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Nuclear DNA Damage as a Direct Cause of Aging

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ABSTRACT

Evidence is presented that damage to nuclear DNA (nDNA) is a direct cause of aging in addition to the effects of nDNA damage on cancer, apoptosis, and cellular senescence. Many studies show significant nDNA damage with age, associated with declining nDNA repair. Evidence for decline of nDNA repair with age is reviewed. Mammalian lifespans correlate with effectiveness of nDNA repair. The most severe forms of accelerated aging disease in humans are due to nDNA repair defects, and many of these diseases do not exhibit increased cancer incidence. High rates of cellular senescence and apoptosis due to high rates of nDNA damage are apparently the main cause of the elderly phenotype in these diseases. Transgenic mice with high rates of cellular senescence and apoptosis exhibit an elderly phenotype, whereas some strains with low rates of cellular senescence and apoptosis show extended lifespan. Age-associated increases of nDNA damage in the brain may be problematic for rejuvenation because neurons may be difficult to replace and artificial nDNA repair could be difficult.

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ABBREVIATIONS

8-OHdG 8-hydroxydeoxyguanosine (oxidative DNA damage), BER Base Excision Repair, GGR-NER Global-Genome Repair (subtype of NER), HGPS Hutchinson-Gilford Progeria Syndrome, HR Homologous Repair, MMR Mismatch repair, nDNA nuclear DNA, NER Nucleotide Excision Repair, NHEJ Non-Homologous End-Joining, oxo8dG 8-oxo-7,8-dihydroguanine (equivalent to 8-OHdG), PARP Poly(ADP-ribose) polymerase, SENS Strategies for Engineered Negligible Senescence, TCR-NER Transcription-Coupled Repair (subtype of NER), TTD Trichothiodystrophy, WS Werner's Syndrome

INTRODUCTION

Nuclear DNA is subjected to a constant barrage of damage, mostly hydrolysis, oxidation, and alkylation.[1] Bases are deleted, bases are mutated, bases are crosslinked with proteins or with other bases, nDNA sequences can be deleted or frame-shifted, and one or both nDNA strands may be broken. Nuclear DNA repair enzymes and associated cell cycle

checkpoint enzymes protect nDNA by either fixing nDNA damage or forcing cells into apoptosis or senescence. But there is considerable evidence that nDNA damage causes cellular dysfunction that manifests as aging independent of apoptosis or cellular senescence.

DNA repair enzymes are not infallible. DNA repair enzyme genes can themselves be damaged. And nDNA damage that leads to mutation is not recognized by DNA repair enzymes at all. Mutated DNA is as faithfully replicated as non-mutated DNA. Cells with increasing levels of nDNA damage, mutation, and epimutation can become increasingly dysfunctional.

The efficiency of nDNA repair enzymes might be expected to decline with age, but whether this actually happens has been controversial. Reviews of the literature between 1985 and 1990 concluded that there is not sufficient evidence to justify the claim that nDNA repair declines with age. The earliest of these reviews[2] examined nDNA repair of ionizing radiation, X-rays, and ultraviolet light, concluding that aging cannot be a direct consequence of a decline in nDNA repair capability and that all aging cannot be a consequence of accumulated nDNA damage. The next review[3] concluded lifespan has not been proven to be dependent on overall nDNA repair efficiency — while acknowledging the limited experimental approaches that had been used (particularly an excessive focus on repair of pyrimidine dimers resulting from ultraviolet light). The last of the reviews[4] concluded that age-related accumulation of nDNA alterations occur "at all levels", but that there is no evidence for a drastic decline in nDNA repair during aging. This latter review may have suffered from the same limitation noted in the previous one — relying excessively on data concerning repair of ultraviolet light damage in rat skin.[5] A more recent review — covering more recent research and a wider range of nDNA repair types — has concluded that "all pathways of DNA repair... become less efficient with age."[6] The conclusions of that review are supported by additional evidence provided in this one.

