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Nuclear genomic instability and aging

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Abstract

The nuclear genome decays as organisms age. Numerous studies demonstrate that the burden of several classes of DNA lesions is greater in older mammals than young. More challenging is proving this is a cause rather than consequence of aging. The DNA damage theory of aging, which argues that genomic instability plays a causal role in aging, gained momentum recently. Support for this theory stems partly from progeroid syndromes in which inherited defects in DNA repair increase the burden of DNA damage leading to accelerated aging of one or more organs. Additionally, there is growing evidence that DNA damage accrual triggers cellular senescence and metabolic changes that promote a decline in tissue function and increased susceptibility to age-related diseases. Here, we examine the evidence that nuclear DNA damage plays a causal role in aging. We then consider how mechanistically DNA damage in the nucleus could contribute to the aging process.

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INTRODUCTION

Damage to DNA occurs with surprising frequency. DNA lesions can cause mutations, block transcription and replication, and trigger DNA damage response (DDR), which arrest cell cycle progression and activate signaling pathways that impact cell fate: repair, apoptosis or cellular senescence. DNA damage is widely recognized as a cause of cancer and there is now strong evidence that links it to aging and diseases associated with aging.

Endogenous and exogenous factors elicit DNA damage. In addition, DNA is chemically unstable under physiological conditions. In fact, the most abundant DNA lesions are unavoidable products of hydrolysis (deamination or depurination) (1). Damage to the phosphate backbone of DNA can occur during replication or repair. Endogenous genotoxins are products of normal cellular metabolism that react with various bonds in DNA causing oxidation, nitrosylation and alkylation of the deoxynucleosides. These chemical changes can be small, leading to miscoding, or large, bulky additions that block transcription or replication. Bifunctional genotoxins can crosslink bases on the same or opposite strands and even crosslink proteins to DNA. Exogenous genotoxins include ionizing radiation (IR), ultraviolet (UV) irradiation, and alkylating agents, which can cause single- or double-strand breaks (SSB and DSB) and DNA adducts. Any type of DNA lesion can be detrimental to cellular function if not repaired.

Fortunately, many repair mechanisms exist to ensure that the 10^4-10^5 alterations that occur per nuclear genome on a daily basis are virtually all repaired. These include base excision repair (BER), nucleotide excision repair (NER; global and transcription-coupled), interstrand cross-link repair (ICL-R) repair, non-homologous end-joining (NHEJ) and homologous recombination (HR). Each pathway recognizes and repairs a specific class of DNA lesions, *in toto* resolving most but not all DNA lesions. Defects in each of these repair mechanisms can have dire health consequences, illustrating the importance of genomic stability to human health. Inherited defects in DNA repair mechanisms can cause congenital anomalies, immunodeficiency, dramatically increased risk of cancer, or rapid onset of degenerative diseases, depending on the pathway affected and the severity of the defect. Each of these repercussions of DNA damage occurs by a distinct mechanism. Here we focus on how DNA damage contributes to aging and age-related disease, focusing exclusively on damage to the nuclear genome.

EXTREME EXAMPLES ILLUSTRATING THAT DNA DAMAGE CAN DRIVE AGING

Defects in DNA repair accelerate aging

In all fields of biology, creating mutants is a powerful tool for the identification of important gene regulators. By this approach, genome maintenance emerged as a key regulator of aging, often with

discovery occurring first in humans. The majority of human diseases clinically defined as progeroid syndromes, or diseases of accelerated aging, are characterized by hypersensitivity to genotoxins and defects in genome maintenance (Table 1). *Vice versa*, most genome instability disorders are characterized by accelerated aging of multiple organ systems with or without cancer predisposition.

Werner syndrome is caused by a mutation in *WRN*, a gene that encodes a RecQ DNA helicase important for managing replication stress and telomere stability. Loss of WRN leads to growth retardation, premature graying of hair, lipodystrophy and multiple age-related diseases including arteriosclerosis, type 2 diabetes, osteoporosis, and cancer (2). XFE progeroid syndrome is caused by a mutation in *XPF*, which encodes the catalytic subunit of a DNA endonuclease (ERCC1-XPF) critical for NER, ICL repair and the repair of some double-strand breaks. XFE patients show premature aging of the musculoskeletal, sensorineural, cardiovascular, hepatobiliary, urogenital, hematopoietic, and central and peripheral nervous systems (3).

There are also more segmental, or tissue-specific, progeroid syndromes. Xeroderma pigmentosum (XP) is caused by a mutation in one of the XP genes *XPA*-*G* critical for NER or *XPV*, which encodes a translesion synthesis (TLS) polymerase (Pol η) required to bypass unrepaired DNA damage. A major substrate for NER and XPV is UV-induced DNA photoproducts. XP patients are therefore extremely sun-sensitive and have a 10,000-fold increased risk of skin cancers in sun-exposed areas of the skin (4). In addition to dramatic acceleration of skin photoaging, the more severely affected XP patients have premature onset of cerebral atrophy and neurodegeneration.

Cockayne syndrome (CS) is caused by mutations in *CSA*, *CSB*, *XPB*, *XPD*, or *XPG,* which are required for transcription-coupled NER. CS patients exhibit progressive growth retardation, neurodegeneration, loss of vision and hearing, tremors, osteoporosis, age-related organ dysfunction, a prematurely aged appearance and shortened life expectancy (5). There are three forms of CS, severe infantile, childhood (post-natal onset that progresses rapidly) and adult (where CS phenotypes develop more slowly). In the former pre-natal development is affect, while in the latter accelerated degeneration occurs post-natally. A related condition is trichothiodystrophy (TTD) caused by mutations in *XPD*, *XPB* and *TTDA*. In addition to CS-like features, TTD patients display brittle hair and dry scaly skin. Importantly, clinically TTD can occur in the absence of a DNA repair defect. However, these patients do not have premature aging features, stressing the link between genome maintenance and aging.

There are many additional genome instability disorders with multi-organ progeroid symptoms include ataxia-telangiectasia and related conditions, Nijmegen breakage syndrome, both impaired in response to and repair of DSBs, and Fanconi anemia, caused by deficient ICL-R. Mandibular hypoplasia-deafness-progeroid features and lipodystrophy syndrome (MDPL) syndrome and Ruijs–Aalfs syndrome (RAS) are two Werner-like progeroid syndromes. MDPL is characterized by early onset

diabetes, lipodystrophy, steatosis, osteoporosis and loss of hearing. RAS patients have early onset cataracts, grey hair, osteoporosis, sarcopenia, cancer, atherosclerosis, osteoporosis and diabetes. MDPL syndrome is caused by mutations in the replicative polymerase POLD1, required for lagging strand synthesis and for TLS. RAS is caused by mutations in *SPRTN*, which encodes a protein that regulates TLS.

The identification of these diseases spurred the creation of numerous animal models and the characterization of engineered laboratory mutants led to the identification of many new human diseases of systemic of segmental accelerated aging. The animal models are useful for discovering how, when and where (in what tissues) DNA damage contributes to aging, an area where much work is still needed. The models, because of their accelerated aging are useful for rapid hypothesis and drug testing. The models for the large part faithfully model the human genetic diseases. Although, it is notable that mice tend to display a milder phenotype than humans. This might arise from the environmental contribution to human disease, which not well reproduced in experimental model systems. Collectively, however, these human diseases and their conservation in multiple animal model systems strongly support the role of DNA damage as a proximal contributor to aging.

