The evolutionary conflict theory of aging

Gordon Irlam
gordoni@gordoni.com

Los Altos, California, United States

November 20, 2024

Abstract

Why we age is an enduring mystery. This manuscript proposes aging is microevolutionarily opposed, but macroevolutionarily favored. Such a conflict between microevolution and macroevolution is highly unusual since traits that are harmful to the organism are usually harmful to the survival of the species. In the case of aging, however, a shorter lifespan makes a species better able to adapt to a changing environment. Conversely, species that age more slowly, and thus live longer, are less adaptable and more likely to go extinct. Drawing on what is known of aging in vertebrates, pathways of aging are identified that agree with this theory. These pathways involve mitochondrial ROS production causing telomeric DNA damage, which leads to cellular senescence, the senescence-associated secretory phenotype, epithelial-mesenchymal transition, thymic involution, and age-related diseases. The resulting framework is capable of explaining the seeming intentionality of many age-related diseases, and offers a high level theoretical framework for better understanding them.

Keywords: microevolution, macroevolution, aging, programmed aging, evolvability, age-related diseases.

Introduction

Many age-related diseases appear to be caused by cellular senescence and immunosenescence. Cellular senescence appears to be activated by telomeric DNA damage, which in turn appears to be caused by reactive oxygen species (ROS). Immunosenescence at least partially appears to occur as a result of thymic involution, which appears likely to be caused by the epithelial-mesenchymal transition (EMT), which appears to be another downstream effect of cellular senescence.

The core idea of this manuscript is that the production of ROS by the mitochondria and its downstream effects can be viewed as macroevolutionarily intentional mechanisms to cause the individual organism to die. A shortened lifespan will reduce the mean time between successive sexual generations, and thus increase the ability of the population to adapt to a changing environment. Conversely, populations with long lifespans, and thus long times between generations, are more likely to become extinct due to their inability to adapt.

Mortality is troubling to some scientists. Evolution appears capable of producing a myriad of complex organismal forms, but unable to perform the seemingly much simpler task of keeping them working. The fact that two relatively recently diverged species, such as mice and men, have such widely different lifespans suggests mortality may be deliberate. But why? And how?

Theories of aging can be divided into two classes. Non-programmed theories of aging, such as mutation accumulation [1], antagonistic pleiotropy [1], and the disposable soma theory [2] propose aging is an accidental response that results from limited selective pressure for extending lifespans in the evolutionary environment. Programmed theories of aging on the other hand propose that aging is adaptive and that there exist evolutionary pressures in favor of aging. There has been considerable controversy regarding which of these two classes of theories are correct [3].

Programmed theories of aging hold that while aging and eventual mortality are obviously harmful to the individual, some greater good comes from aging and mortality. As such they may at first appear dangerously close to the widely dismissed concept of group selection [4]. Programmed theories of aging include aging as a method to limit the spread of disease [5], clearing the population to make space for new progeny that bear useful traits [6], providing some form of advantage to spatially close kin [7], and enhancing the ability to adapt in a changing environment [8] or enhancing evolvability [9]. Evolvability encompasses accelerating the rate of adaptation by increasing the number of sexually produced organisms that can be tested by evolution.

This manuscript presents a programmed theory of aging taken from the vantage point of macroevolution. Evolvability is a somewhat ill-defined theory of the capacity to evolve [10, 11]. The theory presented here is related to evolvability, but whereas evolvability has been claimed not to involve species-level selection [9], the present theory wholeheartedly makes this claim. Consistent with the criticism of programmed aging theories [12], the theory to be developed does not support the existence and maintenance of aging in a population when analyzed by itself. It is only when analyzed through the lens of macroevolution with multiple branching populations that exist in competition, and that are capable of becoming extinct, that aging is maintained. The theory also does not support aging in asexual populations. This manuscript does not seek to simply present a plausible programmed theory of aging, but also seeks to present a detailed description of the approximate inner-workings of the program in vertebrates. This involves elucidating the role of mitochondrial ROS production in causing telomeric DNA damage, which leads to cellular senes-

cence, the senescence-associated secretory phenotype, epithelial-mesenchymal transition, thymic involution, and age-related diseases.

The first part of this manuscript develops evolutionary conflict theory and its implications as an abstract evolutionary theory. This is followed by an applied examination of the proposed mechanism of aging in vertebrates. The two sections: why we age, and how we age, buttress each other. Finally, the discussion section provides an exploration of the implications of the theory for addressing many age-related diseases.

Evolutionary conflict theory

Mortality

Eukaryotic mortality refers to the existence of an apparent intrinsic limit to which a eukaryotic organism can live, before death occurs. Death occurs even though resources are plentiful and predation is minimal. The intrinsic limit may be measured in terms of time, aggregate metabolic inputs, or some other organismal process. The intrinsic limit will be referred to as the maximum lifespan of the organism, to distinguish it from the typically observed lifespan which might occur when resources are limited or predation is frequent. Here an organism that only dies as a result of extrinsic evolutionarily unavoidable misfortune is not considered to be mortal. Evolutionary conflict theory seeks to explain why eukaryotes are mortal.

Frequent sex increases the ability to adapt to a changing environment

Contemplating asexual and sexual reproduction by species with large genomes, such as eukaryotes, the advantage of sex to the species is two-fold. First, in a changing environment, sex allows the combination of advantageous alleles that were originally created by spontaneous mutation. Second, sex acts to reduce the mutational load; the build up of deleterious alleles created by spontaneous mutation. The shorter the generation time, the greater should be the ability to adapt to a changing environment. Competition between species means the environment is nearly always changing, even when the overall outcome is a stable balance between species.

Generation time creates an upper bound on the pre-post-reproductive time

Generation time and life expectancy are linked. In particular, a specific finite time between sexual generations implies an upper bound on life expectancy, excluding post-reproductive life expectancy. This is derived mathematically in Appendix A. A requirement for a short generation time results in a short lifespan, excluding any post-reproductive lifespan.

Microevolution and macroevolution

Microevolution is the change in allele frequencies over time within a population by mutation, selection, gene flow, and genetic drift. It typically occurs over time frames of, say, less than a million years. Macroevolution is the change in species and higher order taxonomic groups. It typically occurs over time frames of, say, millions of years. The creation of distinct species, speciation, is a macroevolutionary process made up of multiple microevolutionary mutations whose cumulative effect is to render different populations reproductively incompatible.

From a microevolutionary perspective, the longer the reproductive lifespan, the more offspring are possible. Thus the nuclear and mitochondrial genomes can be expected to evolve to support increasingly long reproductive lifespans, and hence longer and longer maximum lifespans.

From a macroevolutionary perspective, the longer the mean time between successive generations of the species, the less adaptable the species will be to a changing environment. Conversely, the shorter the generation time, the more frequently genetic recombination occurs, and the greater the advantage to the species from sex. Other things being equal, this means species with short generation times fitter than competing species with long generation times. Thus macroevolution favors a shorter generation time.

A more complete analysis of the macroevolutionary situation would need to take into account changing organism sizes, population sizes, and other factors associated with a change in generation time. The net effect though is to reduce the odds of a species with a longer generation time surviving.

There will be a macroevolutionary species specific optimal generation time. This length of time relates to how quickly organisms can produce successful offspring, as well as the loss in adaptability that comes with a longer generation time.

Macroevolution favors a specific finite generation time. The simplest way of achieving this is through mortality once that time has been reached. Keeping organisms around for a while after the optimal generation time is reached, but rendering them infertile, is only of interest to the extent it benefits future generations. Consequently, not just the generation time, or even the pre-post-reproductive time, but also the lifespan effectively favored by macroevolution, is finite.

In summary, microevolution strives for near immortality at a cost to the species, while macroevolution favors a specific finite maximum lifespan.

The evolution of maximum lifespans

How do species and maximum lifespans evolve if macroevolution favors a shorter maximum lifespan, but all it is being given to work with by microevolution is longer maximum lifespans?

The precise genetic basis of speciation appears to be somewhat of a mystery, but it is empirically known to occur over relatively short time frames. For instance, the mean duration of speciation for primates has been estimated to be 0.6 million years [13].

Simple maximum lifespan extending mutations fix rapidly

The microevolutionary mutation and fixation of beneficial alleles that extend maximum lifespan occurs rapidly. Consequently we can expect most species to be close to a microevolutionary local maximum. There are almost no simple point mutations or allele frequency changes that will significantly increase lifespan in the evolutionary environment. This is evaluated mathematically in Appendix A.

More complex maximum lifespan extending mutations fix infrequently

Despite a paucity of genes for which simple mutations might extend maximum lifespan, there are likely to be multiple genes for which more complex mutations might extend maximum lifespan. Such mutations might require two or more point mutations occurring at different sites on the same or different genes. This is evaluated mathematically in Appendix A.

Maximum lifespan extension is selected against by macroevolution

Speciation is fast, but the evolution of more complex maximum lifespan extending mutations appears to be relatively slow. Consequently, by the time a species lineage has evolved a new maximum lifespan extending function, there will be multiple similar species that have radiated off from the lineage that do not possess the same or any other maximum lifespan extending function. These species will have a shorter generation time, and thus be better able to adapt to a changing environment. Assuming that these similar species that do not possess the maximum lifespan extension exist in competition with the species bearing the maximum lifespan extension, the non-maximum-lifespan extension bearing species will usually be favored by macroevolution. The species bearing the maximum lifespan extending function will be more likely to go extinct.

In the scenario just considered, macroevolutionary pressure limiting the extension of maximum lifespan was brought about by closely related species that radiated from the original species. More generally, such pressure might be brought about by more distantly related species occupying niches that overlap with that of the species in question.

A recurring debate in evolutionary biology is whether macroevolution is simply repeated rounds of microevolution [14]. Evolutionary conflict theory implies there is more to macroevolution than can be explained by microevolution. Microevolution is unable to explain the persistence of organismal mortality that results from the competition between species. Evolution can only be understood by including species level macroevolution as part of the picture. On the other hand, evolutionary conflict theory doesn't provide support for evolution occurring at taxonomic levels above the species level.

Successful species come from a long line of failures to live longer

The success of a species is tightly bound up with how rapidly it can engage in genetic recombination. Species that fail to evolve to live longer have a shorter generation time and thus an evolutionary

advantage over similar species that do evolve to live longer.

Microevolution is trying to extend species maximum lifespans, but macroevolution keeps pushing it back to its least successful attempt. Successful organisms might come from a long line of successful organisms, but successful species usually come from a long line of failures; failures to live longer that is.

Species that have little need to evolve live longer

Species that have little need to evolve to maintain their position in the environment, might be expected to evolve to live longer. This appears to agree with observation.

The Greenland shark has a lifespan of at least 272 years, the longest of any known vertebrate [15]. The Greenland shark is an apex predator that feeds opportunistically at least in part by scavenging [16, 17]. There may thus be little need for the Greenland shark to evolve. It has no predators, and its prey are often dead or weak. It is also worth noting, the Greenland shark has a very low metabolic rate per unit mass [18]. The significance of this will become apparent once the mechanisms of aging are discussed.

One common reason species need to evolve is as a result of inter-species competition. If a species faces little competition, microevolutionary mutations that increase maximum lifespan will accumulate. On the other hand, the more intense the competition between species, the closer to the species preferred length of time to live species should be found. Anecdotally, consider the long lifespan of the Galápagos tortoise, which probably faces little interspecies competition. Similarly, the naked mole-rat occupies a relatively unique ecological niche, subterranean burrows in the Horn of Africa that often have little oxygen, and it exhibits a very long lifespan for its size [19]. The salamander *Proteus anguinus* is found exclusively underwater in European caves, has an average weight of only 17g, and yet it can live for more than 100 years [20]. And finally, the bristlecone pine, *Pinus longaeva*, generally grows in harsh environments where most other plants are unable to grow, and it appears to have the longest lifespan of all known non-clonal organisms [21].

Similar arguments apply to clades of species. When many species occupy the same niche or overlapping niches, competition between them is likely to keep lifespans in check. Conversely, if the species occupy a relatively unique niche, they are likely to be subject to less macroevolutionary pressure on maximum lifespan. This might go some way towards explaining the relatively long lifespans of bats [22], tortoises [23], turtles [24], and salamanders [20].

Species along a lineage will tend to exhibit increasing lifespans

It might at first appear as if species only ever evolve longer maximum lifespans as there isn't any microevolutionary pressure to evolve a shorter maximum lifespan. This isn't fully true. If lifespan is determined based on aggregate metabolic inputs, then it is possible for a shorter maximum lifespan to evolve in terms of time, if it goes hand-in-hand with an increase in metabolism. For instance there would likely be an increase in metabolism per unit mass if a species of dinosaur evolved into shorter lived warm-blooded birds.

Another example in which shorter maximum lifespans may evolve is if the species in question experiences a temporary bout of heavy predation over an evolutionary time period. Predation can prevent maximum lifespans from being obtained, permitting genes contributing to maximum lifespan to be lost, and thus resulting in a smaller maximum lifespan once predation levels fall.

For the most part though, species appear likely to develop longer and longer maximum lifespans, then to go extinct as a result of being outcompeted by other relatively unrelated and faster evolving species with shorter maximum lifespans. This might even go some way towards explaining how mammals could have ended up replacing large, and thus slowly evolving, dinosaurs.

The trend in maximum lifespans may help explain Cope's rule. Cope's rule is the claim that species within a lineage tend to evolve larger body sizes over time [25]. If species tend to evolve longer maximum lifespans, then all that is required is for there to be a link between maximum lifespan and body size, for Cope's rule to be valid. Such a link appears highly likely.

Aging

Aging is a process of declining ability to respond to stress over time, and an increase in the probability of death.

Almost all eukaryotic organisms appear to age, while under suitable conditions symmetrically dividing prokaryotic populations must be immortal [26, 27]. It seems reasonable to hypothesize that the primary purpose of aging is to cause organism death as a means of increasing the ability of the species to adapt.

Some support for the hypothesis that aging exists to increase the ability of the species to adapt is given by age-related disease susceptibility getting reset concurrently with the process of genetic recombination, which is the very process that increases adaptability, rather than in response to some other biological event. Further evidence will be provided in the section dealing with aging in vertebrates,

The duality hypothesis

If genes that caused aging only caused aging they would be selected against by microevolution, but if such genes also played some separate and important life giving role, they need not be. We should thus expect aging-related genes to be pleiotropic; also exhibiting some beneficial function from a microevolutionary perspective.

Duality hypothesis: Aging-related genes will also exhibit some vital life-enhancing function.

The duality hypothesis applies to both nuclear and mitochondrial genes.

An overview of the operation of the duality hypothesis is given in Table 1. Genes for each aging-related function also appear to play an important life-enhancing role.

Table 1: Overview of the operation of the duality hypothesis. Aging-related genes also exhibit life-enhancing functions.

Genes	Aging-related function	Life-enhancing function
electron transport chain ROS related	produce ROS	produce H^+ gradient
double-strand break response	persistent telomeric DNA damage signaling in senescence	DSB repair
senescence related	multicellular organismal death	organismal development and wound healing [28, 29]
thymus related	thymic involution	T cell maturation
apoptosis related	unicellular organismal death	many and various [30]
epithelial-mesenchymal transition related	promotes cancer, fibrosis, and thymic involution	organismal development and wound healing

Aging-related genes:

- Have an aging-related function that is selected for by macroevolution over macroevolutionary time frames.
- The aging-related function would be selected against by microevolution if microevolution was able to operate over macroevolutionary time frames.
- Pleiotropically selected for by microevolution over microevolutionary time frames.

The difficulty of combating aging

Corollary to the duality hypothesis: Anti-aging interventions based on existing genes can often be expected to exhibit reduced biological fitness in the evolutionary environment.

If the existing gene is aging-related the corollary follows from the duality hypothesis, as attempts to down-modulate the gene will also down-modulate its life-enhancing function. If the existing gene is not aging-related then it purely has a life-enhancing function, and the cumulative effects of microevolution can commonly be expected to have already modulated its expression to a near optimal level.

The corollary to the duality hypothesis suggests that interventions intended to extend maximum lifespan that are based on existing genes will have to tread carefully so as to not interfere with any life-enhancing function.

An approach to aging that may be able to avoid these problems is gene therapy. The targeted manipulation of anti-aging genes may be possible in a way that doesn't affect their vital life-enhancing function. Such successful manipulations are likely to require multiple mutations rather than involving a single point mutation.

