Protagonistic pleiotropy: why cancer may be the only pathogenic effect of accumulating nuclear mutations and epimutations in aging

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Abstract

Since Szilard's seminal 1959 article, the role of accumulating nuclear DNA (nDNA) damage – whether as mutations, i.e. changes to sequence, or as epimutations, i.e. adventitious but persistent alterations to methylation and other decorations of nDNA and histones – has been widely touted as likely to contribute substantially to the aging process throughout the animal kingdom. Such damage certainly accumulates with age and is central to one of the most prevalent age-related causes of death in mammals, namely cancer. However, its role in contributing to the rates of other aspects of aging is less clear. Here I argue that, in animals prone to cancer (such as mammals), evolutionary pressure to postpone cancer will drive the fidelity of nDNA maintenance and repair to a level greatly exceeding that needed to prevent nDNA damage from reaching levels during a normal lifetime that are pathogenic other than via cancer. I term this the "protagonistic pleiotropy of chromosomal damage" (PPCD) hypothesis, because this interaction of cancer-related and –unrelated damage is the converse of the well-known "antagonistic pleiotropy" phenomenon first proposed by Williams 50 years ago. I then consider a selection of recent data on the rate of accumulation of nDNA damage in the context of this hypothesis, and conclude that all presently available evidence is consistent with it. If this conclusion is correct, the implications for the feasibility of greatly postponing mammalian (and eventually human) aging and age-related pathology are far-reaching.

Introduction

The somatic mutation theory (SMT), in its current form, proposes that species-maximum lifespan is substantially influenced by the rate of accumulation of mutations and epimutations that degrade gene expression genome-wide. "Epimutations" refers here to spontaneous changes either in DNA methylation or in histone modifications; these can persist through DNA replication because, for example, enzymatic methylation of a CpG dimer usually depends on prior methylation of the other strand. SMT does not refer to the occurrence of damage that is subsequently removed, either by the action of DNA repair enzymes or by the death of the host cell: it concerns only the accumulation of permanent damage. Moreover, it specifically excludes one age-related cause of death that is certainly caused by mutations and epimutations, namely cancer. For brevity, rather than speaking below of "permanent, accumulating nuclear mutations or epimutations not relevant to cancer and not …" I hereafter denote the classes of chromosomal alteration under discussion by the term "SMT damage."

Most, if not all, cancers harbour multiple mutations and/or epimutations that underlie their evasion of anti-cancer defences and therapies. However, direct evidence for a contribution of SMT damage to mammalian aging remains elusive. If SMT damage accumulates too slowly to contribute to any age-related health problem within a currently achievable lifetime, the task of extending healthy and total lifespan reduces to that of combating (a) cancer and (b) accumulating age-related molecular and cellular damage other than to chromosomes (de Grey et al., 2002; de Grey, 2003) – a task that, while still plainly daunting, may be much easier than addressing general nDNA damage.

The inevitable multiplicity of processes of aging

Aging is a product of evolutionary neglect, not evolutionary intent (Olshansky et al., 2002). While exceptions exist (Finch, 1990), animals generally age "by default" – they possess multifaceted systems for maintaining physiological integrity, i.e. for retarding aging, but none for inducing or accelerating

aging. Species differences in maximum lifespan result from differences in the sophistication of their anti-aging systems and in factors (such as body temperature) that influence the rate of occurrence of molecular and cellular damage.

A key corollary of the unselected nature of aging is that no single process will be solely responsible for aging in any complex organism. The evolutionary argument is as follows:

1. For each process of aging there are metabolic pathways, encoded by genes, which minimise its rate.
2. These genes are at least partly different for different processes of aging.
3. All genes suffer spontaneous mutations in the germ-line at a meaningful rate.
4. Many mutations only mildly impair gene function.
5. Accumulation of mutations over multiple generations is prevented only when those carrying the mutation have fewer descendents.
6. Death from one process of aging precludes death from another.
7. Selection against death from a given process is absent if death almost always occurs at a lesser age from other processes.
8. If, therefore, a process A exists that generates life-threatening levels of damage in a given species by age (say) 10 years, and a second process B is harmless at age 10 but is life-threatening by age 30, genes involved in retarding process B but not A will experience negligible selection against spontaneous germ-line mutations that *modestly* degrade their performance and cause process B to become appreciably pathogenic by age 10 but not sooner.
9. Such mutations will thus accumulate in the population and eventually become fixed, so process B becomes a substantial contributor to the aging of this species.