Cancer incidence increases exponentially with age in humans up to age 80.[1,7] If the subset of nDNA damage/mutation leading to cancer increases exponentially, then the subset of nDNA damage/mutation not leading to cancer would likely also increase exponentially with age (although an exponential increase in cancer does not necessarily imply an exponential increase in nDNA damage/mutation leading to cancer). A decline of efficiency for nDNA repair enzymes, cell cycle control enzymes, and apoptotic enzymes would mean an increasing rate of formation of mutations, epimutations, and unrepaired nDNA damage leading to cellular dysfunction as well as to cancer.

Aging is the result of crosslinking and other kinds of damage to macromolecules and tissues. All aging cannot be due to damaged nDNA, but there is persuasive evidence that damaged nDNA makes a significant contribution to cellular dysfunction associated with aging.

INCREASED NUCLEAR DNA DAMAGE AND MUTATION WITH AGE

Mouse kidney cells show a significant age-associated increase in nDNA damage that correlates with pathology.[8] A comparison of clonal growths of neural stem cells derived from 2-month and 2-year old mice showed no deletions or loss of heterozygosity in the chromosomes of the young mice, but in the old mice more than one third of the samples showed a complete deletion of at least one chromosomal region, and most samples showed loss of heterozygosity on three or more chromosomes.[9] Haematopoietic stem cells from young mice show few signs of phosphorylated H2AX histone (a marker of nDNA damage), whereas 82% of stem cells from old mice exhibited phosphorylated H2AX histone, usually in multiple foci.[10]

A study of rat liver found **8-OHdG** (8-hydroxydeoxyguanosine, also known as **oxo8dG**, a lesion representing about 5% of all forms of DNA oxidation damage) to be present in about one of every 130,000 nDNA bases.[11] A study of mouse heart, liver, brain, kidney, spleen, and skeletal muscle tissues showed significant increases in nDNA 8-OHdG with age for all tissues studied. The authors estimated that the rate of formation of 8-OHdG in brain tissue is more than tripled in old mice compared to young mice.[12] A study of rat brains found total nDNA levels declined about 60% in the cortex, striatum, and hippocampus in old rats as compared to young rats, and the levels of nDNA oxidation damage due to 8-OHdG were nearly double for the old rats in all brain areas.[13]

In the human cerebral cortex nDNA damage in many gene promoters is evident after age 40 and the damage is most pronounced after age 70.[14] One mouse study indicated significant age-associated increases in mutation in the intestine and heart, although for the brain, mutation increase is mainly in the hypothalamus and hippocampus.[15] Another mouse study showed the increase in mutations per cell division in old mice over young mice to be about 15 times greater in liver and about 30 times greater in brain.[16] There are nearly twice as many double-strand nDNA breaks in the cerebral cortex of adult (180 days) rats as in young (4 days) rats — and old (>780 days) rats have more than twice the double-strand breaks as adult rats.[17]

Epigenetic loss of gene expression contributing to progressive physiological dysfunction in older mice can be up to two orders of magnitude greater than somatic mutations.[18] A mouse study of lung and kidney tissue found evidence of nDNA damage due to epigenetic changes and chromatin structure disorders in older mice (and considerably more single-strand breaks).[19] Nuclear DNA methylation in a variety of tissues declined with age about twice as fast for the mouse *Mus musculus* as for the mouse *Peromyscus leucopus* (which has more than twice the lifespan).[20] Gene expression in mouse heart cardiomyocytes becomes significantly more heterogenous with age.[21] Hepatocytes from aged rats displayed less than one-tenth the level of nDNA synthesis in response to epidermal growth factor as is seen in young rats.[22] In both humans and rats assays of a variety of tissues showed an increasing heterogeneity of gene expression with age — indicative of weakening of gene regulation.[23]

