Review

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Defining the toxicology of aging

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Mammalian aging is complex and incompletely understood. Although significant effort has been spent addressing the genetics or, more recently, the pharmacology of aging, the toxicology of aging has been relatively understudied. Just as an understanding of 'carcinogens' has proven crucial to modern cancer biology, an understanding of environmental toxicants that accelerate aging ('gerontogens') will inform gerontology. In this review, we discuss the evidence for the existence of mammalian gerontogens, as well as describe the biomarkers needed to measure the age-promoting activity of a given toxicant. We focus on the effects of putative gerontogens on the *in vivo* accumulation of senescent cells, a characteristic feature of aging that has a causal role in some age-associated phenotypes.

How do toxicants affect the molecular aging phenotype?

Aging is a complex process that reflects the interplay of genetic and environmental factors. In humans, this intricacy underlies a considerable heterogeneity in the pace of physiological aging: while one disease-free 75 year old may play singles tennis and volunteer at pre-school, another may require a walker and be dependent on care providers. A crucial need in gerontologic research is an understanding of how host genetics and environmental exposures interact over the organismal lifespan to produce common phenotypes of aging, such as increased risk of certain diseases, loss of regenerative capacity, and frailty (see Glossary).

To address this need, there has been substantial effort to use classical genetic analyses to study aging across species. Using unbiased approaches, a large number of genes have been identified in *Caenorhabditis elegans, Saccharomyces cerevisiae*, and *Drosophila* that modulate lifespan through an effect on insulin growth factor 1 (IGF-1)/insulin signaling and metabolism [1–5]. These studies have been supported by analyses of genetically engineered mice as well as studies of human progerias, also identifying genes and pathways that are reproducibly associated with lifespan in mammals [6]. Recent human genome-wide association studies (GWAS) focused on age-associated phenotypes have also proven informative regarding human aging [7,8].

In addition to these genetic results, informative studies of pharmacologic agents suggest that number of compounds affect mammalian aging. Unlike toxicology, pharmacology studies the effect of compounds that are intentionally given for a treatment purpose, and in the modern era, such agents usually engage a known 'target' molecule. The National Institute of Aging (NIA) Interventions Testing Program has begun to serially test compounds to examine their effect on the lifespan of both male and female mice. Such efforts have clearly defined a role for metabolic regulators, such as metformin and rapamycin [9,10], in the modulation of mammalian lifespan, or aging. Although these genetic and pharmacologic approaches have made unquestioned contributions to the study of aging, toxicology, the study of unintended exposures to toxicants, has been under-utilized for this purpose.

Glossary

Ames mutagenesis test: a biological assay used to test the mutagenic potential of a specific toxicant using bacteria deficient in histidine synthesis. Toxicant-treated bacteria are plated on low histidine plates, with colony formation evidence of mutation allowing for histidine synthesis. The number of bacterial colonies is related to the toxicant's mutagenicity.
Biomarker: a biological indicator (e.g., expression of a protein or transcript) of a physiologic process of interest. Generally, optimal biomarkers are causally associated with the biological process that they are intended to monitor. For example, low-density lipoprotein (LDL) cholesterol levels are a good biomarker of atherosclerosis because they have a direct role in its pathogenesis. Fever, however, is a poor biomarker of infection because it has no causal role in infection. Measures that reduce LDL cholesterol will reduce atherosclerosis, whereas anti-pyretics will not treat an infection.
Cellular senescence: a phenomenon where replication-competent cells permanently stop dividing in response to a variety of cellular stresses. Several markers of senescence are known, including expression of the $p16^{INK4a}$ tumor suppressor gene and senescence-associated cytokines (see 'SASP'). Generally, senescence is considered to be distinct from terminal differentiation in that the former is stochastic and the latter is a programmed cellular event.
Frailty: in geriatric care, the state of having low physiological reserve in the absence of overt disease. Frailty is characterized by poor wound healing and tissue repair; sarcopenia and decreased exercise tolerance; and increased susceptibility to age-associated diseases.
Gerontogen: an environmental stimulus, exposure, or toxicant that accelerates the rate of molecular aging.
Healthspan: the period of the organismal lifespan spent in good health. Therapeutic measures to extend the healthspan are generally considered to be more desirable than those that extend lifespan only.
Progeria: any of several genetic disorders associated with premature physiological aging. Examples include disorders of DNA metabolism (e.g., a taxia-telangectasia and Werner syndrome) and laminopathies (Hutchison–Guilford syndrome).
Quiescence: the state where replication-competent cells temporarily exit the cell cycle, generally resting in the G0 or G1 state. As opposed to senescent cells, quiescent cells are capable of cell cycle re-entry when stimulated with mitogens.
Sarcopenia: the age-associated loss of skeletal muscle mass.
Senescence-associated secretory phenotype (SASP): a large suite of cytokines, growth factors, and inflammation-associated proteins that are secreted from a senescent cell.