Cancer therapy with genotoxic agents accelerates aging

A second, emerging line of evidence supporting a causal role of DNA damage in aging comes from the oncology literature. Cancer survivors treated with genotoxic agents age several decades faster than individuals not exposed to genotoxins. Radiation and chemotherapy cause vast amounts of DNA damage but have no specificity for tumor cells. Thus, the DNA of normal cells is damaged by these exogenous genotoxins, triggering apoptosis, senescence or mutagenesis. This creates a situation very similar to patients with faulty DNA repair, where there is a greater burden of DNA damage and accelerated aging.

Indeed, survivors of childhood cancer, by the time they are 50-years-old, have a >2.5-fold increased incidence of severe disabling or life-threatening age-related diseases compared to their siblings (6). This includes stroke, myocardial infarction, congestive heart failure, osteoarthritis, renal failure, loss of vision or hearing, infertility and lung fibrosis. Patients with childhood brain tumors requiring therapy targeted to the central nervous system experience neurocognitive decline much earlier than their siblings (7). Chest-directed radiotherapy significantly increases the risk of cardiac disease in childhood cancer survivors with hypertension (8). Treatment with platinum-based chemotherapy and/or radiation therapy significantly increases the risk of insulin resistance and cardiovascular disease within a decade of cancer therapy (9).

Frailty is defined as poor endurance, high risk of falls, disability and hospitalization due to chronic diseases and onset is typically near end-of-life. The incidence of frailty in young adult childhood cancer survivors (mean age 33 years old) is approximately 10%, which is identical to the incidence in the normal population ≥65 years old, illustrating a dramatic acceleration of the onset of frailty following cancer therapy (10). Childhood cancer survivors in their twenties perform the same as 60 year olds in a timed walk and grip strength test (11). The impact of cancer therapy extends to adults, as surviving early stage breast cancer comes with a significant reduction in cardiovascular reserve capacity and therefore increased risk of cardiovascular disease (12). These observations, like those in DNA repair deficiency syndromes, are consistent with DNA damage driving aging.

There is clear evidence in humans and animal models that excessive DNA damage caused by both exogenous and endogenous sources drives accelerated aging. However, for DNA damage to play a causal role in the normal aging process, one expects one or more of the following to be true, that: i) sources of DNA damage, *e.g.*, reactive oxygen species, increase with age; ii) DNA damage and its direct sequelae (activation of the DNA damage response, mutations and senescent cells) increase with age, or iii) the capacity for DNA repair declines with age, and iv) longevity correlates with improved DNA repair. Finally, there should be a plausible mechanism by which DNA damage can drive aging. Here we review the evidence currently supporting each of these predictions.

EVIDENCE THAT DNA DAMAGE INCREASES WITH AGE

Sources of damage increase with age

The free radical theory of aging posits that aging is caused primarily by oxidative damage incurred by reactive oxygen species (ROS) that chemically modify critical cellular biomolecules (13). This theory has evolved over the years to become the oxidative stress theory of aging but the principle is the same, in that the accumulation of oxidative damage drives aging. In support of this theory, there is a large body of literature indicating that oxidative damage to all cellular macromolecules increases with age. Furthermore, overexpression of antioxidant enzymes that detoxify ROS, such as Cu/ZnSOD, MnSOD or catalase, increase the lifespan of *Drosophila melanogaster* by as much as 30% (14). Additionally, most long-lived mutants in *D. melanogaster* and *C. elegans,* have increased resistance to oxidative stress. In mammals, the role of oxidative stress is less clear because overexpression of catalase, SOD1 (superoxide dismutase 1, pancellular expression) or SOD2 (mitochondrial) does not extend lifespan of mice (15). However, overexpression of catalase specifically targeted to the mitochondria does extend the lifespan of some mice up to 20% (16). Additionally, treatment with an antioxidant, nordihydroguaiaretic acid (NDGA) and an activator of NRF2 (master regulator of antioxidant response) extends median lifespan in male mice (17).

The free radical theory of aging evolved to the mitochondrial theory of aging by implicating mitochondria as the primary source of ROS. Electrons leaked from the electron transport chain at the inner mitochondrial membrane can react with molecular oxygen to produce superoxide radical, which can be converted by SOD to yield hydrogen peroxide (H_2O_2). In the presence of transition metal ions (e.g. Fe^{2+} or Cu^{+}), H_2O_2 can be further converted to the highly reactive hydroxyl radical via the Fenton-type reaction. These ROS react locally to damage genes or proteins necessary for oxidative phosphorylation, leading to further uncoupling of electron transport and increased ROS production in a feed forward manner. There is abundant evidence that ROS and oxidative damage increase as organisms, but which cellular target of these damaging radicals and other reactive molecules is health- and life-limiting? If the answer is DNA then one expects DNA damage to accumulate with age.

Levels of DNA damage increase with age

An endogenous oxidative DNA lesion that is commonly measured in mammals because it is relatively abundant is 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG). There is tremendous variability in the absolute level of 8-oxodG reported primarily due to differences in methods of sample preparation and detection (18). Nevertheless, there is some value in measuring relative levels, for instance between young and old animals if the samples are processed and analyzed in parallel.

Several methods have been employed for measuring 8-oxodG levels in nuclear DNA isolated from tissues of animals at different ages (Table 2), each method with different levels of specificity and sensitivity. For instance, 8-oxodG was measured in the nuclear DNA of liver, heart, brain, kidney, skeletal muscle and spleen of mice and rats using high-performance liquid chromatography coupled with electrochemical detection (HPLC-EC) and shown to increase significantly with the age of the animals (19). Using the same method, a 2.75-fold increase in 8-oxodG levels was detected in aged rat liver compared to young (20), In genetically heterogeneous mice, 8-oxodG is significantly increased with age in heart > kidney > liver > lungs (21).

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is also used to measure 8-oxodG. Adduct levels increase with age in the kidney, testes, liver, cerebral cortex, cerebellum and hippocampus of rats (22) and brain liver, lungs, heart, kidneys and testes of mice, with brain having the greatest lesion burden (~2 x 10^{-5} of dG residues being modified) (23). The levels of 8-oxodG are also significantly higher in the lung, heart, spleen, kidney and brain of aged hamsters compared to young controls (24). Notably, 8-oxodG levels are significantly decreased in the liver, kidney and lung of long-lived dwarf rats (25). 8-oxodG levels measured by immunofluorescence detection, are also blunted by a brief period of dietary restriction in rats (26). In contrast, ELISA failed to detect an age-related increase of 8-oxodG in rat kidney (27).

In humans, there are more 8-oxodG adducts in skeletal muscle and peripheral blood mononuclear cells (PBMCs) from older individuals than young (28). 8-oxodG levels also correlate with the presence of age-related diseases such as neurodegeneration and cardiovascular disease (28). Altogether, the above findings strongly support the notion that 8-oxodG levels, and by inference likely also other oxidative DNA lesions, increase with age.

This is strengthened by the measurements of 8,5′-cyclopurine-2′-deoxynucleosides, including the (5′*R*) and (5′*S*) diastereomers of 8,5′-cyclo-2′-deoxyadenosine (cdA) and 8,5′-cyclo-2′-deoxyguanosine (cdG). This is a group of oxidatively-induced DNA lesions that are chemically stable and their formation is inhibited under aerobic conditions during sample preparation (29), therefore a reliable marker of oxidative damage. cdA and cdG levels are higher in kidney, brain and liver of older mice than young (30). Levels of cdA and cdG increase more rapidly in DNA repair-deficient mice modeling progeroid syndromes.

It is less clear whether DNA strand breaks increase with age. This likely stems from the fact that strand breaks are highly toxic, particularly in replicating cells, and low levels are difficult to measure. DSBs are most commonly measured using the neutral COMET assay or phosphorylated histone variant 2AX foci (γH2AX) is used as a surrogate marker (Table 2). Both methods have the advantage of being applicable to single cells. However, neither is specific for DSBs and therefore may lead to an overestimate of lesions. SSBs are measured by alkaline COMET assay or automated fluorimetric alkaline DNA unwinding analysis, again neither of which is specific for SSBs. A more specific but less sensitive assay employs terminal deoxynucleotide transferase dUTP nick end-labeling (TUNEL) to label broken DNA ends followed by ELISA to detect incorporated nucleotides.