Because most of these studies only compare old and young organisms, an argument can be made that the rate of damage could be leveling off in old age rather than increasing. But a study of human epidermal cell renewal found a relatively constant rate of renewal

up to age 50, followed by a dramatic decline thereafter.[24] Two other studies that examine many intermediate ages support the conclusion of a linear increase in nDNA damage and epimutation. Although individual variation is wide, a linear increase is seen in human leucocyte nDNA levels of 8-OHdG between the ages of 20 and 70.[25] Samples of human bronchial epithelial cells from eight human autopsies showed a significant linear decline in 5-methyldeoxycytidine (epigenetic methylation) between teenage and late age 50s, indicative that aging is associated with dysdifferentiation.[20]

TYPES OF DNA REPAIR

There are many forms of DNA damage and DNA repair,[26] but not all of them are necessarily significant for aging. Mismatch repair (**MMR**) enzymes, for example, recognize and remove mispaired nucleic acid bases, such as pairing of cytosine with thymine. The major consequence of MMR defects is a great increase in microsatellite mutations resulting in hereditary nonpolyposis colon cancer (HNPCC) rather than accelerated aging.[27,28]

Base excision repair (**BER**) repairs damage to single nucleic acid bases (cytosine, guanine, thymine, adenine, or uracil). One of many kinds of **glycosylase enzyme** can recognize and remove the damaged base. A specialized polymerase (**DNA polymerase ß**) attaches the new base. Deletion of essential BER genes in transgenic mice is lethal before birth.[29]

Nucleotide excision repair (**NER**) repairs damage affecting more than one nucleic acid base. Repair of covalently bonded adjacent pyrimidine bases created by ultraviolet light is a classical object of study for NER enzyme activity. NER is more complex (involving more steps and more proteins/enzymes) than BER and is more error-prone than BER. Although BER operates on mitochondrial DNA as well as nDNA, there is no evidence that NER occurs in mitochondria. The two subtypes of NER are: (1) global-genome repair (**GGR-NER**), which recognizes damage throughout the genome, and (2) transcription-coupled repair (**TCR-NER**), which recognizes damage that stalls transcription of RNA from nDNA. Defects in GGR-NER lead to cancer, whereas defects in TCR-NER more readily lead to apoptosis.[30] Cancer cells can’t replicate if transcription is blocked.

BER and NER are utilized for damage to a single strand of nDNA. But for severe forms of damage that cause both strands of nDNA to break, homogenous recombination (**HR**) or non-homogenous end-joining (**NHEJ**) is required. HR can only function when a sister chromatid (during mitosis) or a homologous chromosome (during meiosis) are available to serve as a repair template. Therefore, HR can only function in late S phase and G_2 phase of the cell cycle. NHEJ, by contrast, is a much more frequently used, but highly error-prone form of double-strand break repair. NHEJ is unlikely to result in correct sequences at the site of the break, but NHEJ does succeed in reuniting the chromosome. The large amount of "junk DNA" in the genome apparently accounts for the frequent success of NHEJ.

DECLINE OF NUCLEAR DNA REPAIR WITH AGE

It was noted in the introduction that older reviews were unable to establish whether nucleotide excision repair (NER) declines with age, and that those reviews relied heavily on NER repair of ultraviolet (UV) light damage. A review of more up-to-date studies of human and animal tissues subjected to ultraviolet radiation shows that the question of whether NER repair of UV damage declines with age has still not been resolved, and may vary according to tissue or experimental method. Some studies have found no decline,[31-33] whereas others saw a decline of UV damage repair with age.[34-37]

Many studies have shown a decline in NER for human dermal fibroblasts with age. One study showed NER of human dermal fibroblasts was about half as great for cells from young adults as for infants, and about one third as great for cells from old rather than young adults — an effect attributed to reduced repair protein levels and activity.[38] Another study found the decline in NER to be the result of modified cell cycle factors.[34] But according to yet other studies the decline in NER is due to deficiency of repair synthesis factors.[35,39] Pretreatment of elderly human fibroblasts with oligonucleotides completely corrected the age-associated decrease in NER.[40]

