Perspective

Mitochondrial mutations in mammalian aging: an over-hasty about-turn?

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Abstract: The very low abundance of mitochondrial DNA (mtDNA) mutations in nearly all mammalian tissues even in old age has led most mitochondriologists to reject the idea that such mutations might have a causal role in aging, despite the strong circumstantial (e.g. interspecies) evidence that they do have such a role, the promulgation since 1998 of two detailed mechanisms whereby low levels of mtDNA mutations could be harmful, and the report of a transgenic mouse with cardiomyopathy apparently caused by artificially high levels of mtDNA mutations in the heart. A recent report of a mouse with ubiquitously accelerated accumulation of mtDNA mutations and an array of phenotypes reminiscent of aging has abruptly overturned this consensus, with not only the authors but also many other expert commentators suggesting that the mtDNA mutation theory of aging has risen from the ashes. However, there are compelling reasons to doubt the relevance of this mouse to normal mammalian aging and thus to seek further testing of specific mechanistic hypotheses for how mtDNA mutations could cause age-related dysfunction.

It has been over 30 years since Harman extended his original free radical theory of aging[1] with the idea that mitochondria might be the main victims, as well as perpetrators, of free radical damage, and specifically that the mitochondrial DNA (mtDNA) might be the component whose vulnerability to such attack mediates the role of free radical reactions in limiting lifespan.[2] This hypothesis has received enormous attention within biogerontology over the years, especially after it was more successfully publicised 17 years later by Linnane and colleagues.[3] However, mitochondriologists (and, increasingly, also biogerontologists) have become less enthusiastic about this theory since a decade or so ago, as it became clear that the abundance of mutant mtDNA remains extremely low – below 0.1% – throughout life in almost all tissues. Attempts to revive the theory with proposals for how the focal distribution of mutant mtDNA could allow low levels to be pathogenic[4,5] were generally met with skepticism on account of their complexity as perceived (perhaps not altogether justifiably) by the mitochondriology community. However, the possible role of mtDNA mutations in mammalian aging remains attractive in view of the clear correlation between rate of aging and rate of accumulation of mtDNA damage that is seen across species[6] and also between calorie-restricted and ad lib-fed rodents.[7]

In 2000, Zassenhaus's group reported that elevated levels of mtDNA mutations in the heart, caused by introduction of a transgenic mtDNA polymerase carrying a mutation in its proof-reading domain, induced severe cardiomyopathy in mice.[8] Remarkably little attention has been paid to this work, despite the group's subsequent confirmation[9] that this was not an effect of the promoter, as had been suggested after expression of GFP under this promoter was reported to be toxic.[10] Evidently the community was still not ready to entertain the idea that rare mtDNA mutations could be harmful.

This all changed a few months ago, when Trifunovic et al. published on a mouse also expressing a proofreading-deficient mtDNA polymerase, but this time in all tissues.[11] These mice exhibited elevated (3- to 5-fold) mtDNA mutation levels in numerous tissues (all examined), and a plethora of phenotypes associated with aging appeared prematurely (Table 1); life expectancy was about a year. The publication was accompanied by a commentary by two highly respected biogerontologists, George Martin and Larry Loeb, and attracted considerable attention from the popular science press, in which quoted biogerontologists were more or less unanimous in their opinion that, while a

reciprocal result (extending mouse lifespan by improving mtDNA polymerase proofreading) would be even more decisive, these mice persuasively argue that mammalian aging is partly caused by mtDNA mutations. As one of the more vocal proponents of this hypothesis in recent years, I might be expected to be basking in this – but in fact I feel that my colleagues have been altogether too hasty in their reactions to this work.

Tissue	**Phenotype**	**Relative age of onset**
Blood	Anaemia	Early
Spleen	Hypertrophy	Early
Heart	Hypertrophy	Early
Skin	Alopecia	Midlife
Bone	Kyphosis	Midlife
Liver	None severe	n/a
Kidney	None severe	n/a
Brain, gut, muscle	Not reported	n/a

Table 1. Summary of the phenotypes and ages of onset seen in mice with elevated mtDNA mutations in all tissues.[11]

The problem I perceive is one of timing – the order in which phenotypes appear. The cell types in which unambiguously loss-of-function mtDNA mutations (such as large deletions) are found to accumulate most sharply with age are postmitotic ones such as neurons, cardiomyocytes and skeletal muscle fibres. Trifunovic et al. indeed observed an accumulation of mutations in heart and brain and a clear cardiac hypertrophy. However, in contrast to Zassenhaus's results mentioned earlier, this phenotype cannot confidently be ascribed to the presence of mtDNA mutations in the tissue, because it was temporally accompanied by severe anaemia. One of the tissues affected most profoundly and at the earliest age was the spleen, which was already 25% larger than normal at 12 weeks of age, again indicating an early problem with hematopoiesis, to which cardiac hypertrophy is a highly likely compensatory response. A tissue even more severely affected than the spleen was the testes, which were well below half their normal weight even at 12 weeks of age. A third mitotically active tissue, the epidermis, also showed functional decline from 25 weeks of age, manifest as alopecia. In contrast, mitotically less active tissues such as the liver and kidney showed no abnormality at young ages and relatively little even at older ages. Brain and skeletal muscle weight were not reported, unfortunately, but photographic evidence suggests that any effect must have been mild.

