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RESEARCH HIGHLIGHT

DNA damage, NF-κB and accelerated aging

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The aging process is the major risk factor for disease and disability yet the cellular mechanisms for aging are uncertain. By studying transgenic mice with altered expression of the DNA repair enzyme, ERCC1, it was concluded that DNA damage is an important, if not the primary mechanism for aging. Moreover it was established that altered activity of the transcription factor, NF-κB (nuclear factor kappa B) mediates the effects of DNA damage on aging. Therefore inhibition of NF-κB might have a role in delaying aging.

Old age is associated with evidence of both DNA damage and activation of nuclear factor (NF)-κB, which is a transcription factor involved with mediating many of the cellular responses to tissue damage, inflammation and oxidative stress. Whether these changes are a primary mechanism for aging or simply reflect downstream effects of other processes that are responsible for aging remains unclear. By manipulating NF-κB in a transgenic model of DNA damage, Tilstra *et al.*[1] have provided a strong argument that DNA damage and NF-κB interact to cause aging in their mouse model. In doing so, they have provided a novel therapeutic approach for rare human premature aging (progeria) syndromes and possibly, aging in general.

There are several progeria conditions known to be caused by rare hereditary deficits in the repair and metabolism of DNA and the cell nucleus.[2] The most well known of these are: Werner syndrome, caused by variations in the *WRN* which is a recQ helicase that undertakes repair of double and single strand DNA and; Hutchinson–Gilford progeria syndrome caused by variations in lamin A/C which is involved in maintenance of the inner nuclear membrane.

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There are also some extremely rare human progeria syndromes associated with mutations in a DNA repair enzyme called XPF–ERCC1 (xeroderma pigmentosum group F–excision repair cross-complementing rodent repair deficiency complementation group 1 DNA repair endonuclease). Human mutations in the XPF component of this enzyme are usually associated with xeroderma pigmentosum but there is a single case report of progeria (called XFE progeria).[3,4] There is also a single human case report of a mutation in the ERCC1 component. This was associated with severe developmental defects, some of which possibly resemble progeria.[4] Tilstra *et al.*[1] chose to study mice where the *Ercc1* gene was either knocked out ($Ercc1^{-/-}$, null ERCC1) or mutated ($Ercc1^{-/\Delta}$, hypomorphic ERCC1 with only 10% of the ERCC1 protein expressed) as their models for premature aging caused by deficits in DNA repair and increased DNA damage.

The *Ercc1* gene was in fact the first DNA repair gene to be cloned.[5] Subsequently $Ercc1^{-/-}$ knockout mice were reported and noted to have a very short lifespan of about 1 month,[6,7] while the hypomorphic ERCC1 mouse had a longer lifespan of about 7 months.[8] The changes in gene expression in the livers of these mice overlapped those seen in normal aging and moreover, the mice prematurely developed features of aging.[3] This supported the concepts that the *Ercc1* knockout was a useful model for the study of aging and that random DNA damage might be a proximal step in normal aging. Interestingly, the liver appears to be a focus for many of these aging effects. The morphology, gene expression and metabolic function of the livers in the *Ercc1* mice[3,8,9] resemble those seen in normal aging,[10,11] while reconstitution of *Ercc1* expression in the livers of the *Ercc1* knockout reversed many of the systemic features of aging and dramatically increased their lifespan.[12]

In their study, Tilstra *et al.*[1] started out by establishing that NF-κB was increased and activated in normal aging and that this occurred prematurely in both versions of the *Ercc1* progeria mice. However, they also noted that activation of NF-κB only occurred in some cells but not others which led them to conclude that aging is a random process of cell damage rather than a uniform process affecting all cells equally.

The next step was to determine whether reduction of NF-κB activity slowed aging in these progeria mice. To do this, they used both a genetic method (deletion of one allele of one of the subunits of NF-κB called p65 (deletion of both alleles is lethal)) and a pharmacological method (8K-NBD which inhibits upstream activation of NF-κB). Both methods delayed the onset of many of the phenotypic and pathological features of aging in the *Ercc1* mice, although data on lifespan were not presented.