Much of the reduction in base excision repair (BER) with age is due to a decline in glycosylase activity. Human fibroblasts and leucocytes from old donors show reduced BER glycosylase activity compared to cells from young donors.[41,42] In BER of mixed germ cell nuclear extracts, uracil-DNA glycosylase activity fell 25% for middle-aged mice and 53% for old mice compared to neonatal and young adult mice (a linear decline).[43] Glycosylases that eliminate oxidized and methylated bases in BER have shown many times less activity in old human fibroblasts and leukocytes than in young cells.[41] Although most areas of the mouse brain show little age-related difference in DNA glycosylase activity for selected glycosylases, activity in the cerebellum declined nearly 50% for uracil DNA glycosylase (UDG) and nearly 90% for oxoguanine DNA glycosylase (OGG1).[44] A study of 8-OHdG repair in kidney and liver tissue of young and aged rats showed a significantly lower BER in the older rats.[45] A study of the DNA glycosylase enzyme required for BER of 8-OHdG in lymphocytes of 78 healthy humans ranging from newborn to 91 years of age showed a significant linear decline to less than half newborn values, with very high individual variation.[42]

But BER decline can also be due to reduced DNA polymerase ß activity. An *in vivo* study of mouse tissues (brain, liver, spleen, and testes) found an age-associated decline in BER of no less than 50% in all tissues — attributed to decreased DNA polymerase ß enzyme activity.[46] Young mice expressed 50% more DNA polymerase ß in response to oxidative stress than old mice.[47] A significant decrease in DNA polymerase ß was also seen in aging rat brain neurons.[48,49] Activity of polymerase ß in the rat neurons dropped about 50% — significantly less than the assayed levels — due to accumulation of catalytically inactive polymerase ß molecules.[48] DNA polymerase ß activity in the livers of old rats was found to be half that of young rats.[50] Further study on aging rat brain neurons found that both DNA polymerase ß and DNA ligase were needed to restore BER.[51] But another

study found that polymerase ß addition failed to restore the age-associated decline of BER in mouse liver and brain.[52]

A study of rat liver and kidney tissue found a significant decline in repair of single-strand breaks in old rats as compared to young rats.[45]

Non-homologous end-joining (NHEJ) is significantly reduced in cerebral cortex neurons from adult rats, and even more reduced in the neurons from old rats, compared to neonatal rats.[53] An assay of NHEJ in neurons of rat cerebral cortex found that, compared to neonatal rats, adults showed a 28% decrease in activity and old rats showed a 40% decrease in activity.[54]

ATP-dependent NHEJ peaks in the rat brain at postnatal day 12 and gradually declines thereafter at least up to 210 days.[55] A study of mouse brain tissues concluded that the decline in nDNA repair with age is secondary to accumulated mitochondrial damage and decline in ATP production.[56] Reduced ATP production is associated with aging-associated reduced BER in the human cerebral cortex.[14]

A study of human lymphocytes showed that two proteins required for NHEJ — Ku70 and Mre11 — decline with age.[57] A study of irradiation of human peripheral blood mononuclear cells indicated that decreased nDNA repair in elderly subjects was associated with impaired migration of phosphorylated Ku80 protein from the cytoplasm to the nucleus.[58] A study of human hematopoietic stem cells from healthy donors found that, compared to newborns, there was a nearly 3-fold reduction in Ku70 protein expression in young (30s age) and a more than 6-fold reduction in expression in old (80s age) donors.[59] A study of Ku70 protein in human lymphocytes showed a linear decline between ages 20 and 80 (with wide variation). The same study showed greater longevity in a group of people with higher Ku70 compared to a control group.[57] Note that this study involved a span of ages (not just young and old), which supports the claim that NHEJ capability declines linearly with age.

SPECIES DIFFERENCES IN DNA REPAIR AND LIFESPAN

Evidence of increasing nDNA damage and mutation — and reduced nDNA repair — with age is not necessarily proof that this damage is contributing significantly to aging. But the correlation of nDNA repair activity between mammalian species with the maximum lifespan of those species provides circumstantial evidence for the relevance of nDNA damage repair to aging.