The most parsimonious interpretation of the above data is surely that the only tissues directly affected by the elevated mtDNA mutation rate in these mice are those undergoing rapid cellular turnover. This is consistent with the work of Friedman et al., who showed that lethally irradiated mice could be rescued with stem cells lacking mitochondrial superoxide dismutase (MnSOD) activity (and, thus, possessing elevated mitochondrial oxidative damage, presumably including to mtDNA) but that these mice were permanently anaemic.[12] Similarly, the perinatal lethality of MnSOD knockout mice is associated with cardiac hypertrophy – but also with severe defects in haematopoiesis.[13,14] A tissue that may not fit this pattern is the gut, which has recently been shown to accumulate mtDNA mutations in the crypt stem cells;[15] this discovery merits much further analysis, but unfortunately no analysis of the gut in Trifunovic et al.'s mice has yet been reported.

Why is this so important for the bottom line – the ability to infer from Trifunovic et al.'s data, as so many commentators have done, that mtDNA mutations probably do have a causal role in mammalian aging? After all, if a modest elevation of mutation level causes a wide range of phenotypes of accelerated aging and halves lifespan, who cares which tissues suffer when? Isn't the case proven?

Unfortunately not. First there is the Popperian argument: as I have set out in detail recently,[16] intervention in the rate of aging and in life expectancy can only falsify a given hypothesis in a rather

narrow combination of circumstances: namely, when the intervention (a) is designed to accelerate aging, (b) indeed does so at the level of the process postulated to be important in aging, but (c) fails to reduce mean or maximum lifespan. In particular, even a successful increase in lifespan from retarding a given process cannot be conclusively inferred to demonstrate that that process limits normal lifespan, because the intervention may also have retarded other processes.

But secondly there is the Bayesian argument. Bayesian thinking is a more accurate description of the scientific method than Popperian thinking, because it acknowledges that hypotheses are not simply consistent with the totality of available data or inconsistent with it, but rather sit on a spectrum of plausibility, related, among other things, to how elaborate they are and how often supposed falsifications of them have been repeated. It is fair enough to say that slowing down mtDNA mutation accumulation by improving polymerase proofreading would constitute strong evidence that mtDNA mutations matter considerably in aging, because it is implausible that this manipulation would directly slow down anything else (glycation of long-lived proteins, for example) independently of its effects on mtDNA. (By contrast, caloric restriction, which certainly both slows down the rate of mtDNA mutation accumulation[7] and extends rodent lifespan, does so many mtDNA-independent things in addition that we are none the wiser.) But in order to infer with any confidence from Trifunovic et al.'s data that mtDNA mutations matter in aging, we would have to assume that there is rather close to a linear relationship between rate of accumulation of mutant mtDNA (including both *de novo* mutation and replication of already mutant mtDNA) and rate of aging, since the observed reduction in lifespan was by a factor rather similar to the increase in mutant mtDNA.

Let us now assess this assumption in the context of continuously dividing cells such as haematopoietic stem cells. Cell death in such populations can in principle be compensated by symmetrical cell division, so unless cell division imposes an intrinsic aging effect on such cells (such as telomere shortening, which is certainly irrelevant to aging in mice) they should not show any age-related functional decline other than that arising from having the misfortune to inhabit the same body as a bunch of aging non-dividing cells (and extracellular material). The only simple explanation for a failure of mitotically active tissues in mice that have not yet acquired functional deficits elsewhere is that the cell division response to damage and cell death is somehow inadequate. And the simplest way for it to be inadequate is for it to be too slow. The cell cycle can only go so fast, and stem cells are tuned to divide rather slowly, perhaps to minimise the risk of nuclear mutations. If the rate of cell death exceeds the maximal rate of replacement that the stem cells can achieve, depletion of the stem cell pool will result, and that would certainly give the phenotypes that Trifunovic et al. observed. But if cell death is slower than that, depletion of the stem cell pool will not merely be slower: it will be abolished. Campisi's group showed this in spectacular style recently when they demonstrated that mouse fibroblasts, which senesce in standard culture after fewer than ten doublings, do not senesce at all when grown in low oxygen.[17]

My conclusion, therefore, is that proofreading-deficient mtDNA polymerase is a superb tool with which to investigate the role (if any) of mtDNA mutation accumulation in mammalian aging, but that the results presented by Trifunovic et al. do not of themselves tell us very much. By contrast, as things stand the results of Zassenhaus's group, in which proofreading deficiency was expressed only in the heart and only from birth onward (on account of the promoter used), constitute a much stronger, albeit still circumstantial, body of evidence for a direct effect of mutant mtDNA on the tissue in which it resides (or, indeed, on other tissues) in normal aging. Further illumination of this critical question, so long unanswered despite such widespread interest, will probably not be achieved by creating a proofreading-enhanced mtDNA polymerase, partly because most mtDNA mutations may not arise from polymerase errors anyway, and partly because whether or not mtDNA mutations limit mouse lifespan, other processes probably also do, so that appreciable life extension would not result. Hence, the most promising way forward is to construct a library of tissue- and stage-specific knock-ins of the proofreading-deficient mtDNA polymerase and determine which ones are pathogenic and which are not.