Increased breaks were reported in hematopoietic stem cells isolated from older mice compared to young, both when breaks are measured by γH2AX foci and alkaline comet assay (31). γH2AX foci are increased in several tissues of aged mice, including liver, testes, kidney, lung, brain and skeletal muscle (32). The existence of DSBs is supported by other studies that used more direct methods to measure strand breaks. SSBs, as measured by alkaline COMET, are increased in leukocytes from 2-year-old mice compared to young adults (33). In humans, γH2AX foci are reportedly increased in hematopoietic stem and progenitor cells isolated from the aged compared to young (34), as well as in PBMCs (35) and dermal fibroblasts (36). A meta-analysis of 36 studies measuring DNA damage with aging in humans, revealed a significant correlation between age and DNA damage burden (37).

There many, many variables between exposure to gentoxic stress and measuring the resultant damage that differ between tissues and cell types: level of exposure, capacity to detoxify the genotoxin, different metabolic needs, cell cycles, need for transcription and replication, threshold to activate DNA damage responses and the selection of cell fate. This is an important reminder that cross-sectional

studies, which is largely all we have to date, yield information about the burden of DNA damage and cannot inform as to whether lesions accumulate over time. Longitudinal studies on tissues that can be serially accessed are desperately needed.

DNA repair capacity decreases with aging

There are several lines of evidence suggesting that DNA repair capacity might decrease with age. However, it should be noted that it is challenging to measure DNA repair in tissues and the validity of surrogate markers of repair capacity is not well-established. For example, a reduction in expression of DNA repair genes/proteins is not proven to impact DNA repair. Frequently, the reduction in DNA lesions is measured over time as a surrogate for repair. This approach is limited by the specificity of the method used to measure DNA damage (Table 2). Perhaps the most accurate is measuring the ability of a cell or tissue lysate to repair a synthetic DNA substrate. Here, the limitations are the ability to transfect cells and the quality of the lysate, both of which may be affected by organism age.

In nematodes, long-lived strains, such as *age-1*, *daf-2*, and *age1;fer-15,* have increased capacity to remove UV-induced DNA lesions than wild-type worms (38). In placental mammals, the only way to remove UV-induced DNA damage is via NER, providing a very specific means to measure NER capacity. In a host-cell reactivation (HCR) assay, a UV-damaged plasmid is transfected into cells and expression of a reporter gene used as a readout of NER of UV damage. By HCR on PBMCs or fibroblasts, NER capacity declines by 1% per year as humans age (39). An age-related decline in NER capacity was also discovered using an antibody to detect UV-induced lesions in human dermal fibroblasts acquired from donors of different ages (40) and skin biopsies after UV-B irradiation (41). In hepatocytes isolated from rats, clearance of UV-induced cyclobutane pyrimidine dimers (CPD's) is reduced 40% in cells from old animals compared to young (42).

In mammals, BER capacity also appears to decline with age. Use of a synthetic DNA substrate containing a single G:U mismatch to measure BER capacity in tissue lysates revealed a 85% reduction in BER in the brain of old mice compared to newborn animals and a 50% reduction in the liver (43). Incision of a synthetic oligonucleotide harboring various oxidative DNA lesions was used to compare BER glycosylase activity in brain lysates of young and old mice (44). There was a significant decline in older animals. Similar results were obtained using rat brain, with AP endonuclease, DNA polymerase and ligase activity also decreased (45). Urinary excretion of 8-oxo-7,8-dihydroguanine by rats is increased by caloric restriction (46), suggesting that BER of this lesion is improved by life-extending caloric restriction. Similar, to these animal studies, BER glycosylase activity is reportedly reduced in human fibroblasts and leucocytes from old donors compared to young (47).

DSB and SSB repair are frequently measured by applying ionizing radiation to induce lesions and quantifying lesion attrition over time. Of note, DSB repair, in particular, is at least partially replication-dependent and the proliferation rate of cells decline with age. Residual γH2AX foci appear increased in hematopoietic stem/progenitor cells isolated from old individuals compared to young (34). Measuring NHEJ of DSBs in lysates from rat brain or isolated neurons, using a linearized plasmid as a repair substrate, revealed an age-dependent decline in NHEJ (48). Primary human dermal fibroblasts transfected with reporter plasmids to measure NHEJ- or HR-mediated repair of a DSB created by I-SceI restriction endonuclease, revealed a dramatic, linear decline in both repair pathways as the age of donors increased from 20-80 years (36). *In vivo*, attrition of γH2AX foci induced by a chemical carcinogen declines as mice age (49). Use of a transgenic reporter of NHEJ in mice revealed that DSB repair decreases 2-4-fold between middle- and old-age in a tissue-specific manner (50). However, multiple human studies measuring SSB repair in PBMCs were recently summarized (28). Not one demonstrated a change in SSB repair capacity with age, although study design was sub-optimal in most cases.

Of note, senescent cells accumulate with age in mammals (51). There is compelling evidence that BER (47), NER (52) and NHEJ (53) are reduced in senescent cells relative to earlier passage non-senescent cells. Thus, DNA repair may be reduced in a subset of cells that increase in number as an organism ages. Furthermore, genotoxic stress and *ex vivo* culture conditions induce senescence of cells, which will impact measurement of DNA repair. New tools to measure DNA repair *in vivo* will be needed to determine if diminution of repair occurs in all cells and cell types as an organism ages.

Mutations increase with age

Errors during repair or replication of damaged DNA can lead to mutations, which include base substitutions, small insertions or deletions and larger genome rearrangements. In contrast to DNA damage, most of which is rapidly repaired, DNA mutations are irreversible. Hence, somatic mutations are a stable molecular endpoint indicative of DNA damage and genomic instability. Somatic mutation theories of aging were proposed in the 1950s, but were difficult to test in the absence of reliable assays to detect spontaneous mutations. Only chromosomal alterations could be measured by Giemsa stain of metaphase spreads prepared from dividing cells (54). Currently, the method of choice for identifying chromosomal anomalies is *fluorescence in situ* hybridization (FISH), using locus specific probes or chromosome painting, which provide much higher resolution than classical cytogenetic methods and can be applied to post-mitotic cells. Using these methods, it has now been convincingly demonstrated that the frequency of lymphocytes with chromosomal aberrations increases with age in both humans

and mice, with 5-10% of cells carrying at least one aberrant chromosome at old age (55). Most of these events occur because of errors during mitosis and a role of DNA damage in this process is yet unclear.

The resolution of FISH is in the Mb range and insufficient to detect smaller genome structural variation (SV) events or point mutations, which comprise the vast majority of the spontaneous mutations that accumulate with age. To detect such mutations selectable marker systems were developed. The most widely used assay to detect mutations in human cells is based on the X-linked hypoxanthine-guanine phosphoribosyltransferase (HGPRT) gene. When inactivated through a mutation, the cell becomes resistant to 6-thioguanine and clonal lineages of such cells can be analyzed to identify the mutation. Using this, mutation frequencies were found to increase with age in human PBMCs and kidney epithelial cells (56). In mice and rats, HGPRT mutations also increase with age, but is retarded upon dietary restriction, which significantly extends lifespan (57). Conversely, mutation accumulation at the HGPRT locus of splenic lymphocytes is much higher in the senescence accelerated mice (SAM), a model for accelerated aging (58). Importantly, age-accumulated mutations in both humans and rodents are of all types, including base substitutions, small deletions and genome rearrangements.