Understanding the complexity of the aging-related pathways

Microevolution can be expected to develop genes and proteins to oppose aging and death, while, within limits, macroevolutionary species level selection will seek to promote it. What we may be left with is a large number of only partially successful attempts to limit aging. This may explain the seeming complexity of many of the aging-related pathways. For instance, the apoptotic pathway probably involves the concerted effects of close to 100 proteins. The aging-related pathways are the microevolutionarily hard to repair mechanisms of organismal death.

Further, the duality hypothesis suggests there will be some difficulty in properly determining the aging-related pathways. Each gene can be expected to have both life-enhancing and maximum lifespan reducing functions. Aging-related pathways will be hidden within normal life-enhancing genes.

Finally, evolution dictates that other genes will evolve to oppose the functioning of the age-related pathways, making them very difficult to discern.

The complexity of the aging-related pathways seems abundantly clear, yet it appears to be rarely commented on.

Aging in vertebrates

In many respects, this subsection appears to apply more broadly, but will be focused on the mechanisms of aging in vertebrates. Aging in vertebrates is incompletely understood, and some of the finer details of what follows may be incorrect.

The occurrence of aging, or senescence, in natural vertebrate populations is widespread [31].

The proposed mechanism of aging in vertebrates is shown in Figure 1. This figure will be examined in detail later. At a high level, cellular demands for energy result in electron transport chain activity. As a byproduct this results in the production of ROS. Cumulative cellular lifetime ROS levels are converted into telomeric DNA damage. Once telomeric DNA damage exceeds a threshold, cellular senescence ensues. If the senescent load exceeds the capacity of the immune system to clear, death of the organismal will occur. And the capacity of the immune system to remove senescent cells declines over time due to thymic involution.

The proposed mechanism of aging in vertebrates aligns with evolutionary conflict theory. The path-

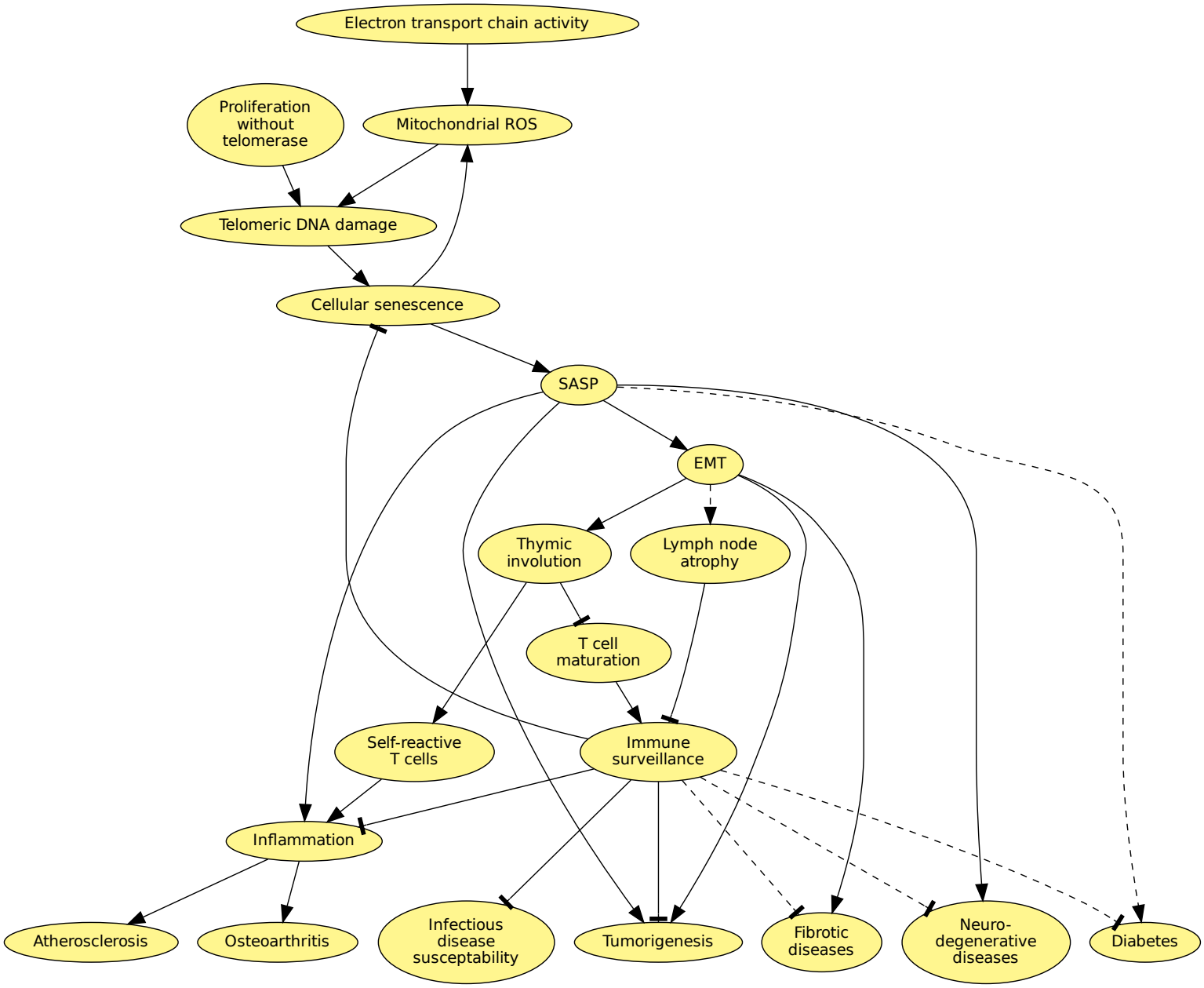

Figure 1: Proposed mechanism of aging in vertebrates. Cellular demands for energy from mitochondria result in the production of ROS. Lifetime ROS exposure causes telomeric DNA damage, which leads to senescence, the senescence-associated secretory phenotype, epithelial-mesenchymal transition, thymic involution, and age-related diseases. Dashed lines are largely hypothetical.

way from electron transport chain activity to cellular senescence and organismal death represents a mechanism whereby the organism's maximum lifespan is finite when measured in terms of energy consumption. This finiteness is in accordance with the needs of macroevolution.

It is challenging to imagine that eukaryotic mortality could have evolved from eukaryotic immortality. This runs counter to microevolution. It seems much more likely that eukaryotic mortality has always existed, and evolution has acted primarily to increase maximum lifespans.

Sex, mortality, and the presence of mitochondria, all seem to have emerged simultaneously as fundamental aspects of the eukaryotic cell. It is reasonable to hypothesize that the mitochondria were partially toxic to the cell, bringing about mortality, but that this mortality could be rescued through sex. The toxicity of the mitochondria is a result of the ROS they produce, and this mechanism of mortality seems to have been preserved across all mitochondria bearing eukaryotes. This places mitochondrial ROS produced by the electron transport chain at the beginning of the mechanisms of aging.

The channeling of ROS into telomeric damage and cellular senescence represents evolutionary refinements to the mechanisms of aging. Cellular senescence, or something like it, in which the aged cell doesn't die but nearby cells do, is necessary to bring about mortality in multicellular organisms.

Surveillance of senescent cells by the cellular branch of the adaptive immune system represents an evolutionary addition present in vertebrates that extends maximum lifespan. Within limits this maximum lifespan extension was probably not selected against by macroevolution because of the concomitant increase in fitness resulting from the ability of the adaptive immune system to combat infectious diseases. The extent to which macroevolution allows this maximum lifespan extension to develop is determined by the occurrence of thymic involution.

To fully understand the proposed mechanism of aging in vertebrates, it is necessary to first briefly review ROS, cellular senescence, immunosenescence, and EMT.

Reactive oxygen species

The mitochondrion is a major source of reactive oxygen species (ROS). Complexes I and III of the electron transport chain both leak superoxide ($O_2^{\bullet -}$), with roughly 0.2-2.0% of all oxygen consumed by the mitochondria ending up as $O_2^{\bullet -}$ [32]. Complex I leaks towards the mitochondrial matrix, while complex III leaks towards both the matrix and the intermembrane space [32]. $O_2^{\bullet -}$ gets converted into the more stable ROS hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD). H_2O_2 is stable by itself, but in the presence of ferrous iron ions (Fe^{2+}) it undergoes the Fenton reaction, producing an extremely reactive hydroxyl radical ($HO^{\bullet}$), a hydroxide ion (OH^-), and ferric iron ions (Fe^{3+}) [33]. As the name suggests, ROS are highly reactive, and unless neutralized by antioxidants, can cause damage to the nucleic acids, proteins, and lipids that make up the cell [34]. Cellular membranes are largely permeable to H_2O_2 [35, 36, 37] with a permeability coefficient of $2{\times}10^{-6}$ m s^{-1} at 37°C [38], and weakly permeable to $O_2^{\bullet -}$ [37] with a permeability coefficient $2{\times}10^{-9}$ m s^{-1} at 37°C [39].

In mammals mitochondrial ROS production is known to increase with age [40].

Despite billions of years of evolution, harmful ROS production by the mitochondria has never been

eliminated. This is consistent with the possibility that ROS production is a macroevolutionarily intentional mechanism of bringing about mortality.

SOD converts $O_2^{\bullet -}$ into O_2 and H_2O_2. Eukaryotes contain several forms of SOD.

Peroxidases break down H_2O_2 to water and oxygen. Three common peroxidases are peroxiredoxins (Prxs), glutathione peroxidase (GPx), and catalase. Catalase has an extremely high turnover rate. In most eukaryotes catalase is only found in peroxisomes, and not in the cytosol. The Ctt-1 gene of *Saccharomyces cerevisiae* and the ctl-1 gene of *Caenorhabditis elegans* are two exceptions. The frequent lack of a cytosolic catalase may be the result of H_2O_2 being used to signal the passage of time against the species maximum lifespan.

Consistent with ROS being the evolutionary mechanism through which lifespan is controlled, comparisons between different species have generally shown a negative correlation between ROS levels and lifespan [41, 42].

Within individual species the overexpression of antioxidant enzymes is generally associated with an increase in lifespan [43] [41][Table 4]. Similarly the deletion of genes coding for antioxidant enzymes generally results in a decrease in lifespan [41][Table 5]. Exposure to antioxidants compounds also often increases lifespan [41][Table 6]. These effects are however by no means universal. Contradicting the theory being developed, mild exposure to ROS generating compounds can increase lifespan [41][Table 7]. Similarly mutations that increase ROS production can sometimes increase lifespan [41][Table 8]. This is known as hormesis. The reason for this lack of universality regarding the effects of antioxidants and ROS may be because the details of what is happening matter. The behavior of H_2O_2 is different from that of free radicals, and ROS are also used by the cell as signaling molecules and for the killing of bacteria [44, 45]. The discrepancy in the behavior of antioxidants is explored further and resolved in the section on antioxidants in Appendix E.

In humans, various mitochondrial haplogroups have been correlated with longevity [46, 47]. It might be argued that this association could simply be the result of correlations between the mitochondrial and nuclear genomes [46]. However, by transplanting different mitochondrial genomes into the same cell line, some of these longevity associated haplogroups have been found to produce less ROS [47]. This suggests that reduced mitochondrial ROS could be the cause of the mitochondrial haplogroup associated longevity.

Cellular senescence

Senescent cells fail to divide, resist apoptosis, and usually exhibit the senescence-associated secretory phenotype (SASP) [48]. The SASP is frequently pro-inflammatory, proapoptotic, and is possibly even capable of inducing senescence in both nearby and distant non-senescent cells [48, 49, 50]. Natural killer cells are often capable of clearing senescent cells [51], as are macrophages [52]. However, with age, the number of senescent cells is found to accumulate, and this is implicated in various age-related diseases [53]. Since the SASP is implicated in various age-related diseases, and senescent cells can induce senescence in other cells while themselves being resistant to apoptosis, the SASP represents an ideal mechanism to ultimately cause organismal death. The precise chemicals that uniquely define the SASP have been difficult to pin down. This is understandable. If the SASP was well defined, organisms might evolve to resist its effects.

Cellular senescence has been partitioned into different types [54]. Replicative senescence limits the number of divisions a cell can make and is linked to mitotic telomere shortening. Oncogene-induced senescence is in response to non-telomeric DNA damage. Stress-induced senescence is the induction of senescence in response to chemicals such as H_2O_2. All three result in growth arrest, the SASP, and morphological changes. Studies of stress-induced senescence are probably the most relevant here, as this closely reflects the action of mitochondrial ROS.

Cellular senescence plays a vital role during development where the clearance of the senescent cells promotes tissue remodeling [28]. Cellular senescence also plays a vital role during tissue repair following injury [29, 52]. Consistent with the duality hypothesis, this makes it difficult to evolutionarily disable the harmful effects of senescence.

Immunosenescence

Immunosenescence is the gradual decline in the efficacy of the immune system with age [55]. Multiple factors contribute to immunosenescence [56]. A major factor is thymic involution [55]. The thymus is the site of T cell maturation. Thymic involution is the gradual atrophy or shrinking of the thymus with age. Thymic involution appears to include an increased thymocyte apoptosis and reduced thymocyte proliferation in the aged thymus [57]. This leads to a reduction in naive T cell output that likely contributes to immunosenescence [58]. Thymic involution is common to nearly all organisms possessing a thymus [59], although the selective pressures for thymic involution appear not well understood. The possibility that thymic involution is intended to cause organism death and therefore promote frequent genetic recombination doesn't appear to have been previously considered.

Another aspect of immunosenescence that may contribute to a reduction in T cell levels is atrophy and fibrosis of the lymph nodes [60]. This atrophy has been shown to be a barrier to the effectiveness of thymic rejuvenation [61]. It has been speculated that cellular senescence is involved in this age-related deterioration of the lymph nodes [62].

Epithelial-mesenchymal transition

The epithelial–mesenchymal transition (EMT) is a process whereby epithelial cells appear to turn into mesenchymal cells [63]. In doing so they acquire a fibroblast-like morphology, become more migratory, and exhibit an extra-cellular matrix producing phenotype [64, 63]. EMT occurs during development, wound healing, and cancer metastasis [63]. EMT plays a key role in fibrotic diseases [65, 66].

The EMT by thymic epithelial cells produces cells that are described as EMT-derived fibroblasts [67]. This process appears to be responsible for thymic involution [67].

EMT can be caused by the effects of the SASP on epithelial cells [68, 69].

Interestingly, the pathways of cellular senescence and EMT share some of the same molecular actors [70].

Likely decline in clearance of senescent cells by the immune system with age

Natural killer (NK) cells are able to kill other cells and have activating NKG2D receptors, inhibitory NKG2A receptors, and inhibitory and activating killer-cell immunoglobulin-like receptors (KIRs). Senescent cells express elevated levels of NKG2D ligands: MHC class I chain-related protein A (MICA), UL16 binding protein 1 (ULPB1), and UL16 binding protein 2 (ULPB2) [71]. Consequently, many senescent cells are probably capable of being cleared by NK cells. Those that aren't cleared display increased levels of the non-classical major histocompatibility complex (MHC) inhibitory ligand human leukocyte antigen (HLA) E of NKG2A [72], and/or MHC I inhibitory ligands HLA-A, HLA-B, and HLA-C for the KIRs [73]. Many of these remaining cells can probably be cleared by T cells:

- CD8+ cytotoxic T (T_C) cells are suspected of being capable of directly clearing senescent cells [74]. This requires the presentation of an appropriate peptide by MHC I, which senescent cells possess [73], a low level of the inhibitory NKG2A receptor on the T_C cell, which is the case [72, 75], and a costimulatory signal, which NKG2D can provide [76]. As for the appropriateness of the peptides in identifying senescent cells, senescent cells appear to express some peptides that are not expressed by non-senescent cells [73].
- CD4+ T helper (T_H) cells are known to be capable of responding to oncogene-induced senescence and clearing senescent cells with the assistance of monocytes/macrophages [77].
- Natural killer T (NKT) cells are a specialized type of T cell that have limited T cell diversity along with features reminiscent of the NK cells of the innate immune system [78]. NKT cells are capable of, at a minimum, coordinating the removal of senescent cells [79]. NKT cells mature in the thymus, and lymphotoxin β receptor (LTβR) knockout in medullary thymic epithelial cells (mTECs) reduces both the number of mTECs in the thymus, and the thymus' production of NKT cells [80, 81]. NKT levels in peripheral blood decline significantly with age [82].

The ability of T cells to clear senescent cells is consistent with the observed shorter mean lifespan for athymic mice raised in a germ-free environment [83].

The ability of the immune system to clear senescent cells likely declines with age. One reason for this is the effect of thymic involution on T cell production by the thymus.