A positive correlation between lifespan and the amount of nucleotide excision repair (NER) in response to ultraviolet light exposure has been seen in fibroblasts of seven mammalian species, with human fibroblasts showing about five times the synthesis as rat fibroblasts.[60] A study which correlated maximum lifespan with NER of ultraviolet light nDNA damage in twelve mammalian species found a six-fold difference in the NER activity of mice and men. A roughly linear correlation was seen between nDNA repair and maximum lifespan for the mammals, with humans having the most active NER.[61]

Poly(ADP-ribose) polymerase (PARP) is a regulator of base excision repair (BER) and also has a role in non-homologous end-joining (NHEJ) as well as in telomere maintenance.[62] A positive correlation between PARP activity and longevity has been seen in mononuclear blood cells of 13 mammalian species, with human cells showing about five times the PARP activity of rat cells.[63] Most nDNA damage due to gamma-radiation is repaired by BER, and mammals with longer lifespans have been shown to have more efficient nDNA repair of gamma-radiation.[64]

The correlation of nDNA repair with mammalian lifespan cannot prove causality, however. It is reasonable to believe that mammals that have evolved to live longer have evolved to have greater protection against cancer.

THE CONCEPT OF ACCELERATED AGING DISEASE

One biogerontologist has challenged the concept of "accelerated aging" on grounds that it is too easy to shorten lifespan with poisons and defective genes – and too hard to validate in the absence of biomarkers of aging that normal aging has been accelerated.[65] Against this view is the claim that the association of generally recognized "elderly phenotypes" with certain diseases or genetic defects justifies the "accelerated aging" classification.[66] The accelerated mortality associated with high blood pressure, HIV infection, or a dangerous occupation cannot be called accelerated aging, but accelerated aging would certainly be expected to increase mortality risk.

Biogerontologists have long searched for "biomarkers of aging" that could be used to distinguish biological age from chronological age.[67] But aging is probably the product of many forms of damage rather than one form. Distinct biomarkers should be required for cell loss, extracellular garbage, intracellular garbage, protein crosslinks, etc. Moreover, because aging damage is due to both heredity and exogenous damaging agents, different tissues would be expected to manifest the different types of aging at different rates. Tobacco smoke, for example, accelerates cardiovascular aging.[68,69] Diabetes and dietary advanced glycation endproducts (AGEs) accelerate the protein crosslinking form of aging damage.[70] If aging is an assortment of forms of damage, increasing each form of damage will increase the damage-specific biological age of the corresponding phenotype and biomarker.

The description of accelerated aging disease as segmented is based on the mistaken notion that there is a single form of aging damage. Because there are many forms of aging damage, ***all*** aging is segmental. Some people get osteoporosis, some get arthritis, some get cancer, some lose their hair, etc. The form of aging manifested in any one person will be the product of the heredity and exogenous damaging agents relevant for that person.

ACCELERATED AGING DISEASES AND DEFECTIVE DNA REPAIR

It is surely no coincidence that so many diseases described as manifesting accelerated aging are due to defective nDNA repair, and that the diseases most strongly associated

with accelerated aging are the ones with the most severe nDNA repair defects. It is worth discussing in some detail the diseases considered the most severe manifestations of accelerated aging.

Dermatologists commonly distinguish **photoaging** from **intrinsic aging** in the skin, attributing most skin aging to photoaging. There is considerable overlap in the pathophysiology of photoaging and intrinsic aging,[71,72] indicating that skin photoaging can be regarded as a form of accelerated aging. Ultraviolet radiation stimulates collagen degradation while inhibiting collagen production,[73] increases oxidative DNA damage,[74] and causes stress-induced premature senescence in skin fibroblasts.[75]

The tissue-specific accelerated aging disease **xeroderma pigmentosum** (**XP**) is due to a defect in one of seven genes/proteins which are required for nucleotide excision repair (NER). XP victims show dramatically accelerated aging only in areas of skin exposed to the sun, and have a skin cancer rate that is more than a thousand times greater than normal.[76] The fact that XP victims show such dramatic photoaging in exposed skin areas indicates that nDNA damage is largely responsible for the accelerated skin aging.[76]