nDNA damage: the special case of cancer

Cancer's main strength is that it has natural selection at its disposal. Typical cancers exhibit high genomic instability and consequent cell-cell gene expression variability, so almost any attack from either the body's natural defences or the clinician will leave a (perhaps small) proportion of the cells in a tumour unharmed. Those cells then proliferate, restoring the cancer's severity – and with resistance to the original treatment.

Evolution can increase lifespan only by postponing death from cancer as well as all other age-related causes. But since the genetic bases of different cancers vary so widely, no gene can easily be determined to be irrelevant to cancer. Thus, enhancing DNA maintenance and repair at selected loci is insufficient: it must be genome-wide, protecting all genes. Referring to the evolutionary argument above, and considering the production of nuclear mutations/epimutations contributing to cancer as process A and the production of SMT damage as process B, step 2 is lacking (there is no easily detectable difference between the genes that protect against cancer and those that protect against SMT damage-mediated pathology) so step 8 is impossible.

Why is this important? Cancers derive from a single cell that possesses the necessary gene expression changes. Dysfunction resulting from SMT damage, by contrast, can become pathogenic only by affecting a substantial proportion of the cells that perform some necessary function – many, many cells. Thus, to avoid cancer until a certain age, a species must possess DNA maintenance and repair machinery that will "unnecessarily" prevent SMT damage-mediated pathology from non-cancer causes until a much greater age. A clear parallel exists between this proposed interaction and the concept of antagonstic pleiotropy (AP) proposed by Williams (Williams, 1957) and now recognised to play a widespread role in aging: while in AP a deleterious phenomenon is selected for because the genes underlying it also confer an outweighing benefit, here a harmless (within the available lifetime)

phenomenon is selected against because the genes underlying it also cause a deleterious phenomenon. Thus, this conclusion is hereafter denoted the PPCD ("Protagonistic Pleiotropy of Chromosomal Damage") hypothesis.

PPCD applies only to species for which cancer is a major cause of death. Wholly postmitotic animals, such as flies and nematodes, do not die of cancer, so PPCD does not claim that SMT damage is irrelevant to aging in those organisms (though the logic *may* hold there too, since the germ line and the larval stages do involve cell division).

Theoretical challenges to PPCD

Distinguishability of genes relevant to cancer

PPCD asserts that no gene can "easily" be determined to be irrelevant to cancer. However, evolution does many things that are not at all easy. Why not this?

If the difference between the two classes of gene is subtle, its recognition will require extensive genetic machinery, maintained by selective pressure if it is to survive over evolutionary time. Maintaining all genes as well as any gene needs does not require this machinery, so it will win out by default unless it has a drawback serious enough to keep the gene-specific maintenance machinery from accumulating germ-line mutations while pan-genome maintenance improves.

Redundancy of anti-cancer defences: an alternative?

Age-related cancers are age-related because they result from the combined misexpression of multiple genes. Most vital cellular processes, by contrast, are lost if *any* of numerous genes fails. Might this not balance the disparity of how many cells must be affected to cause pathology, so that all loss-of-function mutations are, after all, similarly prone to contribute to age-related pathology?

When multiple mechanisms contribute additively to a selected trait, evolution will generally favour improving them all a little rather than improving some of them a lot, simply because at the sequence level there are typically more ways to achieve a small improvement than a large one. Humans have more complex tumour-suppression mechanisms than mice, and indeed more mutations are needed to make a cell fully cancerous in humans than in mice (Boehm et al., 2005). But there will still be an *additional* postponement of cancer from performing genome-wide chromosomal maintenance and repair somewhat better than would be needed for reasons other than cancer. Thus, however great the redundancy in the anti-cancer defence arsenal evolves to be, there will remain selection to maintain global repair and maintenance at a level better than any problem except cancer needs it to be.

