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Perspective

Does Damage to DNA and Other Macromolecules Play a Role in Aging? If So, How?

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One of the most pervasive ideas regarding the causes of aging is that longevity is constrained in large measure by damage to macromolecules. An increasing body of cellular and molecular data, generated over the past decade or so, has generally supported this "damage accumulation" hypothesis of aging. There remain unanswered questions regarding which types of damage are most important for driving aging. In addition, there have been recent challenges to the damage accumulation hypothesis and a new emphasis on the importance of cellular responses and the sequelae to damage, rather damage per se. New tools and approaches are on the horizon and will need to be developed and implemented before we can fully understand whether and to what extent macromolecular damage drives aging phenotypes.

Key Words: Apoptosis—Cellular senescence—Reactive oxygen species.

An Old Hypothesis—Still Alive!

Among the oldest hypotheses regarding the causes of aging is macromolecular damage. The most vulnerable targets were thought to be long-lived molecules, such as extracellular matrix proteins, or template molecules, such as DNA.

Since the formulation of the "free radical hypothesis of aging" in the 1950s, the main sourcse of damage was thought to be reactive oxygen species (ROS) produced by mitochondria (1; and also see article by Van Remmen & Jones, [2]). More than five decades later, the idea that damage plays a central causal role in aging remains an important hypothesis. Now, however, it is clear that there are myriad types of macromolecular damage. This knowledge poses a major challenge: identifying which types of damage are most important for which tissues, identifying the sources of each type of damage, and determining whether damage can be prevented or reversed. Now, there is also a new perspective in understanding the relationship between damage and aging: cellular responses to damage may be double-edged swords. In the short term, these responses protect cells, tissues, and organisms from damage sequela. Over time, however, cellular responses may compromise the integrity and function of tissues and organs and contribute to aging.

Damage and Aging

Chemical damage to macromolecules (eg, lipids, proteins, DNA) appears to be a general characteristic of living organisms. The sources of damage are broad, ranging from the high fluxes of UV radiation to which the first replicating organisms were exposed to mitochondrial ROS. Mitochondrial ROS are still considered a major cause of aging (3), despite data suggesting that not all age-related phenotypes or diseases are caused by ROS (4).

Damage can interfere with molecular function and hence important cellular processes (eg, transcription, translation, metabolism). Alternatively, damaged molecules can accumulate and nonspecifically impair cellular function (eg, in the case of protein aggregates). Macromolecular damage can also drive aging indirectly. Examples include DNA mutations, caused by the replication of damaged templates or imperfect repair, and cellular death or senescence (irreversible cell cycle arrest), which occur when damage loads are overwhelmingly high. The adverse effects of these cellular responses are likely examples of evolutionary antagonistic pleiotropy. That is, their primary role is to protect the organism from early death or loss of fitness, but they can have deleterious effects later in life.

Both the free radical (1) and the disposable soma (5) hypotheses of aging predict that unrepaired somatic damage is a universal proximal cause of aging. In support of these hypotheses, damaging metabolites (eg, ROS), damaged molecules (eg, oxidized proteins, lipids, DNA), and the sequela of damage (eg, DNA mutations) do in fact accumulate with age (6–11). Moreover, severe damage often coincides with tissue or organ dysfunction. Further, long-lived species tend to excel at preventing or repairing damage (see also article by Miller,

this issue), although there are exceptions (eg, high levels of oxidative damage in the long-lived naked mole rat; 12). Nonetheless, the idea that damage is a main cause of aging will remain unproven until conclusive cause-and-effect relations are established. A succinct overview of the status of the field and important avenues for further study are discussed later.

MOVING BEYOND CORRELATIVE STUDIES

Correlative studies have been a backbone of aging research, so there is a wealth of correlative data suggesting that damage causes aging. Unfortunately, by their nature, correlative studies do not address causality or mechanism. Nor do they allow a rational reconciliation between the preponderance of evidence that damage causes aging and the few studies that appear to question this idea. One correlation—between the efficiency of UV-induced DNA excision repair and species-specific life span (13)—is now known to be biased by the fact that rodents have lost a pathway to repair damage they never encounter in the wild (as largely nocturnal animals with fur, they rarely encounter UV radiation). The question now is how important are excision repair pathways (which also repair non-UV–induced lesions) for determining the life span of mammals, including humans? This question will not be answered by correlative studies. Likewise, correlating damage with functional decline will not determine cause and effect. For example, in skeletal muscle mitochondrial DNA (mtDNA) deletions localize to fibers that are also deficient in electron transport activity, and these defective fibers increase with age in humans and rodents (14,15). Although these elegant findings argue that mtDNA mutations cause the age-related decline in skeletal muscle function, they do not formally prove this point. Because there is an abundance of studies linking macromolecular damage and aging by correlation, additional studies of this nature should not be a priority. Rather, new approaches are needed to integrate already available data sets. In addition, more approaches are needed that mitigate specific types of damage. For example, transgenic mice with reduced mitochondrially generated oxidative damage had a modest but significant increase in life span (16,17). Additional studies of this sort are needed. Further, precise aging phenotypes, rather than life span, should be examined because specific types of damage may cause aging only in certain tissues or organs.