A major drawback of HGPRT and most other methods based on endogenous reporters is their limitation to cell types that can be grown in culture and cloned. The development of mice and later flies harboring transgenic reporter genes that can be recovered in *E. coli* to study mutations that occurred in the animal, permitted analysis of age-related accumulation of mutations in different organs. Studies with several reporter mice revealed that mutations accumulate with age in many organs, albeit at vastly different rates. Mice with accelerated aging due to increased DNA damage accumulate mutations faster than normal mice (59), while long-lived Ames dwarf mice have lower mutation frequencies (60).

Mutation spectra appear to differ between tissues. Using transgenic mice with a lacZ reporter, a small, chromosomally-integrated construct, a broad range of mutations are detected. In small intestine, a tissue with a high rate of cell turnover and mutation accumulation, point mutations predominate, while in liver and heart, with substantially less cell proliferation, genome structural variations are more common. In young mice, the spectra of point mutations is very similar between tissues, but at older age tissues develop unique mutation spectra (61).

Direct measurement of somatic mutations is challenging, except when they were very frequent, as occurs at repeat loci. Telomeric DNA loses repeat sequence copies as humans age in PBMCs and many other tissues (62). At microsatellite loci, mutations increase 2-fold with every couple of decades of life in human lymphocytes (63). A considerable part of the mammalian genome consists of retrotransposon sequences, in particular LINE-1 repeats, and a fraction of these elements retain their activity, enabling insertional mutagenesis. Increased activity of LINE-1 elements is observed in multiple

tissues of aged mice (64), as well as yeast and flies, suggesting a mechanism for increased mutagenesis in old age.

The reason it is difficult to directly analyze somatic mutations is their low abundance and heterogeneous nature. Since *de novo* somatic mutations are unique to an individual cell, analysis of DNA from bulk cell populations reflect more the germline genotype. Occasionally somatic mutations occur that are enriched through clonal outgrowth and an age-related accumulation of clonally expanded, copy number variants is found in human lymphocytes (65, 66). To obtain a quantitative measure of somatic mutations, sequencing of single cells or clones derived from a single cell is necessary. Whole genome sequencing of clonal organoid cultures derived from human stem cells isolated from multiple tissues of donors age 3-87 revealed that in all organs mutations accumulate at a rate of 40 *de novo* events per year (67). Similar results were obtained in mice (68). Single cell assays are also being used to detect genome structural variations including copy number variations and retrotransposition. However, to date, estimates of private (non-clonal) mutational events vary dramatically, and no studies address the impact of age on mutation accumulation.

In summary, there is overwhelming evidence that somatic mutations accumulate with age, supporting the notion that DNA damage, and its consequences, occur over the lifetime of an organism with regular frequency. The question remains whether the number of somatic mutations in any tissue ever reaches levels high enough to cause age-related organ dysfunction? The first estimates from single cell analysis suggest that by old age each cell has several thousand base substitutions and at least one gross chromosomal alteration, with possibly 5-10% of cells being aneuploid. This burden of somatic mutations is considerable. Hence, the real question seems to be how cells can continue to function with so many mutations? The answer lies in the robustness of genomes due to the diploid nature, redundancy and a distributed functional organization. Initially, therefore, genomes can tolerate many mutations. However, the high level of integration of the many sequence features that encode specific cellular functions could lead to amplification of the effects of multiple random mutations. It is possible, therefore, that even at a linear increase of somatic mutations with age, their effects on health and mortality could be exponential, following Gompertz kinetics. *In toto*, there is a strong correlation between DNA damage, its sequelae, and aging. What remains to be proven is cause and effect.

EVIDENCE THAT IMPROVED DNA REPAIR CORRELATES WITH LONGEVITY

If DNA damage drives aging, one might predict that long-lived species generate fewer endogenous genotoxins and/or have improved genome maintenance. This has been long-debated as part of the free radical or stochastic damage theories of aging. Consistent with that prediction, in *C. elegans*, more than 40 single gene mutations yield an increase in lifespan of at least 20% and all of these mutant strains

are resistant to genotoxins (69). In mammals, the relationship is much less clear. Measuring NER in fibroblasts isolated from seven species yielded a tempting correlation between repair capacity and species' lifespan (70). A more stringent comparison using pairs of size-matched species of bats, rodents and primates with very different lifespans failed to detect a correlation between lifespan and response to UV (71). Comparison of five studies analyzing 40 mammalian species in which NER was rigorously measured by unscheduled DNA synthesis in UV-treated fibroblasts yielded a modest correlation between species lifespan and NER capacity (72).

Several studies report up-regulation of genes required for DNA repair in fibroblasts or tissues from long-lived species compared to short-lived. However, DNA repair is largely regulated post-translationally. It is also important to note that primary fibroblasts differ in their response to culture conditions, in particular, atmospheric oxygen. Thus, a significant challenge to interspecies comparisons is the health of fibroblasts *ex vivo*.

Within a species, long-lived humans (centenarians) are reported to have the same amount of spontaneous DNA breaks as younger people (73) and cells from centenarians appear to respond better to challenge with oxidants (reviewed in (74)). This is often interpreted as improved DNA repair, but may equally well reflect improved response to oxidative stress.

Thus, this is an area where substantially more work is needed to establish whether longevity is driven by nuclear genomic stability. There are diverse and unexpected bits of evidence that support a relationship. For example, a disproportionate number of genes identified in unbiased and targeted genome-wide associated sequencing (GWAS) associated with longevity are involved in genome maintenance (75). One study involved age of natural menopause in ~70.000 women and led to the identification of 44 genetic variants associated with early or late menopause, a strong biomarker of healthy aging (76). Approximately 2/3rd of these are associated with genome maintenance genes. Seven of ten significantly associated pathways are involved in DNA repair. The highly significant overrepresentation of DNA repair pathways indicates an intimate connection between genome maintenance and aging phenotypes. In unrelated studies, we know that reduced expression of the repair endonuclease ERCC1-XPF causes accelerated aging (3), while *ERCC1* is one of the top genes under positive selective pressure in the longest-lived mammalian species, the bowhead whale (77). Intriguingly, while hepatocytes from old rats have impaired NER, caloric restriction, which extends longevity, restored the NER capacity of old rats to that of youthful levels (42). In a human interventional study, brief caloric restriction increased NER capacity in PBMCs of individuals who had low NER prior to dietary intervention (78). Therefore, increased DNA repair capacity could promote longevity and may even prove amenable to improvement.

MECHANISMS BY WHICH DNA DAMAGE DRIVES AGING

If DNA damage causes aging, there must be a logical, universal, underlying mechanism. Spontaneous damage is stochastic. But the response to DNA damage is highly conserved, genetically controlled and with evolution exceedingly more complex. DNA damage triggers activation of signaling pathways termed the DNA damage response (DDR), which facilitates repair and arrests cell cycle progression until repair is complete. If DNA damage is extensive or irreparable, DDR effectors trigger cell death (apoptosis) or cell senescence. These are potent tumor suppressor mechanisms. However, the pay-off is aging.

DNA damage drives cells to senescence

Cellular senescence was first described 50 years-ago by Leonard Hayflick and Paul Moorhead (79) as a state of “irreversible” cell cycle limiting the number of cell divisions (Hayflick limit). Senescent cells are viable but fail to proliferate despite access to nutrients and growth. Later, telomeres were found to shorten with every cell division and, it became clear that critically short telomeres activate the DDR and the creation of Telomere Dysfunction-Induced Foci (TIFs). It is now apparent that multiple stressors, including mitochondrial dysfunction (SOD2 deficiency, inhibition of Complex I, or by SIRT3 knockdown), oxidative stress, strong mitotic signals (oncogene expression or loss of a tumor suppressor) and genotoxic stress also drive cells to senescence. Genetic attenuation of the DDR enables reversal of cellular senescence (80). In contrast, introduction of DSBs in mouse liver, using a tet-inducible SacI endonuclease system, increases the burden of senescent cells *in vivo* and triggers hallmarks of liver aging (81), illustrating a clear path for how DNA damage can play a causal role in aging.