The ability of T cells to clear senescent cells at young ages is likely relative rather than absolute. This is illustrated by periodontitis. Periodontitis is associated with senescent cells in periodontal tissue [84]. Signs of periodontal disease exist in 7 year old children, but the incidence of significant periodontal disease increases greatly around the age of 30-40 [85, 86].

Macrophages in the salamander *Notophthalmus viridescens* appear able to effectively clear senescent cells [87]. This is interesting because salamanders also possess extremely long lifespans for their size [20]. In addition salamanders lack any obvious signs of aging [88].

Mechanism of aging in vertebrates

The proposed mechanism of aging in vertebrates was shown in Figure 1. The cell's demand for energy results in the mitochondrial electron transport chain also producing ROS. The ROS goes on to cause irreparable telomeric DNA damage. Persistent DNA damage response (DDR) signaling results in cellular senescence. Mitochondria of senescent cells exhibit a decrease in mitochondrial membrane potential, and an increase in the production of ROS [89]. This creates a feedback mechanism strengthening the commitment to senescence.

Cellular senescence involves the production of the SASP. The SASP is implicated in a wide variety of age-related diseases including atherosclerosis [90], osteoarthritis [91], tumorigenesis [92], Alzheimer's disease [93], and possibly diabetes [94, 95]. The SASP builds up over time.

The SASP may be responsible for EMT, which plays a key role in tumorigenesis and fibrotic diseases. Alternatively, since senescence and EMT share some of the same molecular actors, senescence may promote EMT in a more tightly linked manner.

In addition to directly causing diseases, EMT appears to cause thymic involution and speculatively, lymph node atrophy. Both of which lead to a reduction in T cell surveillance by the adaptive immune system. Thymic involution also results in the production of inflammation causing self-reactive T cells. The loss of immune surveillance both leads to the failure to clear senescent cells, and is implicated in many age-related diseases including atherosclerosis [96], susceptibility to infectious diseases, tumorigenesis [97, 98], and possibly fibrotic diseases [99].

EMT causes the thymus to involute, which impairs the ability of the immune system to clear senescent cells, causing the senescent load to increase, and further promoting EMT. It is a vicious cycle, which will eventually result in organismal death.

The role of senescence, immunosenescence, and EMT, in age-related diseases is examined in more detail in Appendix B.

Moderate doses of certain antioxidants are known to inhibit cellular senescence [100]. Perturbations of the electron transport chain are known to promote senescence [101]. This is consistent with mitochondrial ROS leading to senescence.

Detailed, but still simplified, tentative molecular pathways of aging in vertebrates are described in Appendix C.

The aging brain

Many cells in the body are short lived, and derived from telomerase expressing stem cells [102]. These cells are less likely to undergo cellular senescence. On the other hand neurons and astrocytes in the brain are very long lived. It is therefore important to understand whether these cells undergo senescence, and the effects of the SASP on these cells.

Neurons naturally exhibit cell cycle arrest and are capable of exhibiting many other features of senescence, including production of the SASP [103]. However, because of the blood-brain barrier, senescent neurons may fortunately not usually be surveilled by T cells [104]. The full effects of

the SASP on neurons appear unclear. However, the SASP component IL-6, which is usually pro-inflammatory [105], has multiple effects on neurons in the brain including promoting neuronal survival [106].

In vitro, astrocytes have been shown to undergo replicative senescence as well as H_2O_2 induced senescence. So astrocytes are clearly capable of undergoing senescence. However, astrocytes may have a trick up their sleeve to reduce the likelihood of becoming senescent. Astrocytes produce ATP by breaking glucose down to pyruvate by glycolysis as usual. This produces some ATP and NADH. However, not all of this pyruvate enters the citric acid cycle and oxidative phosphorylation. Astrocytes ferment some of the pyruvate to lactic acid and consume the NADH [107]. The lactate is then exported from the astrocyte by monocarboxaylate transporters [108]. These mechanics are well known, but the fact that this reduces the dependence of astrocytes on ROS producing oxidative phosphorylation appears to have been overlooked. Consistent with this, proliferating astrocytes have been shown to barely be affected by the inhibition of electron transport chain complex I or ATP synthase [109]. Senescent astrocytes have been hypothesized to play a role in Alzheimer's and Parkinson's disease [110]. Thus any reduction in astrocyte oxidative phosphorylation is likely to be partial, rather than complete. As to the SASP, IL-6 appears to have no effect on astrocyte morphology [111].

In summary, neurons and astrocytes seem to have found mechanisms to either avoid being cleared when they become senescent, or delay becoming senescent, as well as avoid some of the ill-effects of the SASP.

Discussion

As with any scientific theory, the evolutionary conflict theory of aging must be subjected to scrutiny, and if need be refined, modified, or rejected. Findings that challenge the theory are explored in Appendix E. So far, plausible alternative explanations exist to these challenges that don't require changes to the theory.

Addressing age-related diseases

Today there exist many one-disease-at-a-time approaches for addressing age-related diseases. These approaches are likely to only be weakly effective. The elimination of all forms of cancer for instance is only expected to extend lifespan in the U.S. by 3 years [112]. If one age-related disease doesn't kill you, another one will.

Proposed multi-disease approaches for addressing age-related diseases are split across the nine different hallmarks of aging: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication [113]. There isn't a clear consensus on the relationship between the different hallmarks of aging, and what causes what.

Age-related diseases and their mechanisms may be divided into three classes. Those that exist downstream of mitochondrial ROS production; these may be considered fundamental and of

macroevolutionary origin. Those that exist due to an evolutionary trade-off between the nuclear genome's desires for immortality and reproduction; these are probably rare, and may also be considered fundamental, but of nuclear origin. And those that exist merely because they occurred infrequently enough in the evolutionary environment to be selected against; these may be considered as residual. These residual diseases may exert a significant toll if the maximum lifespan for the species is increasing, or if most of the fundamental diseases have been cured.

If the fundamental macroevolutionary origin age-related diseases were eliminated, and mortality rates dropped to match those of a U.S. 20 year old in 2019, based on the Social Security Administration life tables, the lifespan of men would increase to 927 years, and for women it would increase to 2,469 years. These lifespans are probably unobtainable due to residual age-related diseases, but they provide an upper bound on what might be possible. In the short run, extensions to lifespan are likely to be far more modest. Even a 10 year increase in lifespan might be an ambitious goal. This is because it isn't until the final major cause of mortality is eliminated, that lifespans will really take off.

It must be remembered that humans live much longer than mice. Many of the pathways for promoting age extension in mice have probably already been found by evolution in humans. This means many promising interventions in mice will fail when translated into humans.

Other approaches to aging that may initially appear unrelated to the mechanisms proposed here are worth considering. If the pathway proposed here is correct it should largely be possible to align the other approaches with this pathway. This is done to good effect in Appendix D.

Feasible current interventions

The evolutionary conflict theory of aging suggests several possible simple interventions that might be able to extend lifespan:

- Try to avoid hydrogen peroxide. H_2O_2 is sometimes used as an antiseptic to treat wounds, as a mouthwash, or as a tooth whitening agent in toothpaste. The safety of H_2O_2 has been assessed in long term animal trials, but these appear to have focused on the question of whether H_2O_2 is a carcinogen [114], and not whether it plays a broader role of promoting senescence and reducing lifespan. The role of H_2O_2 in telomere shortening suggests it is best avoided where possible.

- Possibly consider a relatively low iron diet. Iron is key to the Fenton reaction that produces the extremely reactive radical $HO^{\bullet}$ which can cause telomeric damage. To reduce the prevalence of iron deficiency anemia, many foods are iron fortified. Lower iron intake might help explain the healthspan and lifespan advantages of a vegetarian diet [115]. Iron is an essential nutrient, and so consuming a low iron diet shouldn't be taken to extremes.

- Consider experiencing intermittent hypoxia. As discussed in Appendix D, a lack of oxygen increases mitochondrial biogenesis which reduces ROS production. One way to achieve intermittent hypoxia might be through aerobic exercise. Permanent hypoxia might also be beneficial for lifespan, such as through living at a higher altitude. This may explain why amphibians live longer at higher altitudes but not at higher latitudes [116], and why humans

may exhibit greater longevity at higher altitudes [117]. Unfortunately, permanent hypoxia appears to be associated with slower cognitive functioning [118], suggesting more research in this area is first warranted.

- Reduce caloric consumption. As discussed in Appendix D, fewer calories mean less ROS production. Caloric restriction, or GLP-1 receptor agonists, are likely to increase lifespan.

- Maintain good oral hygiene. Oral bacteria lead to the generation of H_2O_2 by cells of the innate immune system [119]. This is harmful to the bacteria, but it is also harmful to the periodontal tissue where it is hypothesized to cause telomeric DNA damage that leads to cellular senescence [84]. This may explain why periodontitis appears to be an independent risk factor for cardiovascular disease, cerebrovascular diseases, certain cancers, diabetes, and rheumatoid arthritis [120]. In one study the relative risk of all cause mortality for individuals with periodontitis compared to no periodontal disease was 1.46 [121]. This was after adjusting for many other demographic, social, and health factors that may have influenced the outcome.

- Breathe clean air. Both air pollution and smoking are associated with an increased prevalence of age-related diseases [122, 123]. Particulate matter is associated with increased secretion of H_2O_2 by mucosa [124]. H_2O_2 secretion is one of the innate cellular defense mechanisms of the mucosa [124]. ROS produced by particulate matter has also been identified as a crucial mediator of particle toxicity [125]. Consistent with the mechanisms of aging elucidated here, air pollution and smoking are associated with both telomeric shortening [126, 127], and accelerated thymic involution [128, 129]. Smoking is associated with an increased risk of not just cancer in general, but lung cancer in particular. Presumably, in addition to their role in causing telomeric shortening, ROS in the lung microenvironment also causes non-telomeric DNA damage to proto-oncogenes.

Future interventions

The proposed molecular pathways lead to several predictions. Certain ROS inhibitors, telomere repair, senolytics, senomorphics, EMT inhibitors, and thymic regeneration may be able to prevent or delay certain age-related diseases. Hypothesized effects are shown in Table 2. Many anti-aging interventions appear likely to be most effective when started at an early age. This is because they only decelerate the rate of incidence of various age-related diseases, rather than reduce their rates. It may be necessary to combine multiple interventions, such as senolytics and thymic regeneration, for maximum effect. By the duality hypothesis, all such interventions that are based on existing genes must be careful not to interfere with any vital life-enhancing role. In addition, the neurodegenerative diseases and apoptosis of pancreatic β-cells may be delayed or prevented by anti-apoptotic factors. Whether these anti-apoptotic factors should be classified as senomorphics isn't clear.

New molecular entities that don't bear any resemblance to existing proteins, but appear to have a beneficial effect on lifespan are highly promising.

Table 2: Hypothesized effects of different interventions on the rates of incidence of different diseases with age. Decelerating effects are those for which the rates of increase in disease incidence with age are reduced. Reducing effects are those for which there is an absolute reduction in the rate of disease incidence following the intervention. Interventions are assumed not to be capable of reversing thymic involution. Thymic regeneration is assumed not to have an effect on the functioning of the immune system due to lymph node atrophy.

	Inflammatory diseases	Cancer	Fibrotic diseases	Infectious disease susceptibility	Neurodegenerative diseases
ROS inhibitors	decelerate	decelerate	decelerate	decelerate	decelerate
Telomere repair	reduce	reduce	reduce	decelerate	reduce
Senolytics	reduce	reduce	reduce	decelerate	unknown
Senomorphics	reduce	reduce	reduce	decelerate	no effect
EMT inhibitors	decelerate	reduce	reduce	decelerate	unknown
Thymic regeneration	reduce	reduce	no effect	no effect	unknown

Conclusion

For a long time microevolutionary selection has prevented scientists from reaching the conclusion that age-related diseases are intentional, but if the process is being driven by macroevolution, intentionality suddenly becomes plausible. Scientists might have overlooked the fact that a maximum lifespan extending gene might benefit the organism, but be harmful to the organism's descendants as it implies less genetic recombination is occurring over time. And genetic recombination is key to maximizing fitness in a changing environment.

Mortality is opposed by microevolution, but favored by macroevolution. This is a highly unusual situation. It leads to the following predictions: aging-related genes are pleiotropic, simultaneously exhibiting both aging-related and life-enhancing functions, successful species commonly come from a long line of failures to live longer; species that have little need to evolve will evolve long lifespans; and, species along a lineage will tend to exhibit increasing lifespans. These predictions appear to agree with the available evidence.

Long term, if it was possible to increase human lifespan to say, 150 years, this would, assuming no change in female reproductive time span, result in a doubling of the planet's population. This would have many serious social and environmental implications. Despite this it appears desirable. Otherwise why else would we today be investing heavily in finding cures to many age-related diseases through one-disease-at-a-time approaches. It is just that the one-disease-at-a-time approaches are only likely to be weakly effective, while targeting the core mechanism of aging has the long term potential to make major gains in healthspan and lifespan.

The evolutionary conflict theory of aging makes an important clinical prediction: certain ROS inhibitors, telomeric interventions, senescence interventions, EMT inhibitors, and thymic regeneration may be capable of preventing, treating, or curing many age-related diseases. The heavy burden of age-related diseases argues for a Manhattan project-like effort to better understand the fundamental biology of aging and to invest in the development and clinical trial of drugs and other interventions so as to delay, prevent, treat, and cure these age-related diseases.

Appendices

A: Mathematical support

Generation time creates an upper bound on the pre-post-reproductive time

Consider those organisms of some species that survive to effective sexual maturity. Let T_b be the mean time from fertilization until birth, T_m be the mean time from birth to effective sexual maturity, T_r be the mean effective reproductive time span, and T_s be the mean time from the end of the effective reproductive time span until death occurs.

The total life expectancy of organisms that reach effective sexual maturity, T, is given by,

$$T = T_m + T_r + T_s$$

with the first two terms representing the pre-post reproductive time.

Successful offspring can be expected to be distributed more or less at random over the reproductive lifetime of an organism. Mathematically then, the mean generation time, g, is given by,

$$g = T_b + T_m + \frac{T_r}{2}$$

If g is finite, the above equation means that T_b, T_m, and T_r are all bounded from above. Since T_m and T_r form the pre-post-reproductive time, this means the pre-post-reproductive time is bounded from above.

Simple maximum lifespan extending mutations fix rapidly

Imagine the existence of a site that if mutated and fixed would extend the maximum lifespan. Suppose the heterozygous selection coefficient per generation, s, is 10^{-2}. The spontaneous mutation rate in higher eukaryotes, μ_s, is around 10^{-8} per base per sexual generation [130]. Let the population size, N, be 10^6. Mutations of a particular genomic base pair in diploids are created at the rate $2N\mu_s$. And the probability that the mutation fixes is $2s$ [131]. So the mean time for a mutation that is destined to fix to occur, that is the establishment time in generation, τ_e, is,

$$\tau_e = \frac{1}{4sN\mu_s}$$

Plugging in the above numbers, results in 2.5×10^3 generations for τ_e.

Adapting the analysis of an asexual population [132], to a diploid sexual population, the mean time for a mutation that is destined to fix, to actually fix, τ_f, is roughly,

$$\tau_f = \frac{2 \log 2Ns}{s}$$

Resulting in 2.0×10^3 generations for τ_f.

Both the mutation establishment and fixation times are small. Since the occurrence of new maximum lifespan extending mutational prospects is likely to be a rare event, this means, there will be few simple maximum lifespan extending mutational prospects that have not already been found. Those that do exist will have come into existence recently.

More complex maximum lifespan extending mutations fix infrequently

For the sake of argument, consider a gene for which the combined effect of two particular mutations would extend maximum lifespan, but either mutation alone is harmful. Quantifying this, suppose the heterozygous selection coefficients per generation for the single and double mutations, s_1 and s_2, are -10^{-2} and 10^{-2}, respectively.

Assume the two mutations that need to occur to extend lifespan are nearby, so that the effects of recombination are negligible. Single mutations of a particular genomic base pair are created at the rate $2N\mu_s$. The mean number of generations that a single mutation will exist is $-1/s_1$. The chance of the second mutation occurring per generation is μ_s. And the probability that the double mutation fixes is $2s_2$.

Then, since we don't care which order the two mutations occur, there is an additional factor of 2 in the rate, and mean time for a double mutation to occur that will fix, τ_2, is given by,

$$\tau_2 = \frac{-s_1}{8 s_2 N \mu_s^2}$$

Plugging in the above numbers, results in 1.3×10^9 generations for τ_2.

Even if there were 1,000 complex mutational opportunities like this, it seems likely speciation would occur before any of the more complex mutations had been found.