Werner's syndrome (**WS**) is associated with early onset of very many aging-associated diseases. WS first becomes evident in the late teens or early 20s, and typically results in death by age 50 from cardiovascular disease. Osteoporosis, premature hair graying, alopecia, high blood pressure, stroke, cataracts, severe atherosclerosis, and type 2 diabetes are extremely common in WS.[77] Defective homologous recombination is believed to be the primary reason for the chromosomal abnormalities of WS victims.[78,79] Defective recombination leads to genomic instability and thus greatly increased risk of cancer, particularly sarcomas.[80] Whereas normal human fibroblasts experience replicative senescence after about 60 divisions, the fibroblasts of WS patients senesce after about 20 divisions, resulting in many senescent cells.[81] The accelerated senescence of fibroblasts from WS patients is associated with an accelerated accumulation of double-strand breaks.[82] Much of the accelerated aging phenotype in WS is probably due to increased levels of the inflammatory cytokines produced by senescent cells.[83,84] Conversely, proinflammatory cytokines have been shown to induce cellular senescence.[85] Microarray profiling of human fibroblast genes showed 91% of the genes were common to both WS and aging cells, 6% were unique to normal aging, and 3% were unique to WS.[86] But WS victims show no increased tendency for neurodegeneration, prostate problems, or Alzheimer's Disease — and the immune system remains normal.[77] The progeroid symptoms of WS have been attributed to both increased cellular senescence[87] and increased apoptosis.[88]

In **Hutchinson-Gilford Progeria Syndrome** (**HGPS**, "childhood progeria", in contrast to the "adult progeria" of Werner's Syndrome) a child is born with abnormally short telomeres. Victims are characterized by short stature, early hair loss, cardiovascular problems (stroke and coronary dysfunction are common), and an elderly facial phenotype, but normal cognition and immune function, and no disposition to cancer.[89] The disease is caused by a point mutation in the gene for **lamin A**, a filament protein in the nuclear matrix and nuclear lamina that is required for nDNA replication and nuclear

organization. Cells with the lamin A mutation show an impaired ability to form foci for the recruitment of DNA repair proteins during nDNA replication, resulting in defective homologous recombination (HR).[90-92] A study which compared HGPS patient cells with the skin cells from young and elderly human subjects found similar defects in the HGPS and elderly cells. These defects included down-regulation of certain nuclear proteins, increased nDNA damage, and demethylation of H3 histone leading to reduced heterochromatin.[93] Most often HGPS children die of myocardial infarction or stroke (average age of death is 13). The premature atherosclerosis is without the usual association with high blood pressure or high blood cholesterol. HPGS victims do not have the high rates of presbyopia, cataracts, osteoporosis, or Alzheimer's Disease often seen in the elderly.[89] Like Werner's Syndrome, HGPS is primarily a disease of proliferative tissues characterized by high rates of cellular senescence and apoptosis.[94]

Trichothiodystrophy (**TTD**) is due to defects in the **TFIIH** protein required for both NER and normal transcription. Most often these defects are in the subunits of TFIIH. One type of mutation leads to xeroderma pigmentosum, whereas other mutations lead to TTD.[95] TTD patients do not show increased incidence of cancer. The aging phenotype (which includes osteoporosis, early graying, cachexia, and neurological abnormalities) is attributed to increased apoptosis as well as impaired cell functioning.[96]

XFE progeria is created in transgenic mice which are null for both XPF and ERCC1 genes, rendering the mice defective in both NER and nDNA interstrand crosslink repair. Repair of interstrand crosslinks requires NER followed by HR.[97] Defective crosslink repair leads to double-strand breaks in these mice. [98] In XFE progeria there is increased cell senescence and apoptosis, but reduced mutation and telomere loss.[99] These mice show accelerated aging, but suppressed carcinogenesis.[99]