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The concept of a 'carcinogen', environmental toxicants that promote cancer, has been a crucial component of modern cancer biology. For example, the observation by Sir Percival Pott that chimney sweeps in London exhibited an increased risk of scrotal cancer provided the foundation for cancer epidemiology [11]. Additionally, work showing that tobacco exposure significantly increases the risk of lung cancer led to the identification and characterization of numerous carcinogens in tobacco smoke [12]. The appreciation that environmental agents can have an important causal role in carcinogenesis has led to an advanced set of carcinogenicity assays, both *in vitro* and *in vivo*, to assess the cancer-promoting potential of any new compounds or chemicals released into the environment. Although our understanding of the nature of carcinogens is highly sophisticated, our understanding of how toxicants may promote aging is nascent.

The concept that environmental exposures can promote aging is not new. For example, Tom Perls noted that the rate of physiological aging is determined largely (50–75%) by non-genetic factors [13]. Moreover, George Martin reasoned that there must be environmental agents that accelerate the rate of molecular aging, and coined the term 'gerontogen' for such age-promoting toxicants. He presciently suggested that tobacco smoke might represent a gerontogen, based on its ability to promote disparate age-associated conditions (e.g., cancer, atherosclerosis, emphysema, and pulmonary fibrosis) [14]. According to this theory, differential exposure to largely unknown gerontogens explains much of the non-genetic variation in the rates of human physiological aging.

There has been some work to address the toxicology of aging, with the bulk of this effort focused on DNA-damaging agents. This focus has been reasonable given evidence suggesting that DNA damage modulates mammalian aging, although the precise mechanistic link between DNA damage and aging remains unclear [15,16]. Likewise, there are well-recognized *in vitro* assays for the DNA damage response (e.g., phospho-H2AX, quantification of specific DNA adducts) and mutagenic potential (e.g., Ames mutagenesis test), but it is not known how to use these results in the context of aging. The approach of serially testing compounds in rodents long-term and then phenotyping for aging has not been widely used, given that the design and interpretation of such experiments is challenging [17]. The limited amount of prior work in this area brings into sharp relief the fact that the identification of gerontogens has been hampered by a lack of good biomarkers for molecular age (not due to lack of effort), which in turn reflects an incomplete understanding of the basic science of mammalian aging.

In this review, we summarize efforts in mammals to understand how environmental exposures accelerate or retard aging. The concept of biomarkers features prominently in this discussion because a means to measure various aspects of aging is crucial to this line of research. We discuss how a new biological understanding, particularly the role of cellular senescence in aging, has facilitated the development of aging biomarkers. These methods will translate to human studies aiming to define how unintended environmental exposures contribute to the pace of human aging.

Aging, senescence, and p16^{INK4a}

No single molecular pathogenic pathway accounts for all aspects of aging. Several lines of evidence, however, suggest that activation of *p16^{INK4a}* expression and/or cellular senescence are important contributors to some age-associated conditions. Of relevance to this review, the accumulation of cells with characteristics of senescence *in vivo* is now measurable, providing a means to determine whether a noxious exposure accelerates these aspects of aging mediated by senescence. While it is certainly true that not all gerontogens influence senescence leading to an incomplete view of aging toxicology, we have decided to focus on this concept because biomarkers for senescence independent aging exist and can be applied to the study of gerontogens as described in this review.

Cellular senescence, which was first described in the 1960s by Hayflick and colleagues, is a permanent form of cellular proliferative arrest that is thought to be important in tumor suppression [18]. There are many factors that cause senescence *in vitro*, including telomere shortening [19,20], induction of oncogenes [21,22], oxidative stress [19], DNA damage [23,24], and epigenetic alterations [25], but the importance of these with regard to senescence induction *in vivo* has not been clearly defined. Senescent cells are characterized by phenotypic changes; for example, increased β-galactosidase (β-gal) activity and increased expression of pro-inflammatory cytokines [e.g., interleukin 6 (IL-6), IL-8, macrophage inflammatory protein 1 (MIP1), and vascular endothelial growth factor 1 (VEGF1)] which comprise the senescence-associated secretory phenotype (SASP) (Figure 1). Although initially viewed as an *in vitro* artifact, recent work suggests that senescence occurs *in vivo* in response to certain insults and that senescent cells accumulate with aging, although unequivocal resolution of this issue has proven troublesome owing to the limited nature of *in vivo* markers of senescence.

Recent work in mice and humans, in particular, has suggested that expression of the *INK4a/ARF* (or *CDKN2a*) locus is intimately associated with senescence and aging. This locus, which encodes p16^{INK4a}, is generally repressed in normal adult tissues, but in the presence of certain cellular stresses, expression is potently induced. Expression of p16^{INK4a} inhibits the activity of proliferative cyclin-dependent kinases 4 and 6, which in turn activates retinoblastoma (RB) family proteins to inhibit cell cycle progression, inducing a proliferation arrest at the G1 to S boundary. Importantly, even in cell types in which sustained growth arrest is induced by other pathways (e.g., p53), the prolonged expression of p16^{INK4a} is observed in almost all senescent cells. Silencing of the locus requires the coordinated and persistent activity of the Polycomb group (PcG) complexes, which covalently modify histone tails to place repressive marks [histone H3 lysine 27 (H3K27) and H2AK119] at the *INK4a/ARF* locus (Figure 2). Expression of the *INK4a/ARF* locus requires loss of this silencing and is also associated with binding of transactivating transcription factors [26,27]. However, it is not known how the myriad of cellular stresses that activate