B: Cellular senescence, immunosenescence, EMT, and age-related diseases

Many age-related diseases involve senescence, immunosenescence, EMT, or the SASP:

- Cardiovascular disease. Myocardial infarction (heart attack) and stroke are both the result of atherosclerosis. Age is an independent risk factor for the development of atherosclerosis and premature biological aging such as in patients with Werner syndrome or Hutchinson Gilford progeria syndrome accelerates the development of atherosclerosis [133]. The SASP

is implicated in atherosclerosis [90]. The thymus is also suspected of playing a key role in atherosclerosis [134].

- Cancer. EMT is key to cancer's ability to metastasize [135]. Age is a primary risk factor for most cancers. One model of tumorigenesis holds that the immune system is capable of resolving many cancers in the young, but that immunosenescence leads to reduced ability to do so in the elderly [97, 98]. Oncogene-induced senescence is widely considered a tumor suppressor. However, the SASP can both promote and inhibit tumorigenesis [136, 92]. In addition senescent cells may be able to escape oncogene-induced senescence leading to tumor progression [137]. Perhaps senescence in the context of a premalignant lesion should be viewed as a decision to leave it up to the immune system to decide upon the organism's fate.
- Alzheimer's disease. Alzheimer's disease is a disease of the elderly that results in neuronal apoptosis. SASP astrocytes may play a role in Alzheimer's disease [93].
- Diabetes. Insulin promotes the cellular absorption of glucose. Type 2 diabetes involves a combination of inadequate insulin production by β-cells in the pancreas and cellular insulin resistance. The production of insulin by β-cells appears to be limited in type 2 diabetes, at least partially because some β-cells have committed apoptosis [94]. Insulin resistance is a reduced ability to absorb insulin and use it to take up glucose. Thymic dysfunction due to aging is hypothesized as a cause of insulin resistance [138]. Senolytics are drugs that kill senescent cells. Senolytic drugs are known to be able to prevent and alleviate insulin resistance in mice [95].
- EMT and its endothelial cousin, endothelial-mesenchymal transition, likely play a vital role in fibrotic diseases including cirrhosis of the liver [139], kidney fibrosis in chronic kidney disease [140], and cardiac fibrosis in heart failure and other heart diseases [141].
- Infectious diseases. Increased susceptibility and death due to infectious diseases with age seems likely to be the result of immunosenescence including thymic involution.

The picture that emerges is of many age-related diseases having cellular senescence, immunosenescence, and EMT as common mechanisms, and different age-related diseases merely being different tissue or organ specific expressions of cellular senescence, immunosenescence, and EMT.

C: Simplified tentative molecular pathways of aging in vertebrates

Evolutionarily, it is unclear whether it makes little sense to speak of pathways of aging. The default outcome for eukaryotes was presumably to die as a result of the toxic effects of ROS. Over time pathways evolved to extend lifespan. Those that proved too successful resulted in species going extinct. This left behind residual mechanisms that caused aging and death. It is probably an issue of semantics whether these should be described as pathways.

Pathways of cell fate in vertebrates

ROS are toxic. However, the mechanism by which ROS prove toxic to the cell has become highly stylized by evolution. Whether H_2O_2 proves toxic to a particular cell will be highly context depen-

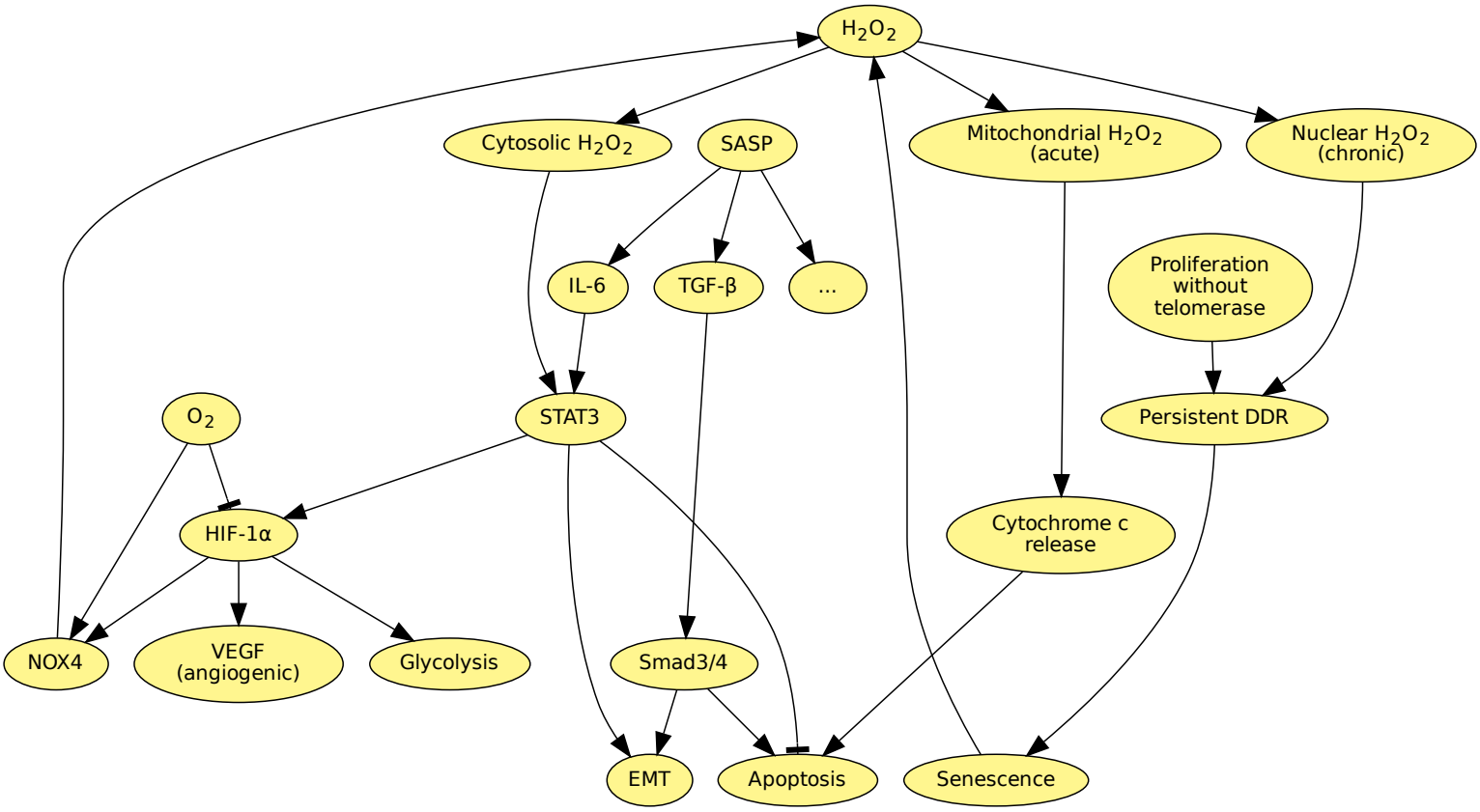

Figure 2: Pathways of cell fate. Proposed pathways leading from intracellular hydrogen peroxide and the SASP to cellular EMT, apoptosis, or senescence in vertebrates and possibly other species.

dent, depending upon factors such as the external environment, the internal physiological state of the cell, and the cell type. A proposed molecular pathway leading from intracellular H_2O_2 to cell fate is shown in Figure 2.

The two cell fates we are most concerned with are cellular senescence and EMT. As discussed earlier, aging-related diseases largely seem to be a consequence of these two cellular modalities. The role of apoptosis in aging-related diseases is less clear, although arguably apoptosis plays an important role in neurodegenerative diseases, the loss of pancreatic β-cells in diabetes, and in sarcopenia.

Figure 2 will be explored briefly below, with EMT and cellular senescence, explored in more detail later.

EMT related pathways

It is hypothesized that H_2O_2 plays a role in the cytosolic determination of cell fate through the promotion of EMT. This is consistent with the observation that H_2O_2 can induce EMT [142, 143, 144].

The SASP plays a key role in EMT. SASP components such as IL-6 inhibit apoptosis and promote EMT. If IL-6 is part of the macroevolutionary mechanism of EMT induced aging, then perhaps transforming growth factor-β (TGF-β) as an early stage SASP component [145], represents a mi-

croevolutionary response to IL-6 induced EMT, attempting to prevent it by instead steering the cell towards apoptosis.

TGF-β is known to play a dual role in cancer, preventing uncontrolled cellular proliferation, but at the same time promoting metastasis. This is known as the TGF-β paradox. This may be a result of the EMT requiring both signal transducer and activator of transcription (STAT3) and Smad3/4 signaling. In the absence of IL-6, TGF-β promotes apoptosis, but in its presence it promotes EMT.

Apoptosis related pathways

H_2O_2 is well known as an inducer of apoptosis [146]. During apoptosis H_2O_2 oxidizes cardiolipin found in the inner membrane resulting in it releasing bound cytochrome c [147]. Oxidized cardiolipin also helps open the mitochondrial permeability transition pore in the outer membrane [148, 149, 150]. Opening of the pore leads to a swelling of the mitochondrial matrix, rupturing the outer mitochondrial membrane, and the release of apoptotic intermembrane proteins into the cytosol, including cytochrome c [151]. Apoptosis may also be initiated from outside the mitochondria.

Senescence related pathways

As will be explored later, either chronic nuclear H_2O_2 or proliferation in the absence of telomerase leads to telomeric damage and a persistent DNA damage response (DDR). The DDR induces cellular senescence.

The mitochondria of senescent cells display increased H_2O_2 production [89], further committing the cell to senescence.

HIF-1 alpha, NOX4, and the Warburg effect

STAT3 upregulates the transcription factor hypoxia-inducible factor 1-α (HIF-1α) both transcriptionally and by stabilizing the protein against ubiquitin mediated degradation [152].

HIF-1α functions as a hypoxia sensor, and is responsible for the upregulation of vascular endothelial growth factor (VEGF) and genes promoting glycolysis when intracellular oxygen is low [153, 154, 155].

HIF-1α upregulates NADPH oxidase 4 (NOX4) [156]. NOX4 converts O_2 into H_2O_2 [157]. Thus the HIF-1α/NOX4/H_2O_2/STAT3 circuit appears to provide a positive feedback mechanism for intracellular H_2O_2 that is governed by the effect of the O_2 concentration on HIF-1α. Speculatively, the production of H_2O_2 concurrent with the promotion of glycolysis may be a macroevolutionary mechanism to ensure vertebrates can't avoid the aging effects associated with oxidative phosphorylation by instead using glycolysis.

Cancer cells frequently rely on glycolysis, even in the presence of oxygen [158]. This is known as the Warburg effect [158]. Cancer cells frequently display high levels of HIF-1α activation, in part due to the hypoxia of the tumor microenvironment [159]. NOX4 expression levels are upregulated

in a wide variety of cancers [160]. In addition cancer cells frequently display high levels of H_2O_2 [161]. Very speculatively, the occurrence of the Warburg effect, and the activation of the HIF-1α/NOX4/H_2O_2/STAT3 circuit in cancer cells could help prevent their apoptosis. Cancer cells that lack the Warburg effect may be more likely to undergo apoptosis as a result of a relative lack of STAT3 activation.

Molecular pathways of cellular senescence in vertebrates

A proposed molecular pathway leading from mitochondrial $O_2^{\bullet -}$ production to senescence is shown in Figure 3 and expanded upon below. The molecular biology of senescence is still being elucidated, and other plausible pathways exist. This is especially true of the lower portion of the figure which shows the activation of the senescent phenotype. The complexity of aging-related pathways creates some difficulty in determining the relevant pathways with certainty.

ROS

As shown at the top of Figure 3, it is proposed that mitochondrially produced $O_2^{\bullet -}$ gets converted into the stable ROS H_2O_2 by SOD. For $O_2^{\bullet -}$ occurring in the intermembrane space, it might first need to pass through the outer mitochondrial membrane. This would probably be possible because the outer membrane contains pores with a diameter of 1.2nm [162]. $O_2^{\bullet -}$ could then be converted to H_2O_2 by the cytosolic SOD, SOD1. For $O_2^{\bullet -}$ directed to the matrix, $O_2^{\bullet -}$ will be converted to H_2O_2 by the matrix resident SOD, SOD2. In the matrix, peroxidases may reduce some of the H_2O_2 to H_2O. H_2O_2 is largely membrane permeable and should over the course of perhaps a few seconds be capable of migrating to the nucleus [35, 36]. The Fenton reaction then produces the highly reactive $HO^{\bullet}$ from H_2O_2.

The Fenton reaction involves the oxidation of Fe^{2+}. In humans, genome wide association studies have found the heme metabolism pathway is related to lifespan, and that serum iron has been found to correlate negatively with lifespan [163]. Generally speaking, mild iron deficiency and iron chelators have been found to increase lifespan in various species, while excess iron has been found to promote aging [164]. This is understandable if increased iron leads to increases in the production of $HO^{\bullet}$.

Interestingly, the Fenton reaction is known to be greatly enhanced in the presence of the DNA sequences AGGG and GGGG [165]. AGGG forms part of the telomeric repeat for many multicellular organisms, with TTAGGG being the sequence for vertebrates [166].

Telomeric damage

As further shown in Figure 3, $HO^{\bullet}$ is capable of producing a range of DNA damage, including frequently converting guanine, G, into 8-oxoguanine (8-oxo-G) [167]. 8-oxo-G is detected and removed by the base excision repair (BER) machinery. In BER, 8-oxoguanine glycosylase (OGG1) removes 8-oxo-G and creates a single strand break (SSB) in the DNA backbone, which is normally immediately filled with the correct base and ligated [168]. In telomeres the SSB repair steps appear

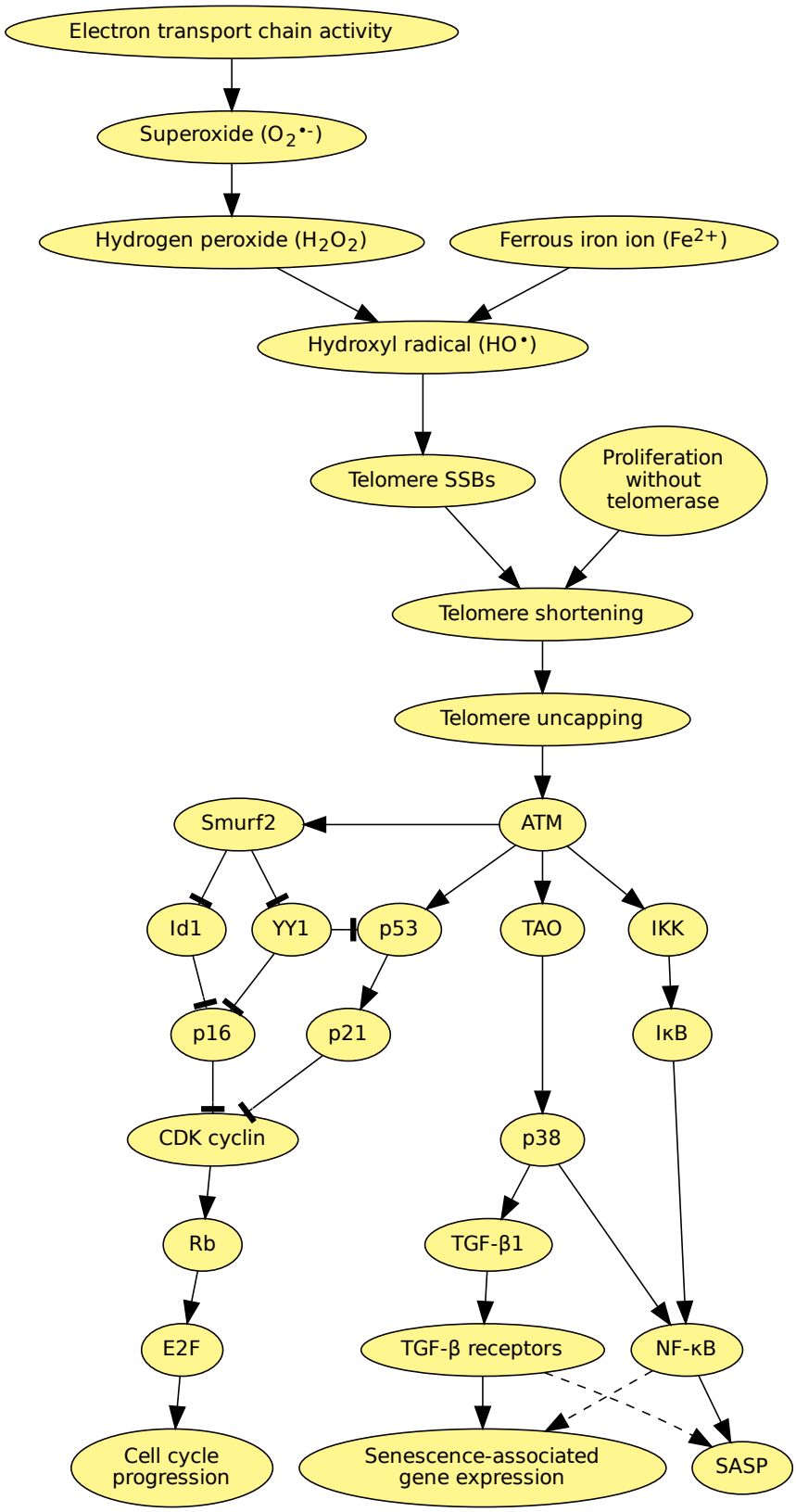

Figure 3: Pathway of senescence. Proposed molecular pathway leading from mitochondrial superoxide production to senescence in vertebrates and possibly other species. The lower portion of the figure showing the activation of the senescent phenotype is both simplified and not fully understood.

impaired [169]. This may be due to the action of telomeric repeat-binding factor 2 (TRF2) which associates with the telomeres [170]. Thus $HO^{\bullet}$ is capable of producing longer lasting SSBs.