Although no modification of nDNA repair enzymes has increased longevity in a mammal, transgenic *Drosophila* with decreased and increased excision repair show "accelerated aging" and "decelerated aging", respectively.[100]

ACCELERATED AGING DISEASES AND SENESCENCE/APOPTOSIS

An argument can be made that the accelerated aging diseases cited in the previous section are due to high rates of cellular senescence and apoptosis resulting from high rates of nDNA damage. Cellular senescence isn't simply a result of shortened telomeres, it is often the result of unrepaired nDNA damage throughout chromosomes,[101,102] although telomere-initiated senescence is probably also an nDNA damage response.[103] Foci of nDNA damage as markers of senescent cells provide the highest estimates (15% of cells) of cellular senescence in aged animals.[104] Senescent cells are prominent in osteoarthritis and atherosclerosis. But even where not prominent, senescent cells contribute to cellular dysfunction and carcinogenesis of adjacent cells by secretion of cytokines, growth factors, and other damaging agents.[105-107]

The **p53 protein** (which is mutated in more than half of cancer cells) arrests cell growth (cell cycle arrest), induces cell senescence, or triggers apoptosis — typically as a

response to nDNA damage, oncogene activation, or other cell stresses.[108,109] Transgenic **p53$^{+/m}$** mice with enhanced p53 protein activity show an accelerated aging phenotype and only live 80% as long as normal mice. Markers of accelerated aging included hair sparseness (hair growth decreases linearly with age in mice), slowing of wound healing, reduced dermal thickness and subcutaneous adipose (both of which normally decline with age), lordokyphosis (hunchbacked spine), muscle atrophy, and reduced vigor. Cancer is exceedingly rare in these mutants.[110-112] These mice support the view that cellular senescence/apoptosis is a defense against cancer and that cellular senescence/apoptosis can lead to aging of the organism as a whole. Conversely, transgenic mice with mutated p66^{shc} gene show impaired p53, reduced apoptosis in response to stress, and "decelerated aging" (lifespan extended 30%).[113]

Both **p16^{INK4a}** and **Arf** proteins inhibit the cell cycle.[114] A study of rodent organs found an average 10-fold increase in p16^{INK4a} gene expression and an average of 3.5-fold increase in **Arf** expression with age, concluding that the proteins are biomarkers — and possible effectors — of both cellular senescence and mammalian aging.[115,116] Increased p16^{INK4a} expression with age may lead to increased senescence of pancreatic ß-cell stem cells in non-insulin-resistant type 2 diabetes[117] — and increased stem cell senescence associated with declining neurogenesis in some (but not all) areas of the brain.[118] Mice that were transgenic with extra genes of both p53 and Arf (with normal activity of both) had strong cancer resistance, increased levels of reduced glutathione (GSH) antioxidant, and lifespan increase of 16%.[119]

NUCLEAR DNA REJUVENATION

The Strategies for Engineered Negligible Senescence (SENS) rejuvenation program describes many forms of damage associated with aging,[120] but regards nuclear DNA (nDNA) damage as mainly leading to cancer, apoptosis, and cellular senescence. Residual nDNA damage is regarded as so negligible by SENS that the nuclear genome is treated as a safe haven for genes in mitochondrial DNA. The claim has been made by SENS proponents that evolution has required such strong defenses against cancer that residual nDNA damage, mutation, and epimutation are negligible.[121] Critics of this SENS view have replied that cancer requires so many mutations that the probability of cell dysfunction in non-cancerous cells is high.[122]

The standard terminology of DNA repair distinguishes between what is called DNA damage and mutation, although damage can lead to mutation. As an analogy, the letter “K” in the word “TAKE” can be **mutated** to “P” giving the word “TAPE”. Or “K” can be **damaged** to “#” giving the non-word “TA#E”. With the exception of mismatch repair, DNA repair enzymes do not fix mutations. In SENS terminology, however, mutations and epimutations are counted as damage insofar as they can lead to cell dysfunction — and therefore need to be fixed if cancer-free rejuvenation is to be achieved.