Markers of senescence are detected at higher levels in tissues of older mice, primates and humans, including in skin, liver, pancreatic islets, bone marrow, intestine, kidney, ovary, heart and retina. Senescent cells have altered metabolism (82). They also secrete pro-inflammatory factors and proteases able to alter the local tissue environment (83), providing plausible mechanisms by which senescent cells could promote aging and age-related degenerative diseases. Indeed, senescent cells are found at sites of numerous tissue-specific, age-related diseases, including atherosclerosis, osteoarthritis, sarcopenia, ulcer formation, cancer and Alzheimer’s disease, suggestive of a causative role. However, the most convincing evidence that senescent cells cause aging comes from recent genetic (84) and pharmacologic studies (85) revealing that clearance of senescent cells can prevent or delay tissue dysfunction and extend healthspan.

Genotoxic stress-induced senescence-associated secretory phenotype: Senescent cells induce autocrine as well as paracrine signaling by secretion of pro-inflammatory cytokines, chemokines and proteases, collectively termed senescence-associated secretory phenotype (SASP) (83). SASP

proteins include IL-6, TGF-β, IL-1α, TNFα, MMPs, IGF binding proteins (IGFBPs), plasminogen activator inhibitor-1 (PAI-1), monocyte chemoattractant protein-1 (MCP-1) (86), all of which are known to be upregulated in many tissues with aging. Depletion of IL-6 is sufficient to block senescence in a cell autonomous mechanism, whereas depletion of TGF-β and IL-1α is sufficient to prevent senescence of neighboring cells (87). This is consistent with historical studies on the bystander effect of radiation exposure. X-irradiation induces secretion of factors, including IL-6 and IL-8, which confer DNA damage and death to unirradiated cells (88). Overall, these studies reveal a critical role for SASP factors in the establishment, maintenance and spread of cellular senescence.

SASP occurs only in response to persistent DNA damage signaling, and is dependent upon DDR proteins ATM, NBS1 and CHK2 (89). In fact, overexpression of p16 or p21 can drive senescence in the absence of DDR activation, but this does not induce SASP (90). An early response to senescence stimuli is the induction of IL-1α (91), which in turn, controls expression of SASP factors at the transcriptional level through activation of NF-κB and C/EBPβ (92). Interestingly, inactivation of p53 in senescent cells leads to an increase in SASP, suggesting p53 suppresses the SASP phenotype (83, 89).

Secretion of SASP factors can be favorable or detrimental, depending on the physiological context. For example, SASP likely contributes to early tumorigenesis (83), as well as chemoresistance (93), and potentially neurodegenerative diseases (94). However, SASP is also important for mammalian development (95), tissue repair (96) and wound healing (97). SASP plays an important role in stimulating clearance of damaged, senescent cells by the innate immune system (98). However, inefficient immune clearance of senescent cells in aged organisms is thought to contribute to chronic inflammation of aging.

Pathways involved in senescence:

DNA Damage Response (DDR): DDR signaling consists of three components: DNA damage sensors, signal transducers and protein effectors. Ataxia telangiectasia mutated (ATM) is a very early signal transducer, in particular, in response to DSBs, while a related protein ATR is the signal transducer for replication stress. These kinases initiate events that affect DNA metabolism, cell cycle and cell fate decisions. Short or damaged telomeres (99) and persistent unrepaired DNA damage (89) leads to the accumulation of numerous ATM and ATR effectors at the sites of damage creating TIFs or DNA-SCARs (DNA segments with chromatin alterations reinforcing senescence), which are a hallmark of senescent cells. The proteins are activated by phosphorylation and modulate local chromatin structure and facilitate DNA repair (100). In a similar fashion, activation of oncogenes, such as RAS or RAF, triggers DNA replication origin firing leading to the accumulation of incomplete replication intermediates and DSBs, robust activation of the DDR, and accumulation of DNA damage foci (101).

Initially, it was thought that dysfunctional telomeres drive human cell senescence while damage at non-telomeric DNA drives senescence in organisms with longer telomeres, like mice. However, senescent cells from mice and men have damage foci in both regions of the genome, suggesting that both contribute to establishing and maintaining a senescent state.

ATM and ATR also phosphorylate a number of effector proteins not directly involved in DNA repair, including p53, CHK1, CHK2, and IKKγ, the regulatory subunit of IκB Kinase (IKK) that activates the transcription factor NF-κB. Persistent DNA damage and prolonged activation of the DDR proteins ATM, NBS1 and CHK2 are required to establish and maintain the secretory phenotype of senescent cells (89). ATM and ATR stabilize the transcription factor GATA4 to initiate and maintain SASP factors during senescence (102). Genetic studies provide clear evidence that p53 contributes as well. Expression of a mutant allele of *p53* that is constitutively activated in mice causes an increase in cellular senescence and premature onset of age-related changes including osteoporosis, sarcopenia, loss of subcellular fat, and death (103). Conversely, an additional copy of the *p53* gene under endogenous regulation improves tissue function and extends median lifespan (104). A key target of p53 transcriptional activation is $p21^{Cip1}$, an inhibitor of cyclin-dependent kinases critical for cell cycle arrest and initiation of replicative senescence (105). Under persistent DNA damage, $p21^{Cip1}$ maintains senescent cell viability by inhibiting caspase signaling (106)

Another DDR signaling pathway that induces senescence is regulated by a second cyclin-dependent kinase inhibitor CDKN2A/$p16^{INK4A}$. Transcriptional activation of $p16^{INK4A}$ is induced by the DDR (107). Transcriptional activation of the *INK4a* also leads to expression of $p14^{ARF}$, which binds and inactivates MDM2, preventing proteasomal degradation of p53. Thus, the pathways are not completely independent, although $p21^{Cip1}$ may be more critical for establishing senescence, while $p16^{INK4A}$ is required to maintain it (108). Normally, $p16^{INK4a}$ binds and inhibits CDK4 and CDK6, preventing them from phosphorylating and inactivating the retinoblastoma protein (RB). Activation of $p16^{INK4a}$ and RB causes the formation of dense heterochromatic foci (a hallmark of senescent cells) and permanent repression of E2F responsive genes critical for the G1 to S phase transition (109). Thus, when $p16^{INK4a}$ levels are high and RB is active, senescence is irreversible (80).

$p16^{INK4A}$ levels increase in several tissues in mice and humans with age (110, 111) and increased $p16^{INK4a}$ expression is associated with impaired cellular and tissue function. Genetic ablation of $p16^{INK4a}$ restores function and regenerative capacity in multiple tissues. Clearance of p16-positive cells from aged wild-type mice, increases the healthspan and median lifespan, consistent with these $p16^{INK4A}$-positive, senescent cells playing a causal role in aging (84).

Insulin/IGF-1: Insulin/IGF-1 signaling was the first pathway linked to regulation of aging and lifespan through pioneering work in *C. elegans* (112). *daf2* is a hormone receptor in the nematode *C.*

elegans with homology to insulin and IGF-1 receptor. Mutation of *daf2* in the worm increases lifespan two-fold (112). This is dependent upon DAF-16, a FOXO family transcription factor, HSF-1 a heat shock transcription factor, and SKN-1 a NRF-like xenobiotic response factor. The role of insulin/IGF-1 and FOXO seems to be well conserved. In *Drosophila*, FOXO also modulates insulin signaling and lifespan (113). Moreover, genetic depletion of IGF-1, GH, or the insulin, IGF-1 or GH receptor extends lifespan in mice. Mutations and/or polymorphisms in the IGF-1 receptor, AKT and FOXO3A (components of the same pathway) are associated with longevity in humans (114).