Unrepaired telomeric SSBs will lead to telomere shortening when the cell next divides [171]. In non-proliferating cells, two unrepaired SSBs within approximately 1 or 2 turns of the DNA double helix (10 to 20 base pairs) located on opposing strands are likely to lead to a double strand break (DSB) [172, 173], creating telomere shortening.

Telomere shortening will also occur if the cell is dividing in the absence of telomerase. This is due to the end replication problem. The DNA replication machinery is unable to replicate the last few bases of a linear chromosome.

The DNA damage response (DDR) might view chromosome ends as DSBs and attempt to randomly repair them by joining chromosomes together [174]. TRF2 binds to telomeres and usually prevents the induction of the DDR at chromosome ends [174]. If telomeres shorten sufficiently they become uncapped, adopting a linear conformation, in which the remaining TRF2 appears sufficient to prevent end joining, but insufficient to prevent DDR signaling by ataxia telangiectasia mutated (ATM) [175], leading to persistent ATM DDR signaling by the telomere.

The occurrence of multiple persistent DDR signals from multiple telomeres is sufficient to induce cellular senescence [176].

Support for persistent ATM DDR signaling by telomeres as the indicator of age for the cell is provided by a number of observations. Telomeric damage irreparably appears to be evolutionarily conserved; it occurs in both yeasts and humans [177, 178]. Live-cell imaging experiments show all persistent DNA damage foci to be associated with telomeres [179]. There is an age-dependent increase in the number of telomere-associated foci that occurs irrespective of telomere length [179]. Shortened telomeres are associated with aging, as well as mortality risk [113]. Telomere lengths of mammalian species correlate inversely with their lifespans [180]. Intracellular H_2O_2 levels are known to accelerate telomere shortening [181]. Extracellular SOD, SOD3, is known to reduce the rate of telomere shortening [182]. And all eukaryotes appear to have linear chromosomes with telomeres rather than circular chromosomes or circular genomes like bacteria and archaea.

ATM

The DSB DDR in the form of persistently phosphorylated ATM appears to be at the hub of the senescent phenotype. Activated ATM appears to be responsible for cell cycle arrest, the expression of a number of genes associated with senescence, and the SASP.

Arguing for the model of activated ATM as the cause of senescence, elevated levels of activated ATM have been found with age in naturally aged and acceleratedly aged mice. and reducing ATM activity has been found to reduce senescence [183]. Similarly inhibition of ATM has been found to ameliorate senescence [184]. In this latter result, ATM was hypothesized to phosphorylate a component of an ATPase responsible for acidification of the lysosome leading to lysosomal dysfunction. Seemingly contradicting these findings, decreased ATM levels along with reduced p53 activity have been found in older mice [185]. Similarly, declining levels of ATM have been reported with replicative passage, knocking down ATM has been reported to accelerate senescence, and activation of ATM

has been reported as being capable of clearing replicative senescence [186]. Part of the reason for the seeming discrepancy in these results may be due to the difference between ATM expression levels and phosphorylated and activated ATM, and the study of replicatively induced as opposed to DNA-damage-induced or stress-induced senescence.

p53, p16, p21, and cell cycle arrest

As shown in the lower left part of Figure 3, activated ATM is able to phosphorylate and stabilize p53, a key regulator of cell fate [187].

Activated ATM is also able to phosphorylate and activate Smurf2 [188]. Smurf2 is a ubiquitin ligase, and its targets include the transcriptional repressors inhibitor of DNA binding 1 (Id1) and Yin Yang 1 (YY1) [189, 190]. Id1 and YY1 repress the transcription of cyclin-dependent kinase inhibitor p16 [191]. The pathway from ATM's activation to activation of p16 doesn't appear to be well studied, and it is possible other pathways exist different from this one.

Supporting a role for p16, p16 increases with age, and has even been proposed as a biomarker of aging [192, 193]. p16 expression is also significantly elevated in senescent cells [194].

p16 binds specifically to cyclin dependent kinases (CDKs) 4 and 6 preventing them from phosphorylating retinoblastoma protein (Rb) [191]. In its phosphorylated form Rb would have changed conformational form releasing bound E2F transcription factors [191]. The E2F transcription factors are responsible for the transcription of the genes necessary for the G1 to S phase transition, or in the event of prolonged E2F expression, apoptosis [191, 191].

Both the p16 protein and the p14ARF protein are encoded by the CDNK2A locus, but use different open reading frames [195]. This is highly unusual, but is consistent with the duality hypothesis. Instead of the aging-related function and the life-enhancing function being two different parts of the one protein, they may be two separate proteins coded for by a common stretch of DNA. Whereas p16 appears to lead to cell cycle arrest and senescence, p14ARF appears to block the degradation of p53, and the buildup of p53 is known to result in cell cycle arrest or apoptosis [196]. The mouse equivalent of p14ARF is p19ARF. Having two separate proteins would make the therapeutic inhibition of p16 much simpler than that of most other aging-related genes. Unfortunately, p16 blocks cell cycle progression rather than say production of the SASP, and so p19ARF positive p16 knockout mice are tumor prone [197].

In addition, YY1 acts as a negative regulator of p53 [198].

p53 positively regulates transcription of the cyclin-dependent kinase inhibitor p21 [199]. p21 binds to and non-specifically blocks the activity of CDKs again preventing the G1 to S phase transition [200].

Thus, activated ATM is able to arrest the cell cycle through multiple means.

p38 and senescence-associated gene expression

As shown in the lower central part of Figure 3, in addition to arresting the cell cycle, ATM is also capable of phosphorylating and activating thousand and one amino acid (TAO) kinases [201]. TAO kinases are MAPK kinase kinases (MAP3K), which activate MAPK kinases (MAP2K) kinases, which activate p38 MAPK [202]. Activated p38 is known to both mediate apoptosis and in specific circumstances cell survival [203]. Activated p38 is also known to cause overexpression of transforming growth factor-β1 (TGF-β1) [204]. Osteonectin, apolipoprotein J, and fibronectin are commonly overexpressed in senescence [205]. TGF-β1 appears to cause an increased expression of mRNA for these three genes, as well for its own receptor [206]. This increased expression is eliminated by antibody neutralization of TGF-β1 or its receptor. Thus activated ATM may be capable of producing part of the phenotype associated with senescence.

NF-κB, and the SASP

Finally, as shown in the lower rightmost part of Figure 3, the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is capable of being activated through several mechanisms. NF-κB appears responsible for part of the SASP [207].

The first mechanism of activating NF-κB is by cytosolic ATM activating IκB kinase (IKK), which then phosphorylates IκB leading to IκB degradation via the ubiquitin-proteasome pathway, freeing NF-κB from its association with IκB, and allowing NF-κB to enter the nucleus [183].

A second mechanism of NF-κB activation is through the activity of p38 [208].

Taken together these pathways show a route leading from mitochondrial ROS production to cellular senescence. This provides evidence for the claim that mitochondria ROS enforce mortality, and in so doing improve the ability of the species to adapt.

Molecular pathways of EMT in vertebrates

A proposed molecular pathway leading from the SASP and cytosolic H_2O_2 to EMT is shown in Figure 4 and expanded upon below. The figure is a gross simplification of reality. In particular only the effects of a single inflammatory SASP component (IL-6) and a single anti-inflammatory SASP component (TGF-β) are shown.

IL-6 and STAT3 related pathways

Cytosolic H_2O_2 regulates the transcription factor signal transducer and activator of transcription 3 (STAT3) which will dimerize and translocate to the nucleus where it can bind DNA. H_2O_2 does this through at least two pathways. PTPs are protein-tyrosine phosphatases. H_2O_2 oxidizes the catalytic cysteine residue of SH2 domain-containing PTPs (SHPs) inactivating them [209]. Were they not deactivated SHP-1 would dephosphorylate STAT3 inactivating it [210]. Second, H_2O_2 oxidizes peroxiredoxin 2 (Prx2), which goes on to cause disulfide-linked STAT3 oligomers, reducing

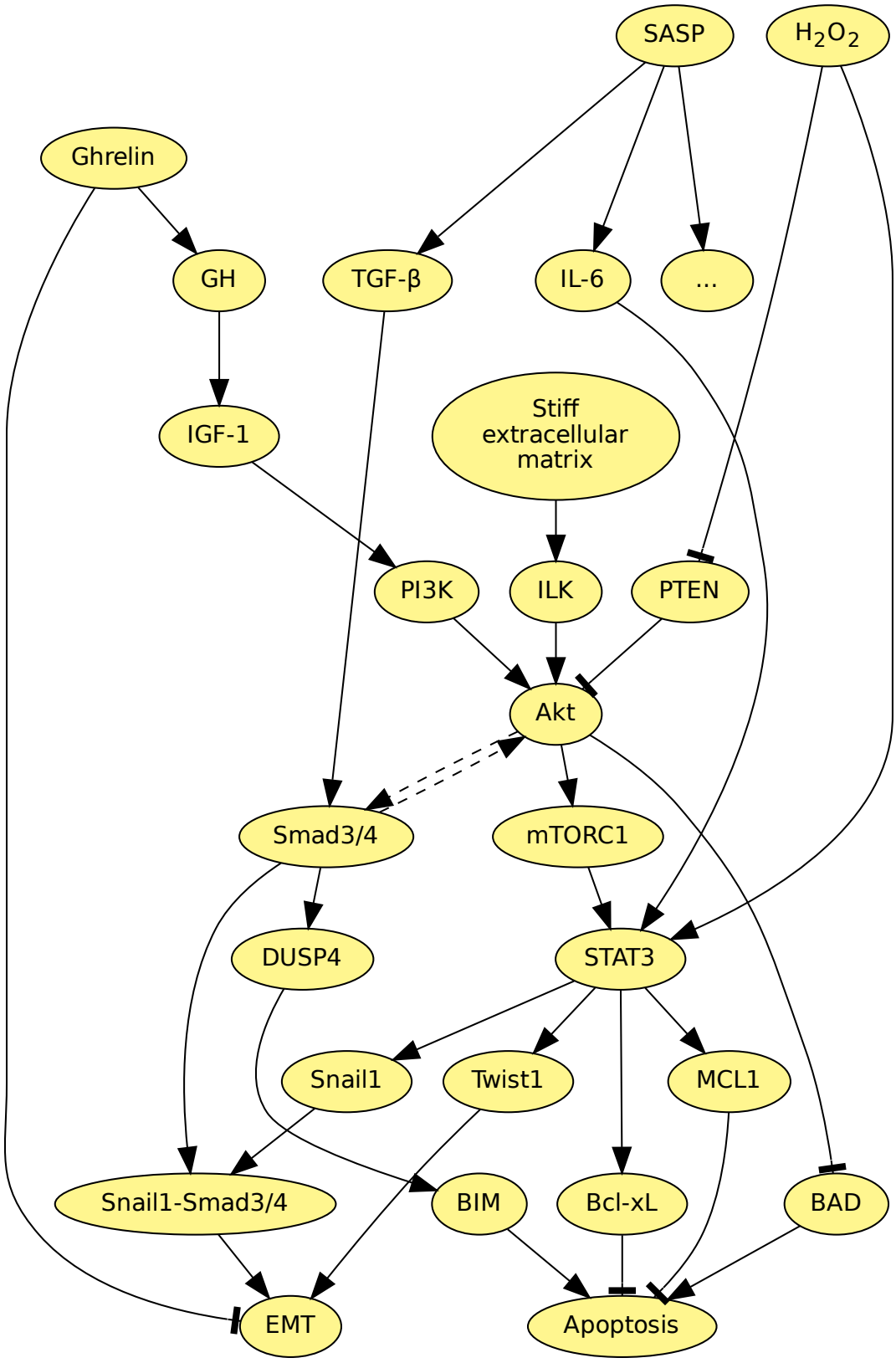

Figure 4: Pathway of EMT. Proposed molecular pathway leading from the SASP and cytosolic hydrogen peroxide to cellular EMT in vertebrates and possibly other species. The figure is a gross simplification of reality.

their transcriptional activity [211, 212]. These two pathways conflict. One increases STAT3 activity, another reduces it. This conflict is known [213], and is to be expected. Aging-related pathways are likely to be opposed by other genes. The question is which is the aging-related pathway, and which is the evolutionary response. On the basis that activated STAT3 promotes EMT [214, 215], the activation of STAT3 by H_2O_2 is viewed as the aging-related pathway. This conclusion is by no means definitive. It is adopted only because it fits with the broader framework of ROS being harmful to the organism.

Twist1 is a transcription factor known to promote EMT [216]. Twist1 expression is induced by STAT3 [217].

Snail1 is a transcriptional repressor. Snail1 expression is activated by STAT3 [218].

Snail1 combines with cofactors Smad3 and Smad4 to form the Snail1-Smad3/4 complex which represses the expression of E-cadherin [219]. E-cadherin is a key protein for cell-cell adhesion, and its downregulation is a key step in EMT [219].

In addition to promoting EMT, STAT3 simultaneously suppresses apoptosis by promoting expression of the anti-apoptotic myeloid cell leukemia 1 (MCL1) and B-cell lymphoma-extra large (Bcl-xL) [220, 221].

STAT3 can also be activated by exogenous IL-6. IL-6 is a key component of the SASP. IL-6 can combine with soluble IL-6 receptor (sIL-6R) and bind to glycoprotein 130 (gp130) which is present on many cell types [222]. Gp130 activates the Janus kinase (JAK) - STAT3 pathway [223].

ILK and Akt related pathways

Akt, aka protein kinase B (PKB), is a kinase that promotes cellular survival. Akt phosphorylates, and thereby deactivates, Bcl-2 associated agonist of cell death (BAD) thereby inhibiting apoptosis [224].

Akt also activates the mechanistic target of rapamycin complex 1 (mTORC1) pathway [225]. STAT3 can be phosphorylated at Ser727 by a number of kinases, including mTORC1, thereby enhancing STAT3's activity [226].

Thus IL-6 or Akt activation makes the cell more likely to invoke EMT [227, 228].

One way in which Akt may be activated is by the integrin-linked kinase (ILK). ILK is activated by the presence of a stiff extracellular environment [229]. Activated ILK phosphorylates and activates Akt [230]. Thus the presence of a stiff extracellular environment will tend to promote EMT, and its absence will tend to promote apoptosis.

EMT as a result of ILK signaling is known to occur in cancer metastasis [231].

Regulation of Akt signaling by PTEN and PI3K

Phosphatase and tensin homolog (PTEN) catalyzes the conversion of phosphatidylinositol (3,4,5)-trisphosphate (PIP_3 to phosphatidylinositol (3,4)-bisphosphate (PIP_2) [232]. Since PIP_3 activates Akt [232], PTEN upregulation inhibits Akt.

PTEN is vulnerable to oxidation by H_2O_2, inactivating it, and agonizing Akt [233]. This represents a second mechanism whereby H_2O_2 may activate STAT3.

Phosphoinositide 3-kinases (PI3Ks) catalyze the reverse reaction from that of PTEN, converting PIP_2 to PIP_3 [234]. As a result PI3K upregulation activates Akt.