If regenerative medicine through the use of stem cells reaches its full potential, then for most tissues of the body intracelluar and extracellular junk and crosslinking will not be so important for rejuvenation because of the ease with which those tissues can be replaced.

Brain tissue may not be easily replaced, however, because personal identity and memory are believed to be the result of neuropil connectivity and synaptic strength,[123] and possibly neuronal epigenetic structure.[124] The significant recovery of cerebral cortex function that often occurs following stroke[125-127] may be due to redundant information storage in the brain. Neural stem cells could be used to regenerate brain tissue.[128] But relying on brain redundancy is a slippery slope to loss of critical information. The most conservative approach is to preserve brain tissue as much as possible. The intra- and extracellular repair strategies of SENS would thus become of utmost importance for neurons, while regenerative medicine could be adequate for most other cells, tissues, and organs.

Much of the decline in nDNA repair with age can be attributed to factors that SENS intends to fix, including decline in nDNA repair proteins and decline in ATP required by nDNA repair enzymes. These SENS repairs would be of more benefit for maintaining people in a youthful condition than for rejuvenating them because the nDNA damage which had resulted from reduced nDNA repair would remain unrepaired.

Not surprisingly, neurons that are defective in nDNA repair easily become apoptotic.[129] However, neuronal apoptosis is more characteristic of neurodegeneration than of normal aging. As recently as the 1980s it was widely believed that normal aging is associated with extensive neuron loss, but it is now established that functional decline in the aging brain is associated with increased neural dysfunction rather than with neurodegeneration.[130]

Numerous studies implicating nDNA damage in brain tissue aging have been cited in this review. The rate of 8-OHdG formation in brain tissue nDNA is more than tripled in old mice compared to young mice. [12] Double-strand nDNA breaks are doubled in the cerebral cortex of adult rats — and quadrupled in elderly rats — as compared to young rats.[17] Significant age-associated increase in mutation observed in the hypothalamus and hippocampus of mice[15] is likely associated with nDNA damage. Because neurons are post-mitotic, they cannot senesce, but it could be instructive to look for signs of cellular senescence in neurons.

It would not be easy to disentangle the effects of nDNA damage on neuron dysfunction from the relative contributions of ATP decline, protein and lipid damage, and “junk” accumulation — or from the effects of those contributions on nDNA repair capability. But the high overlap of gene expression between Werner’s Syndrome victim cells and normal aging cells[86] is evidence that nDNA damage contributes to neuron dysfunction. The predominance of nDNA damage over damage to other macromolecules in the photoaging of xeroderma pigmentosum[76] demonstrates how large a role nDNA damage can play in producing an aging phenotype. The greater longevity seen in people with more Ku70 protein[57] — which is required for nDNA double-strand break repair — supports the contention that nDNA damage significantly affects aging. The fact that aging in transgenic *Drosophila* can be accelerated or decelerated with decreased or increased excision repair undermines the claim that accelerated aging due to reduced excision repair is simply accelerated mortality resulting from increased defect.[100]

SENS regards repair of nDNA to be of secondary importance — a matter to be addressed after SENS has been implemented. However, substantial evidence has been presented in this review that accumulating nDNA damage contributes significantly to aging. Repair of nDNA damage in neurons could be much more difficult than the strategies SENS might use for repairing other forms of aging damage, but perhaps not too difficult. Neurons and other non-proliferative cells economize on nDNA repair enzyme activity by limiting nucleotide excision repair to transcribed genes (there is no GGR-NER in neurons),[131,132] which could mean that neurons are not much harmed by nDNA damage in non-transcribed portions of the genome. Limiting artificial nDNA repair efforts to transcribed genes in neurons would be a less daunting task than trying to repair the whole genome. If nDNA damage is of significant importance in aging damage, then efforts to achieve rejuvenation will have little success in the absence of nDNA repair strategies.

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