Cell-type specificity of DNA repair and maintenance

It may, however, be easier to adjust the quality of nDNA maintenance from one *cell type* to another than from one gene to another. Constitutively postmitotic cells cannot become cancerous, so maybe their nDNA maintenance could be less efficient and SMT damage thus more relevant.

However, this would again require genetic machinery: this time to link particular differentiated states to particular levels of nDNA maintenance. While that indeed occurs occasionally (such as in the case of testis-specific nDNA maintenance machinery (Walter et al., 1994)), it may be less economical to *lower* nDNA maintenance quality in a few cell types than to raise it in a few.

Cell-cell effects must also be considered here. Senescent cells can arise from nDNA damage and induce pre-neoplastic characteristics in neighbouring cells, probably via secretion of growth factors and matrix metalloproteases (Krtolica et al., 2001). *In vivo*, a pre-cancerous cell may be pushed into full-blown malignancy by a senescent, toxin-secreting neighbour. All postmitotic cells in mammals neighbour mitotically competent cells, so they may need to avoid becoming senescent any more often than mitotically competent cells become malignant.

Consistency of PPCD with experimental data

Accelerated aging

Though reports abound concerning accelerated aging and DNA damage, they say little if anything about SMT versus PPCD. Three classes of such evidence exist.

Genetic impairment of nDNA maintenance causes premature onset of symptoms associated with normal aging, in both laboratory mammals and humans (de Boer et al., 2002; Bachrati and Hickson, 2003). However, this does not prove that SMT damage contributes to normal aging (de Grey, 2004).

The opposite – life extension from slowed accumulation of SMT damage – has never been demonstrated; but, conversely, that does not show that SMT damage is irrelevant to aging, only that it is not the *dominant* determinant of the rate of aging in the organism under consideration.

The most useful type of evidence against theories of aging is when accelerating a type of damage does not accelerate aging (de Grey, 2004). Deleting the gene mainly responsible for repairing 8-hydroxydeoxyguanosine (8OHdG) causes much higher levels of nuclear and mitochondrial 8OHdG but no severe phenotype (Klungland et al., 1999). But even this shows only that 8OHdG is less mutagenic *in vivo* than expected (and thus, incidentally, should be given less prominence than it often is as a surrogate for general DNA damage): it says nothing about the cancer/non-cancer question.

Direct quantification of mutation rates

Recognising the above, several groups have quantified SMT damage in unchallenged animals (Kohler et al., 1991; Grist et al., 1992; Martin et al., 1996; Hill et al., 2004). Vijg's group generated mice with a *lacZ* transgene inserted into the genome that acts as a selectable marker for mutations in a stretch of DNA of known length, and that can also be analysed at the sequence level to characterise those mutations.

The conclusions arising from this research have been striking. An increase in mutant load during adulthood is undetectable in some tissues, including the brain, and is a factor of at most three in any tissue yet examined (Vijg and Dollé, 2002). Also, the incidence of point mutations, even by old age, is only around 10^{-4} per gene per cell – far too few to contribute to age-related decline in tissue function (Dollé et al., 1997). However, large-scale chromosomal rearrangements occur roughly as often as point mutations, and a single rearrangement can potentially alter the expression of many genes. Thus, I now consider the potential effects of rearrangements in detail.

Large-scale rearrangements: position effects?

In *Drosophila melanogaster,* rearrangements can cause position-effect variegation (PEV; Weiler and Wakimoto, 1995). PEV is "action at a distance" – the genes whose expression levels are altered can be hundreds of kilobases from the breakpoints. Thus, even a few rearrangements per genome might affect enough genes to impair tissue function.

However, PEV (in mammals too; Ayyanathan et al., 2003) typically occurs when the rearrangement juxtaposes heterochromatin next to euchromatin: the euchromatin becomes overcondensed, suppressing gene expression. Nearly all the rearrangements identified by Vijg's group place the lacZ gene next to another region far from the centromere, hence probably in euchromatin (Dollé and Vijg, 2002). Such rearrangements should not cause PEV.

