the lacZ transgenic reporter mice, extensively cited by de Grey, indicate surprisingly high numbers of genome rearrangement mutations in organs such as liver and heart (Dollé et al., 2002). In our model genome rearrangements occur as a consequence of a breakpoint in a lacZ reporter gene and a second breakpoint elsewhere in the mouse genome, either on the same chromosome (in which case the event reflects a deletion or inversion) or on another chromosome (resulting in a translocation). Genome rearrangements comprise as much as 50% of all mutations accumulating with age in the heart, but also occur in liver and other organs. It is argued by de Grey that the model we proposed to explain how these rearrangements can exert cell functional loss has two weaknesses. First, it is argued that none of the rearrangements necessarily leads to loss of informational template. Second, based on the breakpoints identified, de Grey argues that these events are unlikely to cause position effect variegation because they do not juxtapose heterochromatin next to euchromatin.

Regarding the first argument, while we do not exclude the possibility that the rearrangement mechanisms proposed by de Grey's may occur, the ones we proposed remain the most straightforward explanation of our observations. They are in keeping with the general

consensus as to how illegitimate recombinations, for example, in the presence of multiple DNA double-strand breaks, can give rise to chromosomal aberrations, such as translocations (Richardson et al., 2000). While this does not preclude the occurrence of mutational events as postulated by de Grey we believe that our model for now is the simplest interpretation of the results obtained.

The second argument by de Grey assumes that heterochromatin and euchromatin are separated and do not intermingle. However, a recent study in which higher order DNA structure in the human genome was mapped globally, revealed that there is no strict correlation between open chromatin and the activity of a gene (Gilbert et al., 2004). Indeed, not every gene in the open areas is being transcribed and active genes in regions of low gene density can be embedded in compact chromatin fibers. It is therefore very difficult to argue that the type of rearrangements indicated by the lacZ reporter do not cause positional effects on gene transcription. Hence, even if none of the genome rearrangements as detected lead to loss of sequence information (an unlikely scenario in our opinion) position effects could still lead to dysregulation of transcription. Therefore, based on our results we would argue that at the present stage of our knowledge it is simply impossible to conclude that genome rearrangements of the type we observed are completely harmless and do not lead to cell functional decline through deregulation of transcription.

Our data are based on a lacZ reporter gene, which serves as a neutral marker for measuring genome instability in different organs and tissues of the mouse. While this model is probably still the only model that allows studying a broad range of somatic mutations in essentially every organ or tissue of an aging mammal, ours are not the only data indicating extensive instability of the genome or epigenome during aging. Using classical karyotyping techniques it has now been well-established that white blood cell populations in mice and humans undergo an age-related, exponential increase in chromosomal aberrations (Ramsey et al., 1995; Tucker et al., 1999). In such cells, possibly because of high spontaneous apoptosis levels, the frequency of aberrant cells is low, i.e., varying from 1–5% at young age to 5–10% at old age. However, in mouse liver cells after partial hepatectomy, Curtis and co-workers reported considerably higher numbers of cells with abnormal chromosomes (i.e., from about 10% of the cells in 4–5 month old mice to 75% in mice older than 12 months) (Curtis et al., 1963). In white blood cells there is also an age-related increase in the frequency of cells mutated at the HPRT locus (Jones et al., 1995). Again, the frequency of such events appeared to be quite low, i.e., around $1\text{--}10 \times 10^{-6}$ in either mouse or human cell populations. However, a more than 10-fold higher frequency of HPRT mutants and an increase with age has been observed for tubular epithelial cells in the human kidney (Martin et al., 1996).

There are also many types of changes that would escape detection in the lacZ reporter model, which is therefore far from a panacea in mutation research. For example, in both mouse and human brain a significant fraction of cells, including neuronal cells, were found to display chromosomal aneuploidy, both loss and gain of chromosomes (Rehen et al., 2001; Rehen et al., 2005). In mouse brain, a dramatic age-related increase in loss of heterozygosity events has been observed by analyzing neurospheres from C57Bl/6 $\times$ DBA/2 hybrid mice at 9 chromosome pairs, using known single nucleotide polymorphisms between these two mouse strains (Bailey et al., 2004). Such instability in neuronal progenitor cells could have important phenotypic consequences for the capacity of the aging brain to compensate aging-related degeneration. Evidence has now also emerged for the age-related instability at loci of mini- and micro-satellites, including telomere shortening, mutation accumulation in the mitochondrial genome, and both hyper and hypo-methylation at multiple epigenomic target sites (for a recent review, see (Vijg, 2006)). Especially epigenomic changes with age are likely to be frequent as suggested by reports on the instability of X chromosome inactivation, loss of genomic imprinting and, most recently, an increased epigenomic divergence between

monozygotic twins when they age (Fraga et al., 2005). Finally, evidence has recently been obtained for active retrotransposition of L1 elements in mouse neural progenitor cells, with evidence that L1 insertion occurred preferentially in or near genes with an effect on differentiation of the neural progenitor cells (Muotri et al., 2005).

Especially if one takes into consideration that all assay systems based on selectable markers are likely to greatly underestimate the mutation frequency, for example, by virtually ignoring mutations that cause only subtle defects, we believe the pattern that is now emerging proffers abundant evidence that the overall mutation load of normal cells and tissues is substantial and increases with age. The exact types of events, their tissue-specificity and functional impact remain to be determined, but given the magnitude of these random events, which is now slowly being revealed, it seems highly unlikely that such a pattern of genomic decay would have no adverse effects at all other than increasing cancer risk. Indeed, one could argue the opposite, namely that many more mutations are required to cause cancer, which is based on a highly specific set of alterations necessary to evade restraints on growth, tissue invasion, immune evasion and drug resistance. By contrast, virtually in all case when random genomic or epigenomic alterations are not neutral their effect is detrimental. Because of the network structure of gene functional organization adverse effects are probably more easily induced by random events than imagined in the past when genes were often considered to act in a stand-alone fashion. This offers many different target sites for random genome alterations to adversely affect (not necessarily disrupt) cellular functions.

Cancer presumably arose as a major aging-related adverse phenotype with the evolution of renewable tissues (Campisi, 2003). While as yet it has not been possible to directly compare the accuracy of genome maintenance systems between animals with and without cancer, it seems more likely that the major anti-cancer systems are the gatekeeper tumor suppressors, including apoptosis, replicative senescence and immune surveillance. To further improve DNA repair significantly is energetically costly. Indeed, to maintain all systems of information transfer at levels that are significantly higher than strictly needed for maintaining cell viability is much less economical than co-opting existing pathways of apoptosis or cellular senescence for removing genetically aberrant cells from the population.

In summary, while highly unlikely that spontaneous instability of the genome can explain all possible aging-related forms of cellular degeneration and death there is simply no evidence that the adverse effects of the wealth of mutations and epimutations that are now being discovered to increasingly plague the aging genome are strictly limited to neoplastic transformation.

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