Prolonged IGF activation stabilizes p53 and leads to premature senescence, while IGF-1R knockout in cardiomyocytes delays senescence, SASP and age-associated cardiac remodeling (115). *Klotho* is a gene that regulates lifespan in mice. The secreted form of KLOTHO suppresses insulin and IGF-1 signaling, while the intracellular form inhibits DDR-dependent SASP. Deficiency of KLOTHO causes premature onset of age-related diseases and death (116) and the accumulation of senescent cells (117), whereas overexpression leads to lifespan extension (118). This creates a link between IGF-1 signaling, DDR, senescence and aging.

Genetic depletion of growth hormone/IGF-1 in rodents improves the response of cells to genotoxins as measured by a variety of methods and reduces propensity for cells to undergo cellular senescence (119). Curiously, in murine models of genome instability disorders with accelerated aging, serum IGF-1 is low, yet cellular senescence is increased (3). Chronic exposure of wild-type mice to genotoxic agents also suppresses IGF-1 signaling, while inducing senescence. Collectively, this suggests that reduced IGF-1 signaling protects against the consequences of genotoxic stress, including cellular senescence.

NF-κB*:* NF-κB is a family of transcription factors regulating proliferation and survival in response to a number of stressors, including DNA damage and oxidative stress. Upon nuclear DNA damage, the ATM kinase phosphorylates NEMO/IKKγ, which is the regulatory subunit of the IκB kinase (IKK) complex. IKK then phosphorylates IκB leading to its proteasomal degradation and the translocation of NF-κB to the nucleus to activate transcription (120). It has also been reported that the p65/RelA subunit of NF-κB is a direct substrate of ATM kinase, leading to increased expression of a subset of NF-κB target genes (121). NF-κB activity is greater in aged organisms compared to young (122). NF-κB is also activated in murine models of progeroid syndromes, including the *Zmpste24*-deficient and $Lmna^{G609G}$ models of Hutchinson-Gilford progeria and the $Ercc1^{-/\Delta}$ model of XFE progeroid syndrome (120, 123). Attenuation of NF-κB activity, either genetically or pharmacologically, reduces senescence, delays functional decline and age-related histopathological changes, as well as increasing median lifespan (123). Numerous NF-κB transcriptional targets are SASP factors that play a role in

maintaining and spreading cellular senescence (124). These studies link DNA damage, DDR, NF-κB, senescence and aging.

Sirtuins: Silent Information Regulator 2 (Sir2) was initially identified as a genetic silencing factor and later found to extend the replicative lifespan of yeast (125). Sirtuins are nicotinamide adenine dinucleotide (NAD+)-dependent protein deacetylases that help maintain the replicative lifespan of yeast and contribute to the beneficial effects of caloric restriction (126). The balance between protein acetylation and deacetylation is critical for maintenance of several key cellular processes such as metabolic flux and DDR.

There are seven sirtuins (SIRT1-7) expressed in mammals. Overexpression of SIRT1 in mice suppresses DNA damage and $p16^{INK4a}$ expression, but does not confer longevity (127). SIRT6 overexpression does extend the lifespan of male mice (128). SNPs in *SIRT3*, a mitochondrial sirtuin, are associated with human longevity (129). Sirtuins have many targets, including SIRT1: PGC1α regulating mitochondrial biogenesis and NF-κB, SIRT2: FOXO1 transcription factor, and SIRT6: histone H3 lysine 9 at promoters of glycolytic genes. SIRT1 physically interacts with and attenuates the transcriptional activity of p53 (130). Thus, overexpression of SIRT1 in mammalian cells is able to overcome cellular senescence (131), whereas pharmacologic inhibition of SIRT1 or SIRT2 promotes senescence. These studies link sirtuins with DNA damage and repair, DDR, senescence and longevity.

Exhausting regenerative capacity

DNA damage impacts all cell types in an organism including stem cells, leading to apoptosis, premature differentiation, senescence or the clonal expansion of mutations. DNA damage affects stem cell number and function through cell autonomous and non-autonomous mechanisms, including increased senescence in the stem cell niche. The hematopoietic stem cell (HSC) and its niche, the bone marrow, have been used extensively to examine the effects of DNA damage on stem cell number and function. In a cell autonomous manner, impaired expression or function of the DNA repair enzymes LIG4 (NHEJ), XPD (NER), KU80 (NHEJ) and ERCC1-XPF (NER and ICL-R) reduces the capacity of HSCs to differentiate (132-134). The number of HSCs is also moderately decreased in $Ercc1^{-/\Delta}$ mice, but not $Lig4^{Y288C}$, Xpd^{TTD} and $Ku80^{-/-}$ mice. Interestingly, the HSCs in $Ercc1^{-/\Delta}$ mice label with BrdU, indicating they are no longer quiescent, contributing to their premature depletion. HSCs from mismatch repair-deficient $Msh2^{-/-}$ mice are unable to repopulate the bone marrow of a lethally irradiated mouse, illustrating a cell autonomous loss of function (135).

HSCs transplanted into $Lig4^{Y288C}$ mice graft, renew and differentiate indicating that the niche is not adversely affected by a defect in NHEJ of DSBs (133). In contrast, $Ercc1^{-/\Delta}$ mice engraft poorly (134), indicating the niche is also adversely affected in these DNA repair-deficient mice. Indeed,

ERCC1-XPF deficiency leads to atrophy of the bone marrow stromal call population (136). This illustrates that stem cells are adversely affected by a niche harboring DNA damage.

Muscle-derived stem/progenitor cells (MDSPC) are a heterogeneous population of cells responsible for muscle regeneration. MDSPCs isolated from *Ercc1*$^{-/\Delta}$ mice have an impaired capacity to differentiate and self-renew, identical to MDSPCs isolated from aged mice (137). Interestingly, injecting MDSPCs from young healthy mice into ERCC1-deficient mice extends their lifespan and healthspan, indicating a causal role of stem cell decline in aging.

Neural stem cells (NSCs) are critical for brain plasticity and decline in number and function with age. NSCs are vulnerable to senescence in response to genotoxic stress. NSCs from patients with Cockayne syndrome, caused by impaired transcription-coupled NER, have dramatically impaired differentiation (138). Similarly, NSCs are lost in models of ataxia-telangiectasia caused by mutation of *Atm* (139). Therefore, another mechanism by which DNA damage can promote aging is via loss of stem cell number or function, resulting in loss of tissue homeostasis.

Impacting metabolism and mitochondrial function

Mitochondrial function is dependent upon the integrity of its genome, organelle homeostasis, as well as autophagy/macroautophagy/mitophagy to clear cells of damaged mitochondria. Defects in numerous nuclear DNA repair pathways lead to mitochondrial abnormalities in both patient cells and transgenic mice. In CSB- and ATM-deficient cells, mitochondrial mass is increased, as is ROS (140, 141). It has been suggested that changes in metabolism and decreased mitophagy contribute. CSB and ATM are reported to localize to mitochondria. Thus, it is possible, but not established, that CSB and ATM could play a role in mitochondrial quality control or metabolism that is distinct from their role in maintaining nuclear genomic stability.