So what are the deleterious cell processes that NF-κB activation might be responsible for aging in these *Ercc1* mice? To determine this, Tilstra *et al.* studied the effect of inhibition of NF-κB on gene expression in the liver. Suppression of NF-κB with 8K-NBD suppressed five major biological pathways known to be central to the aging process: immune responses, cell cycle regulation, apoptosis, stress and DNA damage responses, and growth hormone signalling. They also studied the effects of NF-κB inhibition on cellular senescence. Cellular senescence refers to the observation that isolated cells undergo a limited number of divisions before entering a non-dividing stage. Inhibition of NF-κB delayed the onset of cellular senescence and this was associated with reduced production of reactive oxygen species by mitochondria.

This study represents a complex set of experiments focussing on the molecular and cellular biology of aging in a mouse model of a rather esoteric but fascinating type of premature aging. What general conclusions did

the authors make about normal aging after analysing this enormous set of data? They conclude that the first step in aging is random damage to DNA. This leads to increased activity of NF-κB which has a key causal role in many age-related pathologies and processes, in particular, the production of reactive oxygen species by mitochondria. Finally, NF-κB inhibition might have a role in delaying aging.

Does the *Ercc1* mouse model reflect normal aging? It is obvious that the cause of the phenotype of any transgenic model of DNA damage is DNA damage. Moreover, the progeria syndromes caused by errors in DNA repair and metabolism are not premature aging but reflect the early onset of some, but definitely not all, of the pathologies and diseases that are common in old age. It is an oversimplification to conclude that XFE progeria and the *Ercc1* mouse models have some features of aging and a premature death, therefore aging is secondary to DNA damage. Even so, there is accumulating evidence that DNA damage accompanies normal aging[13] and in addition, the gene expression changes in the livers of *Ercc1* knockouts are similar to those seen in normal aging.[3] On the other hand, increased genomic instability was not found to explain all of the reduction of lifespan observed in several types of DNA repair deficient mice.[8] Furthermore, the question remains whether age-related impairment of DNA repair or accumulation of damaged DNA is of sufficient magnitude to have any phenotypic consequences.[13]

The theory that aging is secondary to random DNA damage fits into the major evolutionary view of aging proposed by Sir Peter Medawar in 1952.[14] He argued that natural selection was most powerful for those traits that influence reproduction in early life and therefore the ability of evolution to shape our biology declines with time and aging. Mutations that are deleterious in later life can accumulate simply because natural selection cannot act to prevent them. To put it another way, aging is simply the result of evolutionary neglect. The problem that confronts any such aging theory based on random processes is that the cellular pathways that influence aging and the phenotypes of aging are surprisingly similar between species and individuals.

More recently, the focus of aging research has shifted away from attempting to define the reasons and causes of aging towards understanding how various interventions can influence aging. Overall, there are three groups of interventions that influence aging:[15–18]

1. Genetic manipulation. The genetic pathways that have mostly been studied are those that mediate global cellular responses to nutritional factors (mTOR, sirtuin, AMPK and insulin/IGF-1/GH) or those involved with metabolism and repair of DNA.
2. Dietary manipulation. The most robust effect has been reported with caloric restriction. More recent studies have suggested that the balance of macronutrients might also generate longevity benefits without the necessity to reduce food intake.[18,19]
3. Medications. Resveratrol[16] which activates the sirtuin pathway and rapamycin[20] which inhibits mTOR delay aging and increase longevity in a variety of laboratory models.

Tilstra *et al.*[21] have provided proof of concept for another therapeutic target to delay aging—inhibition of NF-κB. It is of interest that this group using the same mouse model have also shown that inhibition of NF-κB delays age-associated degeneration of the intervertebral discs. NF-κB might prove to be a significant advance in developing therapies that delay aging and increase lifespan,[22] although it should be noted that knock down of NF-κB does not delay normal aging. The development of therapies that genuinely delay aging and progression of age-related diseases will have major consequences for the future health of humans.

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