One means of activating PI3K is through insulin-like growth factor 1 receptor (IGF-1R) signaling. Activated IGF-1R recruits insulin receptor substrate (IRS) proteins [235]. This leads to PI3K activation, and Akt upregulation [235].

The binding of the extracellular hormone insulin-like growth factor 1 (IGF-1) to IGF-1R activates IGF-1R, and thus upregulates Akt. Since Akt activates STAT3, this suggests IGF-1 is likely to be pro-EMT and anti-apoptotic. This appears to be the case. IGF-1 is known to promote the EMT of cancer cells [236, 237, 238], although this effect is by no means universal [239]. Similarly, IGF-1 is known to be anti-apoptotic [240, 241].

The hunger hormone ghrelin stimulates the production of growth hormone (GH) [242], which stimulates the production of IGF-1 [243]. Ghrelin has been associated with cancer cell proliferation, however the literature on the topic has been described as containing inconsistencies [244]. Ghrelin ablation has shown that ghrelin acts to inhibit thymic EMT, although the mechanism doesn't appear to be understood [245]. This ability of ghrelin to inhibit EMT is despite the fact that GH appears to promote EMT [246].

TGF-β and Smad related pathways

Transforming growth factor-β (TGF-β) is an early stage SASP component [145]. The binding of TGF-β to TGF-β receptors (TGF-βR) causes the phosphorylation of Smad3 which then complexes with Smad4 and promotes apoptosis [247]. This apoptosis may be the result of Smad3 inducing the expression of the dual specificity protein phosphatase 4 (DUSP4), which leads to the accumulation of the pro-apoptotic Bcl-2 interacting mediator of cell death (BIM) [248].

As previously mentioned, Snail1 can combine with Smad3 and Smad4 inhibiting the expression of E-cadherin and other genes, and promote EMT.

Interactions between TGF-β/Smad3 and Akt are complex, and highly dependent on the cellular environment and state:

- Akt enhances Smad3 activity by phosphorylating it in mesangial cells, by activating ubiquitin specific protease 4 (USP4) which contributes to the deubiquitination and stabilization of TGF-βR in breast cancer cells promoting EMT, and by inhibiting the Smad3 polyubiquitination promoting glycogen synthase kinase-3β (GSK-3β) [249].

- Akt inhibits Smad3 [250, 251, 252, 253]. Inhibition of Akt by Smad3 is known to occur through Akt binding and sequestering Smad3 in the cytosol in hepatocytes [249].
- TGF-β enhances Akt [254]. TGF-β stimulation results in the phosphorylation of Akt at Ser473 in a Smad independent fashion activating Akt in keratinocytes and mammary epithelial cells, and by causing the expression of microRNAs that activate PI3K in hepatoma cells leading to enhanced EMT [249].
- Smad3 inhibits Akt [255].

Only the two interactions that appear relevant to the determination of cell fate, that is the induction of EMT, are shown in Figure 2.

D: Other approaches to aging

This appendix reviews other approaches to aging, and shows that they can largely be aligned with the molecular pathway proposed for aging in vertebrates. Multiple mechanisms for some of these other approaches have been suggested. In reviewing these other approaches proposed mechanisms that align with the mechanisms proposed in this manuscript are examined.

Senotherapeutics

Senolytic and senomorphic compounds are widely viewed as having much promise as lifespan extending agents [51].

Interfering with the SASP component interleukin-6 (IL-6) appears highly promising; IL-6 antibody has been shown to extend the median lifespan of regulatory T cell deficient mice from around 20 to 50 days [256].

Pharmacological inhibition of the EMT promoting SASP component tumor necrosis factor (TNF) [257] extends lifespan in aging mice [258]. TNF antibodies have also been shown to reverse thymic involution brought about by a TNF transgene [259].

The promise of senotherapeutics is consistent with the pathways of aging explored here.

Thymic transplantation

Transplantation of thymic tissue from young rats to the ocular anterior chamber of aged rats has been shown to increase lifespan by 20-25% [260]. In addition, grafting a newborn thymus under the kidney capsule along with bone marrow transplantation modulates diabetes in a type 2 diabetes mouse model [261].

The effects of thymic transplantation are consistent with thymic involution being a key mechanism of aging.

Thymic regeneration

The treatment of humans with recombinant human growth hormone (rhGH) assists in thymic regeneration [262]. A 1 year course of treatment of rhGH along with dehydroepiandrosterone (DHEA) and metformin produced a 1.5 year reduction in apparent epigenetic age at the end of treatment [262].

Thymic regeneration is consistent with thymic involution being a key mechanism of aging.

Pineal gland transplantation and melatonin

The pineal gland in the brain secretes melatonin into the circulatory system [263]. Melatonin is able to pass through biological membranes [264]. Melatonin can function as an intracellular antioxidant [265]. Circulating melatonin is also able to bind melatonin receptors on the surface of some cell types, while intracellular melatonin is able to bind nuclear melatonin receptors [266]. The thymus contains melatonin receptors [266]. Melatonin promotes the expression of various intracellular antioxidants [267].

Like the thymus, the pineal gland involutes with age [268], and circulating melatonin levels decrease with age [263]. The nighttime administration of melatonin in pineal melatonin producing mice strains (such as C3H/He and CBA/Ms [269]) may possibly extend lifespan [270, 271]. However these results are overshadowed by confusion stemming from the fact that many other lab mouse strains (including C57BL/6, BALB/c, DBA/2, NZB, and Swiss) appear unable to synthesize melatonin [269, 272, 273]. Pinealectomy is known to lead to rapid involution of the thymus in rats, and this involution can be prevented by the administration of melatonin [274].

The lifespan altering effects of pinealectomy are consistent with the mechanism of thymic involution in aging. And the lifetime extending effects of melatonin in melatonin producing mouse strains are consistent with melatonin antagonizing thymic involution.

Aerobic exercise and hypoxia

Aerobic exercise prolongs healthspan and lifespan [275, 276].

Aerobic exercise will likely result in cells in the body being in a state of relative hypoxia. The shortage of oxygen would cause a decline in the production of ATP, which would lead to a compensating increase in mitochondrial biogenesis. Subsequent to the aerobic exercise, the resulting increase in mitochondrial content will reduce the flow of electrons through each individual electron transport chain complex and thereby reduce the production of ROS [277]. Consistent with this hypoxia is known to cause increased mitochondrial biogenesis [278, 279], and to extend lifespan [280]. Confirming this, aerobic exercise is known to increase mitochondrial biogenesis [281], and reduce the production of ROS [276].

The beneficial effects of aerobic exercise are consistent with mitochondrial ROS causing aging.

Preventing stem cell exhaustion

Loss of stem cells represents one proposed cause of aging [282]. The proposed mechanism involves the production of ROS by stem cells causing DNA damage and telomere shortening [282]. In addition, the loss of the stem cell niche provided by progenitor cells is also proposed to lead to stem cell exhaustion, once again as a result of ROS causing DNA damage and telomere shortening [282]. This all aligns with the pathways proposed here.

The SASP has complex effects on stem cells. It can both cause differentiation promotion and differentiation inhibition, depending on the type of stem cell and the SASP factors involved [283]. Whether the SASP can also cause stem cell apoptosis, and thus lead to stem cell exhaustion doesn't appear to have been determined.

It is worth pointing out that stem cells usually express telomerase [102]. This casts some doubt on telomere shortening in stem cells as a cause of stem cell exhaustion. It also means interventions intended to extend lifespan are unlikely to fail due to the shortening of stem cell telomeres causing stem cell senescence.

There are some uncertainties, but the possibility of stem cell exhaustion would be consistent with the ROS – telomeric damage pathway.

Down-regulation of the IGF-1 signaling pathway

Insulin signals to the organism the availability of glucose energy that should be taken up by cells. Insulin-like growth factor 1 (IGF-1) stimulates cell growth, proliferation, and survival [284]. The down-regulation of the insulin/IGF-1 signaling pathway has been proposed as an anti-aging intervention [285].

Adaptability of the species will be maximized if organism lifespans are kept short. If the lifespan is too short however there will be insufficient time for reproduction to occur. If the organismal environment has little energy, it will take longer for the organism to grow and reproduce, and it might be expected that there would be a more permissive mandate regarding the maximum lifespan of the organism. Alternatively, the organism could grow to a smaller size, but a smaller size increases the risks of predation. If the organism is tricked into believing it is in a low energy environment, it might be expected to exhibit an increased maximum lifespan. As discussed below, this appears to be the case: down-regulation of insulin/IGF-1 signaling increases lifespan.

Caenorhabditis elegans has a single insulin/IGF-1 receptor gene, daf-2. daf-2 mutants show increased lifespan [286]. daf-2 mutants exhibit a change in gene expression compared to the wild-type that is mediated by several transcription factors. This includes daf-16 up-regulation, a forkhead subclass O (FOXO) transcription factor [285]. In *Drosophila melanogaster* inhibition of insulin/IGF-1 signaling or increasing FOXO increases lifespan [285]. In mice there is a negative correlation between IGF-1 levels and lifespan [285]. Finally, small dogs have a mutation that decreases IGF-1 levels and live longer [285].

Consistent with the evolutionary theory, in an environment of food abundance and scarcity, the long lived *C. elegans* mutants are outcompeted by the shorter lived wild type [287].

Table 3: Relative catalase mRNA levels of daf-2 mutants. N-fold change in catalase mRNA of *C. elegans* daf-2 mutants versus control. [a] - mapping locus includes both ctl-1 and ctl-2.

Experiment type	Experiment id.	ctl-1	ctl-3
microchip	NCBI GEO DataSets GSE106672 [290]	2.4	2.5
RNA-Seq	NCBI GEO DataSets GSE111338	1.5[a]	2.8
RNA-Seq	NCBI GEO DataSets GSE70117 [291] at 15°C	1.5	1.8
RNA-Seq	NCBI GEO DataSets GSE70117 [291] at 25°C	2.9	2.6
RNA-Seq	NCBI GEO DataSets GSE67975 [292]	1.6	4.2

A possible mechanism by which daf-2 mutants extend lifespan might be through a reduction in the level of H_2O_2. This reduction might occur through the up-regulation of H_2O_2 reducing genes. Unlike humans, which possess a single catalase that is located in the peroxisome, *C. elegans* contains 3 catalase genes. ctl-1 is widely considered to be cytosolic [288], although WormBase WS286 lists its putative location as peroxisomal and mitochondrial [289]. ctl-2 is peroxisomal [288]. ctl-3's location is uncharacterized [288], but predicted to be peroxisomal and mitochondrial in WormBase WS286. Mitochondrial and cytosolic catalases in particular can be expected to reduce cytosolic H_2O_2 levels and reduce telomeric damage. Up-regulation of these catalases in daf-2 mutants has been confirmed by examining the results from a few gene expression experiments as shown in Table 3.

Seemingly antagonizing these findings, IGF-1 is known to enhance thymopoiesis, primarily through thymic epithelial cell expansion [293].

Growth hormone (GH) stimulates the production of IGF-1. GH and IGF-1 overexpression correlates with increased body mass in mice, while GH receptor or IGF-1 deletion reduces body mass in mice [294]. Ames dwarf mice are GH deficient and have a smaller body mass and longer lifespan than normal mice [295]. Treatment of Ames dwarf mice with GH during early life increases body mass and reduces their lifespan [295],

Ames dwarf mice have increased levels of hepatic antioxidants, while mice which overexpress GH have reduced levels of hepatic catalase and shortened lifespans [296]. Regular mice hepatocytes treated with growth hormone show a reduced level of catalase activity and other antioxidants [296].

Besides increased antioxidant activity, a second possible explanation for the benefits of down-regulating the GH/IGF-1 axis comes from the possibility of IGF-1 promoting EMT. This was illustrated in Figure 4. In support of this, an Ecuadorian population with growth hormone receptor deficiency, or Laron syndrome, showed a remarkable reduction in the incidence of cancer [297].

Note that the short run and long run effects of GH/IGF-1 appear to oppose each other. In the short run GH/IGF-1 boosts thymic function [298], which increases organismal survival. In the long run it may promote EMT, which decreases organismal survival.

It seems plausible that repression of the insulin/IGF-1 axis mechanistically extends lifespan by increasing antioxidant levels and thus inhibiting mitochondrial ROS production and/or reducing

EMT. However, interfering with the insulin/IGF-1 axis may only be productive in organisms that have more food security or experience less predation than existed in the evolutionary environment.

Weight reduction and caloric restriction

Body mass, and in particular adipose tissue mass, appears to be a risk factor for the development of age-related diseases [299]. Seemingly related to this, caloric restriction is capable of extending an organism's lifespan [300]. Similarly, GLP-1 receptor agonists promote satiety, reducing food intake, which reduces ROS, reducing cellular senescence and aging-related diseases, and increasing lifespan [301, 302].

Evolutionarily, this may be explained by the same means as down-regulation of the IGF-1 pathway. An organism that appears to be in a low energy environment should be given longer to carry out its biological program.

Mechanistically, the result may be direct. Fewer calories consumed, means less energy burned, means less ROS produced. Furthermore, caloric restriction stimulates ghrelin [303], which may act to inhibit EMT [245]. The inhibition of EMT is predicted to inhibit thymic involution and cancer, thus extending lifespan. Consistent with this obesity appears to accelerate thymic involution [304]. Conversely, caloric restriction results in a reduction in age-related thymic involution [305].

The effects of weight and caloric restriction are consistent with the pathways proposed here.

Down-regulation of mTOR

The mammalian target of rapamycin (mTOR) kinase is an energy and nutrient sensor that stimulates growth and blocks autophagy when nutrients are plentiful [285].

The mTOR pathway has invoked considerable interest as a possible aging mechanism [306]. Inhibition of mTOR has been shown to significantly extend lifespan in a number of species [307].

As previously mentioned, mTOR complex 1 (mTORC1) is one of a number of kinases that can phosphorylate STAT3, enhancing its activity, and STAT3 promotes EMT.

Inhibition of mTOR down-regulates the production of multiple protein synthesis components, including ribosomes, initiation factors, and elongation factors [308]. Thus inhibition of mTOR will reduce the energy needs of the cell. Reducing the energy needs of the cell should reduce the amount of oxidative phosphorylation performed by the mitochondria, and hence reduce the production of ROS. In addition it has been shown that the inhibition of mTOR increases the translation of mitochondrial encoded oxidative phosphorylation subunits, which likely leads to few electrons transiting a given electron transport chain, an oxidized chain, reduced ROS production, and less ROS-mediated cellular damage [307].

Lifespan extension by mTOR inhibition might be linked to mTOR's role in promoting STAT3 and thus EMT, or due to mTOR inhibition functioning as a mitochondrial ROS inhibitor.

Up-regulation of AMPK

Overexpression of the AMP-activated protein kinase (AMPK) activator aak-2 in *C. elegans* has been shown to extend lifespan [309].

AMPK is activated when the AMP to ATP ratio rises [310]. Amongst other things activated AMPK inhibits mTOR and promotes mitochondrial biogenesis [311, 312]. This mitochondrial biogenesis includes production of mitochondrially encoded proteins [313].

Both the inhibition of mTOR and increased mitochondrial biogenesis without a concomitant increase in the energy demands of the cell, might be expected to reduce ROS, and by the mechanisms proposed here extend lifespan.

Up-regulation of sirtuins

Sirtuins are a family of NAD+ dependent deacetylases and ADP-ribosyltransferases [314]. Overexpression of the sirtuins SIRT1 and SIRT6 has been demonstrated to extend lifespan in various species [314].

Mice, unlike humans, express telomerase in somatic cells [315]. In mice SIRT1 expression correlates with telomere length and reduces age-related telomere shortening [316]. In humans a single nucleotide polymorphism in SIRT1 correlates with telomere length and longevity [317].

SIRT1 also deacetylases the autoimmune regulator (AIRE) leading to AIRE's activation in thymic mTECs and thus contributing to T cell development [318].

SIRT6 deacetylates histone H3K9 promoting telomere stability by enabling telomere association with Werner syndrome ATP-dependent helicase (WRN) [319]. Mutations in WRN result in Werner syndrome, a disease exhibiting premature aging [320]. SIRT6 knockout mice exhibit hypersensitivity to H_2O_2 [321]. SIRT6 is also believed to play a role in stimulating DSB repair, with more effective SIRT6 activity correlating with longer lifespan [322]. Finally, SIRT6 deficiency is associated with increased NF-κB signaling [323].

In addition, it has been shown that the TEC specific knockout of SIRT6 drastically reduces the size of the thymic mTEC compartment [324].

In summary, SIRT1 and SIRT6 may extend lifespan by affecting telomere length, assisting in telomere damage repair processes, and/or possibly contributing to thymic mTEC function and development.