Large deletions: degradation or circularisation?

A rearrangement involving two breaks in the same chromosome may be an inversion, in which the segment between the breaks is reversed in its orientation relative to the surrounding regions, or a deletion, in which the inter-break segment is excised. Clearly, deletion of much of a chromosome might have dramatic consequences.

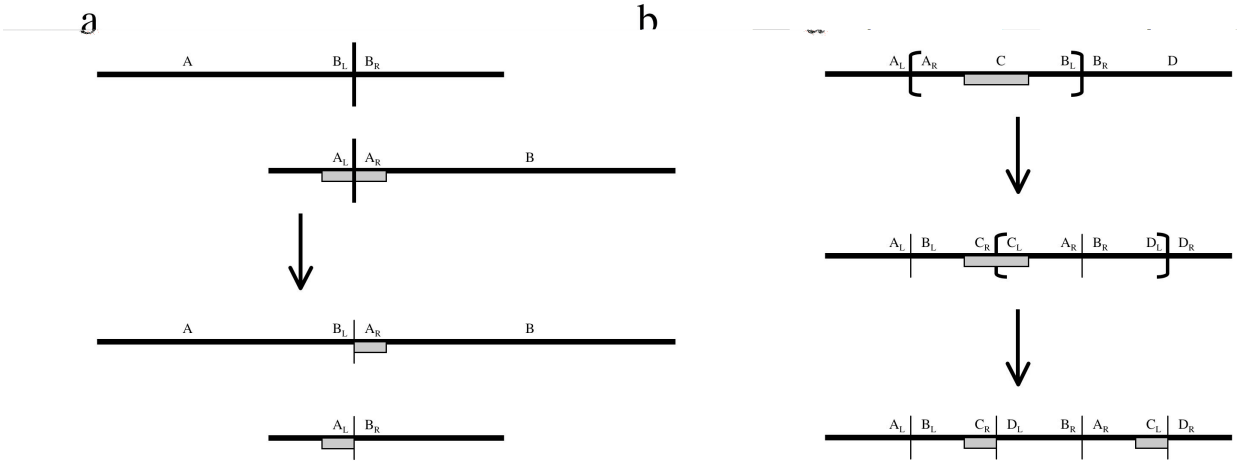

Figure 1. Rearrangements that look like deletions in the Dollé/Vijg assay but are not. The left and right sides of the various breakpoints, as defined in terms of genomic sequence (i.e. on an un-rearranged chromosome), are denoted by L and R subscripts, and the *lacZ* reporter insertion is denoted by the shaded box. (a) An inversion takes place between breaks A and B, and subsequently a second inversion occurs involving breaks C and D. Analysis of the DNA recovered using plasmid rescue will incorrectly suggest a deletion. (b) A translocation takes place between the *lacZ*-bearing chromosome and its homologue. This results in a *bona fide* deletion on one chromosome but a reciprocal tandem duplication on the other, i.e. no overall loss of genetic material.