XPA is critical for NER, which is exclusively a nuclear DNA repair pathway. Surprisingly, in cells from human XP-A patients, there are numerous changes to mitochondria (142). Mitochondrial mass and membrane potential are increased, suggesting accumulation of dysfunctional mitochondria. ATP and oxygen consumption are increased, suggesting altered metabolism. This was attributed a decline in mitophagy driven by NAD^+ depletion driven by PARP-1 activity and chronic activation of the DDR. NAD^+ depletion also impacts SIRT1 activity, which regulates mitochondrial biogenesis and maintenance. A similar mitochondrial defect is seen in *Atm*$^{-/-}$ mice and NAD^+ supplementation partially rescues their declining health (143). In organisms with reduced expression of ERCC1-XPF, which is exclusively a nuclear repair enzyme, mitochondrial structure and function are also affected. Thus, nuclear instability impacts mitochondrial function via mechanisms that remain to be fully elucidated, but could clearly promote aging.

Impacting autophagy and proteostasis

DNA damage also affects autophagy, which is needed to clear cells of damaged mitochondria and debris. The DDR effector p53 increases autophagy via AMPK-dependent inhibition of mTOR. p53 activation also increases expression of phosphatidylinositol phosphatase PTEN (an inhibitor of the PI3K/AKT signaling pathway), which inhibits mTOR via an independent mechanism (144). Other p53 transcriptional targets, such as DRAM (damage-regulated autophagy modulator), play a proximal role in activating autophagy. In contrast, cytosolic p53 binds Parkin, preventing it from translocating to damaged mitochondria to mark them for degradation (145). Accordingly, p53-deficient mice have improved mitochondrial integrity due to increased mitophagy and as a result, greater cardiac reserve (145).

Chaperone-mediated autophagy (CMA) targets specific soluble cytoplasmic proteins to the autophagolysosome for degradation and is mediated by chaperone HSP70. CMA is upregulated in response to DNA damage and is in fact required to terminate the DDR (146). Collectively, these studies suggest that DNA repair is intimately linked to other cellular “repair” mechanisms. In general, it appears that the DDR signaling enhances DNA repair and autophagy to control the level of damage in the cell.

Interestingly, there is evidence, albeit early, that DNA damage is linked to proteostasis. Expression of proteins containing polyglutamine tracts that drive protein aggregation linked to neurodegeneration activates the DDR and γH2AX foci (147). Interestingly, DNA breaks in cells and γH2AX foci in brain of a murine model of Huntington’s disease, are detected before protein aggregation (147). In a murine model of neurodegeneration caused by tau protein aggregation, there is also increased γH2AX foci, specifically in affected neurons (148). In primary neurons from this model, DNA breaks are increased ~2-fold, as measured by Comet assay (148). Similarly, treatment of primary neurons with β-amyloid peptide inhibits DNA-PK (NHEJ activity) causing γH2AX foci (149). These studies suggest that genomic instability can lead to protein aggregation, which can in turn generate more DNA damage, creating a vicious cycle of increased cellular damage.

CONCLUSIONS

Stitching together numerous studies incorporating diverse species and endpoints creates a convincing argument that DNA damage plays a causal role in aging. DNA damage is now firmly linked to all seven pillars of aging (150): i) nuclear genomic instability is the macromolecular damage with many lasting direct and indirect consequences, ii) epigenetic changes includes post-translational modification of histones as well as direct methylation and oxidation of the nuclear genome, which are undoubtedly impacted by genome maintenance mechanisms, iii) inflammation is the senescence-associated

secretory phenotype driven by DNA damage and DDR, iv) adaptation to stress is the DNA damage response, v) proteostasis, vi) stem cells and regeneration, and vii) metabolism are all impacted by DNA damage. In most cases, DNA damage has been shown to directly driving changes in each of these seven pillars, the same changes that occur with aging. This supports a very distal role for DNA damage in driving aging, initiating a common chain of events universally witnessed with aging.

From a legal perspective, the case is closed. There is opportunity: DNA damage is abundant, unavoidable, time-dependent and stochastic. There is motive: DNA damage triggers strong tumor promoter and other stress response mechanisms that are needed to protect multi-cellular organisms with regenerative potential from rapidly succumbing to cancer. And there is means: DNA damage has the ability to promote aging by triggering cell senescence, cell death and other metabolic changes that incur more damage and loss of homeostasis. The challenge comes now in discovering ways to prevent the crime (DNA damage) in order to minimize the deleterious consequences: inflammation, decline and degeneration seen in old age. Also, there is a need to discover at what age criminal intervention is needed. The safest approaches likely lie in modulating distal (damage prevention) and proximal events (removal/replacement of irreversibly damaged cells) in order to maintain highly conserved responses to damage that aim to protect.

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Table I. Diseases of accelerated aging and their link to genomic instability

Human disease	Affected genome maintenance system	Symptoms associated with aging
Xeroderma pigmentosum	Nucleotide excision repair	Premature photoaging of the skin and neurodegeneration
Cockayne syndrome	Transcription-coupled NER	Ataxia, muscle atrophy, cataracts, retinal degeneration
Trichothiodystrophy	Transcription-coupled NER	Ataxia, muscle atrophy, cataracts
Werner syndrome	Telomere maintenance and replication stress	Wrinkles, alopecia, atherosclerosis, cataracts, osteoporosis, diabetes, sarcopenia, increased risk of cancer
Hutchinson-Gilford progeria	Chromatin organization	Alopecia, wrinkles and epidermal atrophy, arthritis, atherosclerosis, loss of subcutaneous fat, osteoporosis and myocardial infarction
XFE progeroid syndrome	NER and interstrand crosslink repair	Loss of vision and hearing, sarcopenia, epidermal atrophy, glomerulosclerosis, neurodegeneration, hypertension, anemia, osteoporosis
Fanconi anemia	Interstrand crosslink repair	Increased cancer risk and premature exhaustion of the bone marrow
Ataxia telangiectasia	DNA damage response	Neurodegeneration and premature exhaustion of the bone marrow
Down syndrome	Base excision repair	Increased risk of cancer, glucose intolerance, osteoporosis, alopecia, hypothyroidism, cataracts and hearing loss
Nestor-Guillermo progeria syndrome	Chromatin organization	Atherosclerosis, alopecia, cardiovascular disease, pulmonary hypertension, osteoporosis, joint stiffness
Bloom syndrome	Resolution of chromatids during DNA replication	Increased risk of cancer, diabetes, chronic obstructive lung disease
Rothmund-Thomson syndrome	Initiation of DNA replication	Cataracts, alopecia, osteoporosis, increased risk of cancer, epidermal atrophy
MDPL syndrome	Post-replication repair of lagging strand and TLS	Diabetes, lipodystrophy, steatosis, osteoporosis and loss of hearing
Ruijs-Aalfs syndrome	Translesion synthesis	Cataracts, grey hair, alopecia, osteoporosis, sarcopenia, cancer, atherosclerosis, osteoporosis and diabetes

Table 2. Methods to measure DNA damage and DNA repair capacity as a function of lesion resolution.