Antioxidants

As discussed in the body of this manuscript, antioxidants are frequently associated with increased lifespan. Furthermore, as explored in Appendix E, those cases where antioxidants don't extend lifespan appear understandable given the framework that has been developed.

The effects of antioxidants on lifespan are thus compatible with the mechanisms of aging proposed here,

Manipulation of redox pathways

Mitochondrial thioredoxin reductase (TrxR) levels are elevated in long lived species of primates, rodents, and birds [325]. Disruption of Trx or TrxR shortens lifespan, increased Trx or TrxR expression can extend it, and allelic variation in cytosolic TrxR has been associated with longevity in humans [326].

NADPH reduces TrxR, which then reduces Trx. The existence of reduced Trx is key to the reduction of peroxiredoxin (Prx), which enables Prx to reduce H_2O_2 to water.

Trx can also be reduced by glutaredoxins, which are reduced by the oxidation of reduced glutathione (GSH) [326]. GSH is generated by glutathione reductase (GR), which is reduced by NADPH. Accordingly, acceleratedly aged mice and naturally aged mice and humans show decreasing levels of the antioxidants GSH and GR with age [327].

Thus by reducing H_2O_2 increases in redox reduction pathways may extend lifespan.

Klotho

The mutation of α-klotho produces an aging phenotype and shortens lifespan [328]. α-klotho overexpression reduces aging and extends lifespan [328].

α-klotho has multiple effects. One way in which α-klotho may exert its effect is through an antagonistic relationship with insulin/IGF-1 signaling. Overexpression of α-klotho has been shown to inhibit the insulin/IGF-1 pathways [329]. And in the reverse direction, insulin/IGF-1 signaling has been shown to down-regulate α-klotho expression [330]. Thus, irrespective of whether α-klotho regulates or is a consequence of the insulin/IGF-1 signaling pathway, α-klotho levels negatively correlate with insulin/IGF-1 signaling. Down-regulation of insulin/IGF-1 signaling has previously been identified as extending lifespan.

Another possible way in which α-klotho may exert its effect is through phosphorylation of FOXO 3 (FOXO3) [331]. This prevents FOXO3 from entering the nucleus where it functions as a transcription factor [331]. In the nucleus FOXO3 would have up-regulated the expression of the SOD 2 (SOD2) gene, whose protein product is found in the mitochondrial matrix [332]. SOD2 converts matrix $O_2^{\bullet -}$ that was leaked by the electron transport chain into H_2O_2. H_2O_2 is partially membrane permeable, and so can migrate out of the mitochondrion. Thus α-klotho expression will reduce SOD2 and the cytoplasmic H_2O_2 concentration.

Thus α-klotho may extend lifespan by down-regulating insulin/IGF-1 signaling, or through reducing intracellular H_2O_2 levels, both of which are consistent with the mechanisms proposed here.

Modulation of germline signaling

The removal of the germ cells in *C. elegans* significantly increases lifespan [285]. Castration of young males is also believed to extend the lifespan of many animals [333]. Countervailing this, the removal of the ovaries is correlated with increased all cause mortality in women [334].

In the case of *C. elegans*, germline loss appears to result in a burst of ROS in somatic tissues in early adulthood [335]. In response to this burst in ROS mitochondrial biogenesis is increased [335]. It is possible, but by no means certain, that this increase in mitochondrial content could lead to reduced ROS production over the long term, and increased lifespan.

Castration of cattle, rats, guinea-pigs, and rabbits causes persistent growth and retarded atrophy of the thymus [336, 337]. Consequently the effects of castration on lifespan are likely the result of improved thymic function.

Women undergo a gradual loss of germ cells as they age. The depletion of germ cells typically occurs earlier than death, and might represent a mechanism to ensure that resources are directed to viable offspring. For women, the presence of germ cells might thus cause the nuclear genes of the organism to seek to resist the aging process.

The lifespan extending effects of the modulation of germline signaling largely agree with the mechanisms of aging developed here.

Enhanced autophagy

Elevated levels of autophagy occur in common with multiple lifespan extending interventions: reduced insulin/IGF-1 signaling, reduced mTOR signaling, germline removal, caloric restriction, and reduced mitochondrial respiration [338]. As such, autophagy is hypothesized as a common mechanism of aging, and interventions to enhance autophagy are hypothesized to extend lifespan [338]. Mechanisms have been proposed here whereby each of these interventions may extend lifespan without having to invoke autophagy as an explanation. These proposed mechanisms might suggest that the link between autophagy and lifespan may be more correlative than causative.

Autophagy related 5 (ATG5) is a key gene of autophagy. The overexpression of ATG5 in mice enhances autophagy and extends lifespan [339]. ATG5 transgenic mice had the same food intake per body weight, but weighed slightly less, and so had less food intake overall [339].

The effect of autophagy could thus be correlative, or it could be to reduce the energy needs of the organism, thereby extending lifespan in a manner similar to caloric restriction.

Parabiosis

Continuous blood exchange between an older and a younger animal, heterochronic parabiosis, increases the lifespan of the older animal [340], and reduces the lifespan of the younger animal [341]. mRNA levels of the senescence markers p16 and p21 and SASP genes are reduced in the older animal as a result of heterochronic parabiosis [342].

A possible mechanism for heterochronic parabiosis is through the modulation of one or more endocrine factors making up the SASP. Both the SASP factors IL-6 and TNF appear capable of exerting endocrine effects [343, 344].

Metformin

Metformin is the first line drug for the treatment of type 2 diabetes [345]. Metformin is also associated with a 30-50% reduction in the risk of cancer among type 2 diabetes patients [346]. Metformin extends lifespan in *Caenorhabditis elegans* and in some strains of *Mus musculus*, but not in *Drosophila melanogaster* [347]. Metformin is proposed to be tested as a drug to increase healthy human lifespan in the TAME trial [348].

The precise mechanism by which metformin exerts its lifespan extending effects has not been fully elucidated.

One of several possibilities by which metformin exerts its lifespan extending effects is it reduces cytosolic ROS concentrations. Metformin has been shown to inhibit complex I of the electron transport chain [349, 350]. A reduction in complex I activity should result in a reduction in the activity of subsequent electron transport chain units, and a reduction in cytosolic ROS. A related mechanism of action for metformin is through the activation of AMPK which is also hypothesized here to reduce ROS [351].

A second possibility is that metformin may scavenge $HO^{\bullet}$ [352].

A third possibility is that metformin increases the production of SOD2 [353]. The herbicide paraquat is an inducer of $O_2^{\bullet -}$. Metformin reduces the effect of paraquat induced ROS and associated nuclear DNA damage, but not H_2O_2 induced nuclear DNA damage [354]. This adds weight to the third possible explanation.

A fourth possible mechanism of action is through the inhibition of thymic involution via metformin's effect on TECs [355].

All of these pathways are consistent with the mechanisms of aging proposed here.

Epigenetic reprogramming

The loss of epigenetic information such as DNA and histone methylation and histone acetylation patterns has been proposed to occur as a part of the aging process [356]. As such, epigenetic reprogramming may be able to treat certain age-related diseases.

It is possible to construct a pathway from mitochondrial ROS production to the loss of epigenetic information via the displacement of SIRT1, which plays a role in the histone deacetylation that maintains epigenetic silencing, and is also involved in DSB repair [357]. ROS are assumed to create DSBs and the recruitment of SIRT1 to this damage may prevent it from playing its role in epigenetic silencing. However these arguments are currently only speculative.

An alternative explanation for the correlation between epigenetic changes and age-related diseases

is that the SASP affects methylation. The SASP component IL-6 has been reported to reduce the level of DNA two DNA methylating enzymes, DMNT1 and DNMT3B [358]. While in ulcerative colitis, IL-6 has been reported to alter the expression of DMNT1 [359]. IL-6 has also been reported to alter methylation patterns in cancer cells [360, 361], and in B cells from patients with lupus [362]. Consequently, aging is associated with changes in methylation.

DNA methylation patterns have been used to construct epigenetic clocks for measuring effective age [363]. One plausible explanation for why these clocks appear to work is if the SASP affects methylation. In this regard, a DNA methylation machine learning model has been successfully constructed based on fluctuating IL-6 levels [364].

Age-related changes in methylation are consistent with the evolutionary conflict theory of aging. However this doesn't imply that artificial changes to methylation can be expected to affect aging. On the other hand, it also doesn't eliminate reprogramming of cells from the arsenal of tools that might be available to fight aging.

E: Challenges for the theory

At first glance the findings that follow may seem challenging for the evolutionary conflict theory of aging to explain. Careful consideration however shows they do not oppose the theory.

mtDNA mutator mice

Seemingly opposing the perspective that ROS cause the cell to age, mice with an error-prone version of the mtDNA polymerase γ displayed an aged phenotype without an increase of ROS in embryonic fibroblast cells [365]. It is as if the mtDNA mutations alone are directly responsible for the aged phenotype, but the natural mtDNA mutation rate appears far too small to have a significant effect [366]. Looking at various tissues it was subsequently shown that mutator mice do show slightly elevated H_2O_2 as they age [367]. It was also shown that age-dependent cardiomyopathy in mutator mice could be attenuated by mitochondrially targeted catalase [368]. The evidence from mutator mice is sufficient to cast serious doubt on the theory that ROS induces more ROS damage creating a vicious cycle, but still leaves open a role for ROS as a residual signaling-like mechanism in aging.

Antioxidants

It has been reported that overexpression of SOD, catalase, and their combination do not extend lifespan in mice [369]. This is understandable. SOD levels might already be high enough that nearly all $O_2^{\bullet -}$ gets converted into H_2O_2. Catalase is peroxisomally targeted, and thus catalase will have little effect on cytosolic H_2O_2 levels.

It has also been reported that supplementation with either of the antioxidants vitamin C or vitamin E reduced lifespan in the short-tailed field vole, *Microtus agrestis* [370]. Vitamins C and E are known to scavenge free radicals, not break down H_2O_2. A lack of effect is thus understandable if nearly all of the $O_2^{\bullet -}$ gets converted into H_2O_2 prior to vitamin C or E having an impact. The

negative effect could be a result of the experimental design, in which the control population was given a small amount of both vitamin C and E, while the test population received a large amount of vitamin C or E, but none of the other compound. Alternatively, and more speculatively, exogenously supplied antioxidants might reduce the organisms production of endogenous antioxidants.

Mitochondrial-targeted catalase

It has been reported that, despite increasing lifespan, a mitochondrial-targeted catalase gene does not inhibit aging-related cellular senescence [371]. Since catalase breaks down H_2O_2, this would represent a challenge to the role of H_2O_2 in the mechanisms of the theory. This conclusion was reached on the basis of two sets of experiments.

In the first set of experiments, the presence of a mitochondrial-targeted catalase gene was found to have no effect on the emergence of senescence in human fibroblasts. To promote senescence the fibroblasts were either exposed to ionizing radiation, or ethidium bromide was used to eliminate mtDNA. The rationale for this being these were two interventions known to both increase mitochondrial ROS levels and produce senescence. Mitochondrial H_2O_2 is theorized to lead to senescence, so a mitochondrial catalase gene should reduce this effect. However, in the case of ionizing radiation it seems likely that this directly causes DNA damage, including telomeric DNA damage, which causes senescence, eventually leading to increased ROS production. ROS may be a consequence rather than the cause of senescence, and so catalase might be expected to have no effect on senescence. Similarly, it is far from certain that the only relevant effect of eliminating mtDNA is to increase mitochondrial ROS production, which then promotes senescence. It will also affect the ADP/ATP and NAD+/NADH ratios both of which are likely to have profound effects on cellular functioning [372].

In the second set of experiments, gonadal adipose tissue from aged mice with a mitochondrial-targeted catalase transgene was shown to exhibit the same senescence markers and have similar SASP factor expression levels as aged mice without the transgene. If the transgene is expressed and active in gonadal adipose tissue mitochondria at sufficient levels, this would represent a challenge to the theory. However, this remains to be established. Expression levels of the transgene differ widely by tissue type and transgene founding organism, presumably reflecting the site of integration [373][Figure S1]. Expression levels for gonadal adipose tissue have not been established. Similarly, aggregate catalase activity potential varies widely between different tissue types in the presence of the transgene; no change for liver and kidney, but a greater than 5-fold increase for heart and muscle [374]. Consequently, one possible explanation of the observed results is that the transgene is only weakly expressed in gonadal adipose tissue. A second possible explanation of the results is that the observed senescence of gonadal adipose tissue is the result of bystander senescence [50].

Lack of DNA damage by mitochondrial hydrogen peroxide

It has been reported that mitochondrially produced H_2O_2 does not directly cause nuclear DNA damage, including via the Fenton reaction [375]. This was determined by artificially generating 10-100 times the normal amount of H_2O_2 in the mitochondria, and failing to observe a DNA damage response. However, this experiment was only performed for 48 hours in human cell lines. This

experiment would have provided the equivalent of 20-200 days of normal mitochondrial respiration. The mechanisms of aging are not expected to operate over such a short time frame in humans.

Author contributions

Gordon Irlam developed the theory and wrote the manuscript.

Acknowledgments

I am very grateful for the time Michael Klüppel, Martin Predavec, and Steven Greidinger spent reviewing early versions of this manuscript, and providing me with feedback. I am also grateful for the time Hina Zain spent reviewing an earlier related manuscript while the ideas here were still in formation.

Conflicts of interest

The author declares they have no financial conflicts of interest in relation to the content of this manuscript.

Funding

Self funded.

References

[1] Michael R Rose. *Evolutionary biology of aging*. Oxford University Press, 1990.

[2] Thomas BL Kirkwood and Michael R Rose. Evolution of senescence: late survival sacrificed for reproduction. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 332(1262):15–24, 1991.

[3] Peter Lenart and Julie Bienertová-Vašku. Keeping up with the Red Queen: the pace of aging as an adaptation. *Biogerontology*, 18(4):693–709, 2017.

[4] Samir Okasha. Why won't the group selection controversy go away? *British Journal for the Philosophy of Science*, 52(1), 2001.

[5] Josh Mitteldorf and John Pepper. Senescence as an adaptation to limit the spread of disease. *Journal of Theoretical Biology*, 260(2):186–195, 2009.

[6] Vladimir P Skulachev. Aging is a specific biological function rather than the result of a disorder in complex living systems: biochemical evidence in support of Weismann's hypothesis. *Biochemistry-New York-English Translation of Biokhimiya*, 62(11):1191–1195, 1997.

[7] Justin MJ Travis. The evolution of programmed death in a spatially structured population. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 59(4):B301–B305, 2004.

[8] André CR Martins. Change and aging senescence as an adaptation. *PLOS One*, 6(9):e24328, 2011.

[9] Theodore C Goldsmith. Aging, evolvability, and the individual benefit requirement; medical implications of aging theory controversies. *Journal of Theoretical Biology*, 252(4):764–768, 2008.

[10] Paul D Sniegowski and Helen A Murphy. Evolvability. *Current Biology*, 16(19):R831–R834, 2006.

[11] Rachael L Brown. What evolvability really is. *The British Journal for the Philosophy of Science*, 2014.

[12] Axel Kowald and Thomas BL Kirkwood. Can aging be programmed? A critical literature review. *Aging Cell*, 15(6):986–998, 2016.

[13] Rampal S Etienne, Hélène Morlon, and Amaury Lambert. Estimating the duration of speciation from phylogenies. *Evolution*, 68(8):2430–2440, 2014.

[14] Todd Grantham. Is macroevolution more than successive rounds of microevolution? *Palaeontology*, 50(1):75–85, 2007.

[15] Julius Nielsen, Rasmus B Hedeholm, Jan Heinemeier, Peter G Bushnell, Jørgen S Christiansen, Jesper Olsen, Christopher Bronk Ramsey, Richard W Brill, Malene Simon, Kirstine F Steffensen, and John F Steffensen. Eye lens radiocarbon reveals centuries of longevity in the Greenland shark (Somniosus microcephalus). *Science*, 353(6300):702–704, 2016.

[16] Julius Nielsen, Rasmus B Hedeholm, Malene Simon, and John F Steffensen. Distribution and feeding ecology of the Greenland shark (Somniosus microcephalus) in Greenland waters. *Polar Biology*, 37:37–46, 2014.

[17] Eric Ste-Marie, Yuuki Y Watanabe, Jayson M Semmens, Marianne Marcoux, and Nigel E Hussey. Life in the slow lane: field metabolic rate and prey consumption rate of the Greenland shark (Somniosus microcephalus) modelled using archival biologgers. *Journal of Experimental Biology*, 225(7):jeb242994, 2022.