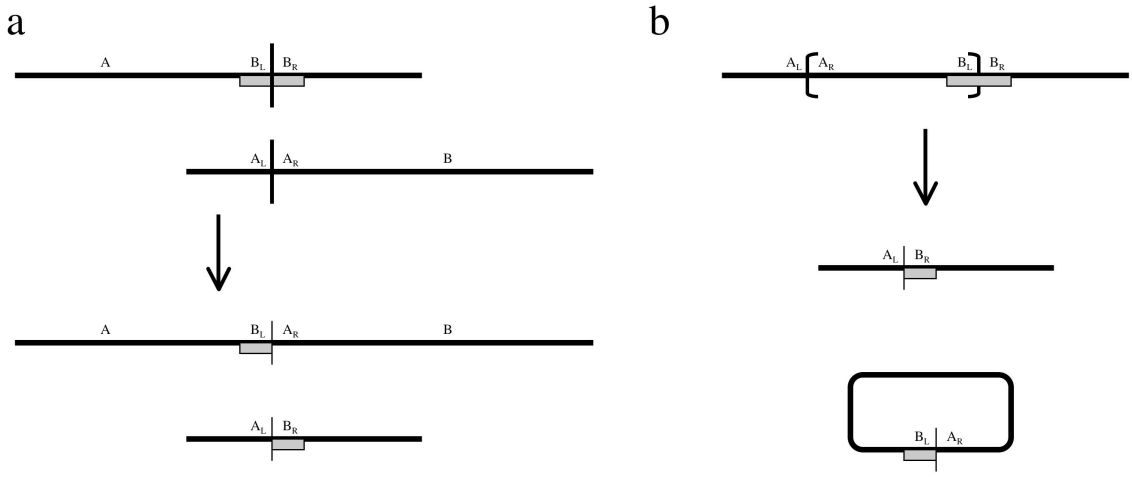

Figure 2. Two-break rearrangements that, when analysed by plasmid rescue of the *lacZ* insertion, look like the (presumably rarer) three-break ones depicted in Figure 4 of (Dollé and Vijg, 2002). Notation is as in Figure 1. (a) A translocation takes place between the *lacZ*-bearing chromosome and its homologue, resulting in a tandem duplication (and a reciprocal deletion on the homologue). (b) A *bona fide* deletion occurs, but the deleted segment is circularised and the transgene recovered from it.

Again, however, Vijg's data seem to argue against this possibility. Firstly, most events either have their other break on a different chromosome than the lacZ insertion – presumably giving no DNA loss – or are inversions rather than deletions (Dollé and Vijg, 2002). Second, the only cell type exhibiting steadily accumulating rearrangements is the postmitotic cardiomyocyte (Vijg and Dollé, 2002), in which expression of the deleted genes may be unaffected, because the excised segment should simply be circularised by the same non-homologous end-joining machinery that successfully joined the

flanking regions (allowing the event to be detected in the first place). Moreover, events that appear to be deletions (because the "other" breakpoint is on the same chromosome and in the same orientation) may in fact be translocations between homologues, or else superpositions of multiple events involving no loss of any chromosomal segment (Figure 1). This would also explain the high level of ostensible three-break events (Dollé and Vijg, 2002): these could more simply be the reciprocal product of a deletion-resembling translocation, or else the circles just described (Figure 2).

Gene expression variability

Recent studies from Vijg's group have emphasised a very different indicator of SMT damage: an increase with age in the cell-to-cell variability of gene expression (Bahar et al., 2006). The variability observed in the heart is typically a factor of about two in young mice and as much as an order of magnitude greater in older mice. These results extend work dating back to Cutler's pioneering work on haemoglobin expression in the brain (Ono and Cutler, 1978) and including a number of recent studies (Lu et al., 2004; Bennett-Baker et al., 2003; Somel et al., 2005).

Striking though these observations are, they only challenge PPCD if two other questions are addressed: what is the degree of variability that is actually pathogenic other than by carcinogenesis, and does the variability increase linearly with age, or exponentially, or does it instead increase asymptotically, remaining within tolerable limits indefinitely (e.g., because when the threshold level is reached the cell dies or elevates repair)? This is a major issue for SMT and conversely PPCD, because tissues are known to exhibit an outstanding degree of "order in the large over heterogeneity in the small,'' again possibly as an anti-cancer defence (Rubin, 2006). Thus far, no clear evidence addressing these questions has emerged.

Conclusion

The possibility that SMT damage contributes to the rate of functional decline with age in mammals is of critical relevance to the feasibility of substantially postponing aging in the foreseeable future. If the only age-related pathology arising from such damage within a normal lifespan is cancer, a total cure for cancer would eliminate nDNA mutations and epimutations as a contributor to human aging. I have argued above that both evolutionary considerations and available data appear to support this welcome conclusion. However, further work is urgently required to resolve remaining uncertainties, especially concerning the consequences of the increasing cell-to-cell variability of gene expression with age.

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