ACCELERATED DAMAGE ACCUMULATION AND PREMATURE AGING

To understand the role of damage in aging, animal models have been generated in which damage accumulates at an accelerated rate, causing the premature appearance of aging phenotypes. In general, these animals are deficient in genes that participate in antioxidant defense or repair DNA or protein damage. Some of these models are invertebrates (eg, *Drosophila melanogaster*) or cells from humans with defects in genes that maintain genomic integrity, but most are mice harboring targeted gene deletions (10).

Most animal models with accelerated damage accumulation show multiple signs of premature aging and a shortened life span. However, there are exceptions. Understanding these exceptions is important—do they challenge the idea that damage drives aging, or do they hint at redundant, adaptive, or compensatory mechanisms that might be harnessed to mitigate damage that causes aging? For example, mice deficient in single antioxidant defense genes do not, in general, age prematurely, although some are cancer prone (4). On the other hand, many mice with engineered defects in DNA repair genes display multiple symptoms of premature aging that are indistinguishable from those displayed by aged wild-type littermates (10). These findings support the idea that the DNA damage, or its sequela, can cause aging. Animals carrying defects in protein maintenance have been less well studied, so more effort is needed in this area. Recently, a defective ubiquitin ligase/co-chaperone (Carboxyl terminus of HSP70-interacting protein) was shown to reduce life span and accelerate age-related pathologies (18), suggesting that impaired protein quality control contributes to aging and mammalian longevity (see also article by Morimoto & Cuervo, [19]).

Although accelerated damage models suggest that damage causes aging, they too do not prove causality. Negative results might be explained by either a lack of careful phenotypic analysis or the need to inactivate more than one of many—often overlapping or redundant—damage control genes. Moreover, temporal and/or tissue-specific regulation of damage may reveal aging only in specific tissues or organs. An important consideration is that aging is variable and differs greatly among even inbred individuals. To understand this variability, future studies might manipulate combinations of genes in the same animal, while providing temporal and tissue-specific control. Likewise, it will be important to generate models that permit titration of specific types of damage—for example, expressing genes encoding damage-generating enzymes or creating chimeras in which damaged cells are mixed with undamaged cells in specific tissues. In principle, tools to create these models are available. Compared with current models, temporal, tissue-specific, titratable, and mosaic models will much more accurately model the damage that accumulates during natural aging.

IMPROVED DAMAGE CONTROL

Conclusions about a causal role for damage in aging will ultimately require increasing the efficacy of damage control and showing that these manipulations extend life span and/or retard specific aging phenotypes. Thus far, there are only few, moderately successful examples of this approach,

which requires careful thought and sophistication regarding the experimental design.

For example, transgenic mice that overexpress the antioxidant enzyme catalase, localized specifically to mitochondria, showed a modest but significant increase in life span (17). However, global overexpression of both catalase and superoxide dismutase, using as transgenic alleles large genomic segments (bacterial artificial chromosomes [BACs], which allow overexpression under normal genetic control), did not increase survival after exposure to paraquat or ionizing radiation, although cells from these mice were resistant to oxidative stress (20). In this case, the antioxidant enzymes may not have been targeted to the most vulnerable sites (eg, mitochondria) or may have had adverse effects (eg, interfering with ROS-mediated signaling, which is important for essential processes such as mitogenesis). Likewise, transgenic mice with constitutively increased activity of p53, a potent tumor suppressor and regulator of damage responses and DNA repair, had a surprisingly short life span, despite very little cancer (21,22). The shortened life span was likely due to constitutively heightened cellular responses to damage. These responses—apoptosis and senescence—may promote aging by disrupting normal tissue function or depleting pools of proliferating cells (23). However, different results were obtained by overexpressing p53 and the Cdkn2a locus, which encodes a positive p53 regulator (Arf) and two other tumor suppressors (p16Ink4a and p15Ink4b), from BACs. These modest overexpressions under normal regulatory control slightly increased life span (24).

Thus, improving damage control by genetic means has the power to establish cause-and-effect relationships, but there are challenges in this approach. These include unanticipated antagonistic effects, such as those discussed earlier. Moreover, upregulation of individual repair pathway components is often toxic because the components often function in large multiprotein complexes, which are disrupted, not enhanced, when only one or a few components are overexpressed. Overcoming this difficulty may require refining methods for introducing mini-chromosomes into animals to allow upregulated expression of entire pathways. Another important problem in this area is the lack of early biomarkers for aging. These are needed because, for the common mammalian models, it is not practical to wait for natural death before knowing whether a manipulation has delayed aging.