Lesion	Method	Description	Specificity	Sensitivity
8-oxodG	HPLC-EC	HPLC separation of nucleosides followed by amperometric detection of 8-oxodG	Low	High
	LC-MS/MS	HPLC separation of nucleosides followed by MS/MS detection of 8-oxodG	High	High
	Immunofluorescence	Lesion-specific antibody	Low	Medium
	ELISA	Lesion-specific antibody	Medium	High
	Quantitative long-range PCR	Detect polymerase-blocking lesions; conversion of 8-oxodG to an abasic site with a glycosylase	Low	Medium
cdA & cdG	LC-MS/MS/MS	HPLC separation of nucleosides and identification of modified nucleosides by MS/MS/MS	High	High
DSBs	DI-PLA[†]	Ligate biotinylated ds oligonucleotide which is detected by anti-biotin antibody	High	High
	Immunofluorescence γH2AX	Use anti-γH2AX antibody to mark DNA DSB sites	Low	Medium
	TUNEL	Label free 3′-hydroxyl group of blunt-end DSBs with labeled dUTPs	High	Medium
	Neutral COMET	Electrophoresis of lysed single cells to extrude broken DNA	Low	High
	Pulsed field gel electrophoresis	Separate large DNA molecules (up to 10 Mb) in solid agarose matrix with an alternating electric field	High	Low
SSBs	Alkaline COMET	Electrophoresis of lysed single cells to extrude broken DNA under alkaline conditions	Low	High
	Fluorometric analysis of DNA unwinding	Unwind of DNA under alkaline conditions and, after staining with Hoechst 33258, measure the amount of remaining ds DNA with fluorometric analysis	Medium	Low
	ELISA	End-label broken strands with antibody detection of incorporated nucleotides	Medium	High

[†]DNA damage *in situ* ligation followed by proximity ligation assay

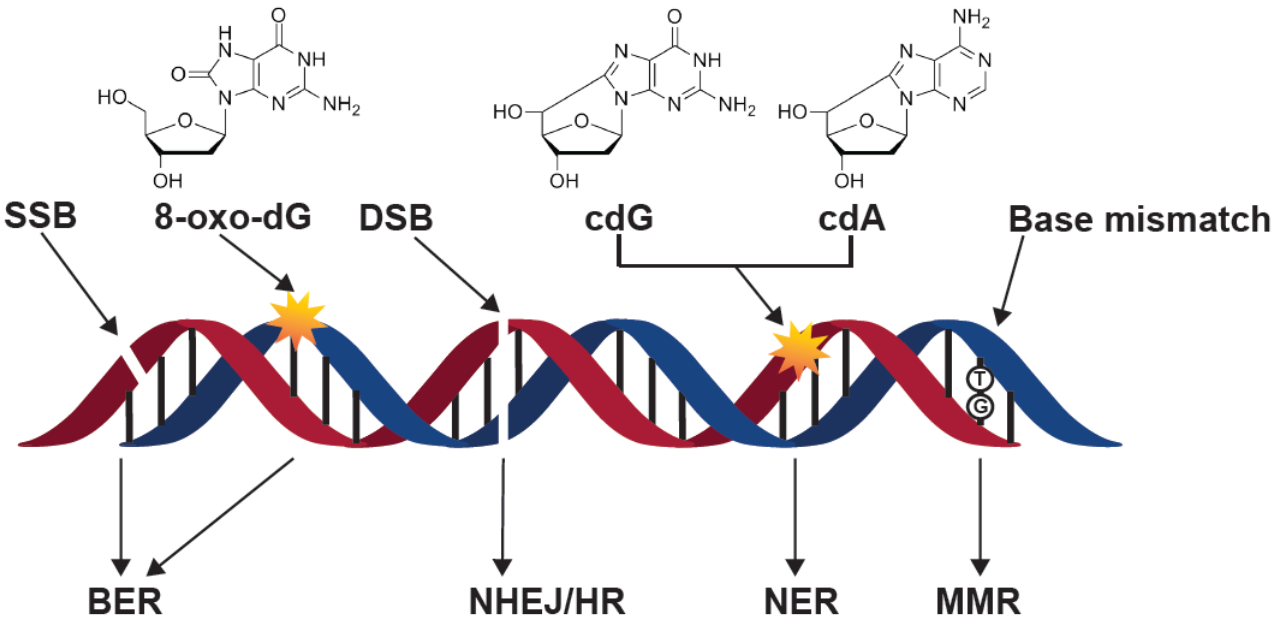

Figure 1. DNA lesions that are implicated in aging and their corresponding repair pathways. DNA damage levels increase with age, which have been demonstrated by the detection of several lesions such as 8-oxodG, cdG, cdA and strand breaks.
Abbreviations: DSB, double-strand break; SSB, single-strand break; BER, base excision repair; NHEJ, non-homologous end-joining; HR, homologous recombination; NER, nucleotide excision repair; MMR, mismatch repair

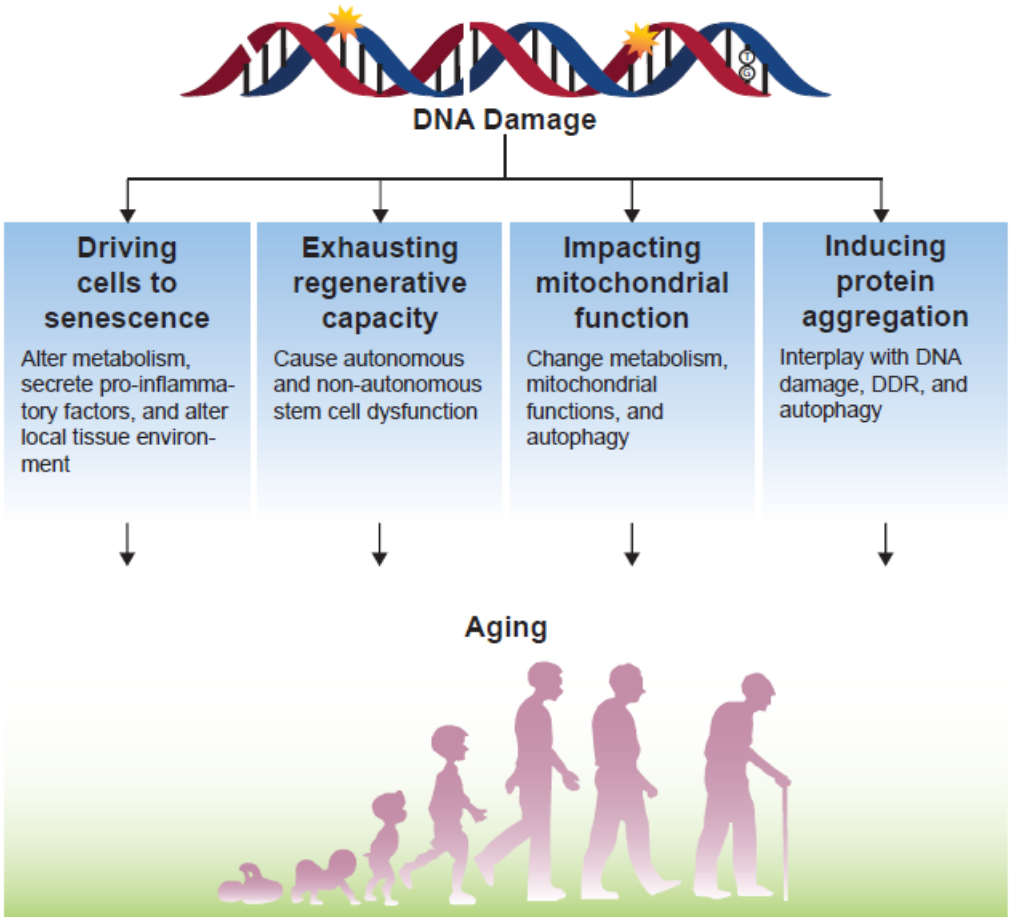

Figure 2. Mechanisms by which DNA damage drives aging. DNA damage plays a causal role in aging by triggering senescence, exhausting replicative capacity, impacting mitochondrial function and inducing protein aggregation. Senescent cells have altered metabolism, and they can secrete pro-inflammatory factors and alter local tissue environment, thus contribute to aging and age-related degenerative diseases. In addition, stem cell function can be impacted by DNA damage by both cell autonomous and non-autonomous mechanisms. Proper function of mitochondria is dependent upon genome integrity, and genome instability can lead to protein aggregation, which can generate more DNA damage in turn.