Understanding How Damage Leads to Aging

Once a causal role for damage accumulation in aging is established, we will need to understand how damage causes aging. This will require the systematic definition of the types of age-related damage that accumulates in each tissue and organ. For this purpose, comprehensive ontologies (defined vocabularies of terms) will be indispensable because they will render phenotypic descriptions of aging computationally accessible (25). Because no single type of damage, or cellular response to damage, is likely to act alone, a systems approach will be needed to connect damage type, underlying cause, and tissue-specific aging phenotypes. These connections will also be necessary to design and assess strategies for mitigating damage to postpone aging phenotypes.

References

1. Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol*. 1956;11:298–300.
2. Van Remmen H, Jones DP. Current thoughts on the role of mitochondria and free radicals in the biology of aging. *J Gerontol A Biol Sci Med Sci*. 2009; doi: 10.1093/gerona/gln058.
3. Balaban RS, Nemoto S, Finkel T. Mitochondria, oxidants, and aging. *Cell*. 2005;120:483–495.
4. Van Remmen H, Ikeno Y, Hamilton M, et al. Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. *Physiol Genomics*. 2003;16:29–37.
5. Kirkwood T. Evolution of ageing. *Nature*. 1977;270:301–304.
6. Lambert AJ, Boysen HM, Buckingham JA, et al. Low rates of hydrogen peroxide production by isolated heart mitochondria associate with long maximum lifespan in vertebrate homeotherms. *Aging Cell*. 2007;6:607–618.
7. Stadtman ER. Protein oxidation in aging and age-related diseases. *Ann NY Acad Sci*. 2001;928:22–38.
8. Pamplona R. Membrane phospholipids, lipoxidative damage and molecular integrity: a causal role in aging and longevity. *Biochim Biophys Acta*. 2008;1777:1249–1262.
9. Hamilton ML, Van Remmen H, Drake JA, et al. Does oxidative damage to DNA increase with age? *Proc Natl Acad Sci U S A*. 2001;98:10469–10474.
10. Hasty P, Campisi J, Hoeijmakers J, van Steeg H, Vijg J. Aging and genome maintenance: lessons from the mouse? *Science*. 2003;299:1355–1359.
11. Vijg J, Dolle ME. Large genome rearrangements as a primary cause of aging. *Mech Ageing Dev*. 2002;123:907–915.
12. Andziak B, O'Connor TP, Qi W, et al. High oxidative damage levels in the longest-living rodent, the naked mole-rat. *Aging Cell*. 2006;5:463–471.
13. Hart RW, Setlow RB. Correlation between deoxyribonucleic acid excision-repair and life-span in a number of mammalian species. *Proc Natl Acad Sci U S A*. 1974;71:2169–2173.
14. Herbst A, Pak JW, McKenzie D, Bua E, Bassiouni M, Aiken JM. Accumulation of mitochondrial DNA deletion mutations in aged muscle fibers: evidence for a causal role in muscle fiber loss. *J Gerontol A Biol Sci Med Sci*. 2007;62:235–245.
15. Bua E, Johnson J, Herbst A, et al. Mitochondrial DNA-deletion mutations accumulate intracellularly to detrimental levels in aged human skeletal muscle fibers. *Am J Hum Genet*. 2006;79:469–480.
16. Lapointe J, Hekimi S. Early mitochondrial dysfunction in long-lived Mclk1+/− mice. *J Biol Chem*. 2008;283:26217–26227.

17. Schriner SE, Linford NJ, Martin GM, et al. Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science*. 2005;308:1909–1911.

18. Min JN, Whaley RA, Sharpless NE, Lockyer P, Portbury AL, Patterson C. CHIP deficiency decreases longevity, with accelerated aging phenotypes accompanied by altered protein quality control. *Mol Cell Biol*. 2008;28:4018–4025.

19. Miller RA. Cell stress and aging: New emphasis on multiplex resistance mechanisms. *J Geronotol A Biol Sci Med Sci*. 2009; doi: 10.1093/gerona/gln072.

20. Mele J, Van Remmen H, Vijg J, Richardson A. Characterization of transgenic mice that overexpress both copper zinc superoxide dismutase and catalase. *Antioxid Redox Signal*. 2006;8: 628–638.

21. Maier B, Gluba W, Bernier B, et al. Modulation of mammalian life span by the short isoform of p53. *Genes Dev*. 2004;18:306–319.

22. Tyner SD, Venkatachalam S, Choi J, et al. p53 mutant mice that display early aging-associated phenotypes. *Nature*. 2002;415:45–53.

23. Campisi J. Cancer and ageing: rival demons? *Nat Rev Cancer*. 2003; 3:339–349.

24. Matheu A, Maraver A, Klatt P, et al. Delayed ageing through damage protection by the Arf/p53 pathway. *Nature*. 2007;448:375–379.

25. Calder RB, Beems RB, van Steeg H, et al. MPHASYS: a mouse phenotype analysis system. *BMC Bioinformatics*. 2007;8:183.