[18] Eric Ste-Marie, Yuuki Y Watanabe, Jayson M Semmens, Marianne Marcoux, and Nigel E Hussey. A first look at the metabolic rate of Greenland sharks (Somniosus microcephalus) in the Canadian Arctic. *Scientific reports*, 10(1):19297, 2020.

[19] Rochelle Buffenstein, Vincent Amoroso, Blazej Andziak, Stanislav Avdieiev, Jorge Azpurua, Alison J Barker, Nigel C Bennett, Miguel A Brieño-Enríquez, Gary N Bronner, Clive Coen, Martha A Delaney, Christine M Dengler-Crish, Yael H Edrey, et al. The naked truth: a comprehensive clarification and classification of current 'myths' in naked mole-rat biology. *Biological Reviews*, 97(1):115–140, 2022.

[20] Maximina H Yun. Salamander insights into ageing and rejuvenation. *Frontiers in Cell and Developmental Biology*, 9:689062, 2021.

[21] Janet L Fryer. Pinus longaeva. In *Fire Effects Information System*. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory, 2004.

[22] Francisco Alejandro Lagunas-Rangel. Why do bats live so long?—Possible molecular mechanisms. *Biogerontology*, 21(1):1–11, 2020.

[23] Sean McKeown. Managing and breeding tortoises in captivity. In *Proceedings of the Northern California Herpetological Society's 1991 Conference on Captive Propagation and Husbandry of Reptiles and Amphibians*, pages 111–116. Northern California Herpetological Society, 1993.

[24] J Whitfield Gibbons. Why do turtles live so long? *BioScience*, 37(4):262–269, 1987.

[25] Joel G Kingsolver and David W Pfennig. Individual-level selection as a cause of Cope's rule of phyletic size increase. *Evolution*, 58(7):1608–1612, 2004.

[26] Krzysztof Ksiażek. Bacterial aging: from mechanistic basis to evolutionary perspective. *Cellular and Molecular Life Sciences*, 67:3131–3137, 2010.

[27] Martin Ackermann, Lin Chao, Carl T Bergstrom, and Michael Doebeli. On the evolutionary origin of aging. *Aging Cell*, 6(2):235–244, 2007.

[28] Daniel Muñoz-Espín, Marta Cañamero, Antonio Maraver, Gonzalo Gómez-López, Julio Contreras, Silvia Murillo-Cuesta, Alfonso Rodríguez-Baeza, Isabel Varela-Nieto, Jesús Ruberte, Manuel Collado, and Manuel Serrano. Programmed cell senescence during mammalian embryonic development. *Cell*, 155(5):1104–1118, 2013.

[29] Joon-Il Jun and Lester F Lau. Cellular senescence controls fibrosis in wound healing. *Aging (Albany NY)*, 2(9):627, 2010.

[30] L Galluzzi, N Joza, E Tasdemir, MC Maiuri, M Hengartner, JM Abrams, N Tavernarakis, J Penninger, F Madeo, and G Kroemer. No death without life: vital functions of apoptotic effectors. *Cell Death & Differentiation*, 15(7):1113–1123, 2008.

[31] Daniel H Nussey, Hannah Froy, Jean-François Lemaitre, Jean-Michel Gaillard, and Steve N Austad. Senescence in natural populations of animals: widespread evidence and its implications for bio-gerontology. *Ageing Research Reviews*, 12(1):214–225, 2013.

[32] Xinyuan Li, Pu Fang, Jietang Mai, Eric T Choi, Hong Wang, and Xiao-feng Yang. Targeting mitochondrial reactive oxygen species as novel therapy for inflammatory diseases and cancers. *Journal of Hematology & Oncology*, 6(1):19, 2013.

[33] Ann E Aust and Jamie F Eveleigh. Mechanisms of DNA oxidation. *Proceedings of the society for Experimental Biology and Medicine*, 222(3):246–252, 1999.

[34] Alugoju Phaniendra, Dinesh Babu Jestadi, and Latha Periyasamy. Free radicals: properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry*, 30(1):11–26, 2015.

[35] Nobuo Makino, Kayoko Sasaki, Kanae Hashida, and Yuki Sakakura. A metabolic model describing the H2O2 elimination by mammalian cells including H2O2 permeation through cytoplasmic and peroxisomal membranes: comparison with experimental data. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1673(3):149–159, 2004.

[36] Gerd P Bienert, Jan K Schjoerring, and Thomas P Jahn. Membrane transport of hydrogen peroxide. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1758(8):994–1003, 2006.

[37] Davis R Mumbengegwi, Qiang Li, Canhui Li, Christine E Bear, and John F Engelhardt. Evidence for a superoxide permeability pathway in endosomal membranes. *Molecular and Cellular Biology*, 28(11):3700–3712, 2008.

[38] Fernando Antunes and Enrique Cadenas. Estimation of H2O2 gradients across biomembranes. *FEBS Letters*, 475(2):121–126, 2000.

[39] Renata A Gus' kova, Ilya I Ivanov, Vitalij K Kol'tover, Victor V Akhobadze, and Andrew B Rubin. Permeability of bilayer lipid membranes for superoxide (O2-) radicals. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 778(3):579–585, 1984.

[40] Maria Luisa Genova and Giorgio Lenaz. The interplay between respiratory supercomplexes and ROS in aging. *Antioxidants & Redox Signaling*, 23(3):208–238, 2015.

[41] Hazel J Shields, Annika Traa, and Jeremy M Van Raamsdonk. Beneficial and detrimental effects of reactive oxygen species on lifespan: a comprehensive review of comparative and experimental studies. *Frontiers in Cell and Developmental Biology*, 9:628157, 2021.

[42] Filippo Scialo, Venkatesh Mallikarjun, Rhoda Stefanatos, and Alberto Sanz. Regulation of lifespan by the mitochondrial electron transport chain: reactive oxygen species-dependent and reactive oxygen species-independent mechanisms. *Antioxidants & Redox Signaling*, 19(16):1953–1969, 2013.

[43] D-F Dai, Y-A Chiao, GM Martin, DJ Marcinek, N Basisty, EK Quarles, and PS Rabinovitch. Mitochondrial-targeted catalase: extended longevity and the roles in various disease models. *Progress in Molecular Biology and Translational Science*, 146:203–241, 2017.

[44] Gerald S Shadel and Tamas L Horvath. Mitochondrial ROS signaling in organismal homeostasis. *Cell*, 163(3):560–569, 2015.

[45] James M Slauch. How does the oxidative burst of macrophages kill bacteria? Still an open question. *Molecular Microbiology*, 80(3):580–583, 2011.

[46] Erhan Bilal, Raul Rabadan, Gabriela Alexe, Noriyuki Fuku, Hitomi Ueno, Yutaka Nishigaki, Yasunori Fujita, Masafumi Ito, Yasumichi Arai, Nobuyoshi Hirose, Andrei Ruckenstein, Gyan Bhanot, and Masashi Tanaka. Mitochondrial DNA haplogroup D4a is a marker for extreme longevity in Japan. *PLOS One*, 3(6):e2421, 2008.

[47] Ai Chen, Nicola Raule, Anne Chomyn, and Giuseppe Attardi. Decreased reactive oxygen species production in cells with mitochondrial haplogroups associated with longevity. *PLOS One*, 7(10):e46473, 2012.

[48] JL Kirkland and T Tchkonia. Senolytic drugs: from discovery to translation. *Journal of Internal Medicine*, 288(5):518–536, 2020.

[49] Yu Sun, Qingfeng Li, and James L Kirkland. Targeting senescent cells for a healthier longevity: the roadmap for an era of global aging. *Life Medicine*, 1(2):103–119, 2022.

[50] Paulo FL da Silva, Mikolaj Ogrodnik, Olena Kucheryavenko, Julien Glibert, Satomi Miwa, Kerry Cameron, Abbas Ishaq, Gabriele Saretzki, Sushma Nagaraja-Grellscheid, Glyn Nelson, and Thomas von Zglinicki. The bystander effect contributes to the accumulation of senescent cells in vivo. *Aging Cell*, 18(1):e12848, 2019.

[51] Selim Chaib, Tamar Tchkonia, and James L Kirkland. Cellular senescence and senolytics: the path to the clinic. *Nature Medicine*, 28(8):1556–1568, 2022.

[52] Sonia S Elder and Elaine Emmerson. Senescent cells and macrophages: key players for regeneration? *Open Biology*, 10(12):200309, 2020.

[53] Jan M Van Deursen. The role of senescent cells in ageing. *Nature*, 509(7501):439–446, 2014.

[54] Estela González-Gualda, Hui-Ling Ou, David Macías, and Daniel Muñoz-Espín. Cellular senescence: from old to new testament. In *Cellular Senescence in Disease*, pages 3–26. Elsevier, 2022.

[55] Rachel Thomas, Weikan Wang, and Dong-Ming Su. Contributions of age-related thymic involution to immunosenescence and inflammaging. *Immunity & Ageing*, 17(1):1–17, 2020.

[56] L Malaguarnera, L Ferlito, RM Imbesi, GS Gulizia, S Di Mauro, D Maugeri, M Malaguarnera, and A Messina. Immunosenescence: a review. *Archives of Gerontology and Geriatrics*, 32(1):1–14, 2001.

[57] Y Bar-Dayan, A Afek, I Goldberg, and J Kopolovic. Proliferation, apoptosis and thymic involution. *Tissue and Cell*, 31(4):391–396, 1999.

[58] Daryl P Shanley, Danielle Aw, Nancy R Manley, and Donald B Palmer. An evolutionary perspective on the mechanisms of immunosenescence. *Trends in Immunology*, 30(7):374–381, 2009.

[59] Danielle Aw and Donald B Palmer. The origin and implication of thymic involution. *Aging and Disease*, 2(5):437, 2011.

[60] Omid Ahmadi, John L McCall, and Mark D Stringer. Does senescence affect lymph node number and morphology? A systematic review. *ANZ Journal of Surgery*, 83(9):612–618, 2013.

[61] Heather L Thompson, Megan J Smithey, Jennifer L Uhrlaub, Ilija Jeftić, Mladen Jergović, Sarah E White, Noreen Currier, Anna M Lang, Afam Okoye, Byung Park, Louis J Picker, Charles D Surh, and Janko Nikolich-Zugich. Lymph nodes as barriers to T-cell rejuvenation in aging mice and nonhuman primates. *Aging Cell*, 18(1):e12865, 2019.

[62] Vivekananda Budamagunta, Thomas C Foster, and Daohong Zhou. Cellular senescence in lymphoid organs and immunosenescence. *Aging (Albany NY)*, 13(15):19920, 2021.

[63] Raghu Kalluri and Robert A Weinberg. The basics of epithelial-mesenchymal transition. *The Journal of Clinical Investigation*, 119(6):1420–1428, 2009.

[64] Jing Yang, Parker Antin, Geert Berx, Cédric Blanpain, Thomas Brabletz, Marianne Bronner, Kyra Campbell, Amparo Cano, Jordi Casanova, Gerhard Christofori, Shoukat Dedhar, Rik Derynck, Heide L Ford, et al. Guidelines and definitions for research on epithelial–mesenchymal transition. *Nature Reviews Molecular Cell Biology*, 21(6):341–352, 2020.

[65] Guya D Marconi, Luigia Fonticoli, Thangavelu Soundara Rajan, Sante D Pierdomenico, Oriana Trubiani, Jacopo Pizzicannella, and Francesca Diomede. Epithelial-mesenchymal transition (EMT): the type-2 EMT in wound healing, tissue regeneration and organ fibrosis. *Cells*, 10(7):1587, 2021.

[66] Rosemarie M Carew, Bo Wang, and Phillip Kantharidis. The role of EMT in renal fibrosis. *Cell and Tissue Research*, 347(1):103–116, 2012.

[67] Jiali Yang, Juan Liu, Jiayu Liang, Fan Li, Wenwen Wang, Huan Chen, and Xiang Xie. Epithelial-mesenchymal transition in age-associated thymic involution: mechanisms and therapeutic implications. *Ageing Research Reviews*, page 102115, 2023.

[68] Remi-Martin Laberge, Pierre Awad, Judith Campisi, and Pierre-Yves Desprez. Epithelial-mesenchymal transition induced by senescent fibroblasts. *Cancer Microenvironment*, 5:39–44, 2012.

[69] Andrea Abaurrea, Angela M Araujo, and Maria M Caffarel. The role of the il-6 cytokine family in epithelial–mesenchymal plasticity in cancer progression. *International Journal of Molecular Sciences*, 22(15):8334, 2021.

[70] Marjon A Smit and Daniel S Peeper. Epithelial-mesenchymal transition and senescence: two cancer-related processes are crossing paths. *Aging (Albany NY)*, 2(10):735, 2010.

[71] Adi Sagiv, Dominick GA Burton, Zhana Moshayev, Ezra Vadai, Felix Wensveen, Shifra Ben-Dor, Ofra Golani, Bojan Polic, and Valery Krizhanovsky. NKG2D ligands mediate immunosurveillance of senescent cells. *Aging (Albany NY)*, 8(2):328, 2016.

[72] Branca I Pereira, Oliver P Devine, Milica Vukmanovic-Stejic, Emma S Chambers, Priya Subramanian, Neil Patel, Alex Virasami, Neil J Sebire, Veronica Kinsler, Alexis Valdovinos, Claude Jourdan LeSaux, João F Passos, Antony Antoniou, et al. Senescent cells evade immune clearance via HLA-E-mediated NK and CD8+ T cell inhibition. *Nature Communications*, 10(1):2387, 2019.

[73] Ines Marin, Olga Boix, Andrea Garcia-Garijo, Isabelle Sirois, Adrià Caballe, Eduardo Zarzuela, Irene Ruano, Camille Stephan-Otto Attolini, Neus Prats, José A López-Domínguez, Marta Kovatcheva, Elena Garralda, Javier Muñoz, et al. Cellular senescence is immunogenic and promotes antitumor immunity. *Cancer Discovery*, 13(2):410–431, 2023.

[74] Larissa GP Langhi Prata, Inna G Ovsyannikova, Tamara Tchkonia, and James L Kirkland. Senescent cell clearance by the immune system: emerging therapeutic opportunities. In *Seminars in Immunology*, volume 40, page 101275. Elsevier, 2018.

[75] Veronique M Braud, Hatice Aldemir, Beatrice Breart, and Walter G Ferlin. Expression of CD94–NKG2A inhibitory receptor is restricted to a subset of CD8+ T cells. *Trends in Immunology*, 24(4):162–164, 2003.

[76] Kushal Prajapati, Cynthia Perez, Lourdes Beatriz Plaza Rojas, Brianna Burke, and Jose A Guevara-Patino. Functions of NKG2D in CD8+ T cells: an opportunity for immunotherapy. *Cellular & Molecular Immunology*, 15(5):470–479, 2018.

[77] Tae-Won Kang, Tetyana Yevsa, Norman Woller, Lisa Hoenicke, Torsten Wuestefeld, Daniel Dauch, Anja Hohmeyer, Marcus Gereke, Ramona Rudalska, Anna Potapova, Marcus Iken, Mihael Vucur, Siegfried Weiss, et al. Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. *Nature*, 479(7374):547–551, 2011.

[78] Jennifer L Matsuda, Thierry Mallevaey, James Scott-Browne, and Laurent Gapin. CD1d-restricted iNKT cells, the 'Swiss-Army knife' of the immune system. *Current Opinion in Immunology*, 20(3):358–368, 2008.

[79] Shivani Arora, Peter J Thompson, Yao Wang, Aritra Bhattacharyya, Hara Apostolopoulou, Rachel Hatano, Ram P Naikawadi, Ajit Shah, Paul J Wolters, Suneil Koliwad, Mallar Bhattacharya, and Anil Bhushan. Invariant natural killer T cells coordinate removal of senescent cells. *Med*, 2(8):938–950, 2021.

[80] Thomas Boehm, Stefanie Scheu, Klaus Pfeffer, and Conrad C Bleul. Thymic medullary epithelial cell differentiation, thymocyte emigration, and the control of autoimmunity require lympho–epithelial cross talk via LTβR. *The Journal of Experimental Medicine*, 198(5):757–769, 2003.

[81] Beth Lucas, Andrea J White, Emilie J Cosway, Sonia M Parnell, Kieran D James, Nick D Jones, Izumi Ohigashi, Yousuke Takahama, William E Jenkinson, and Graham Anderson. Diversity in medullary thymic epithelial cells controls the activity and availability of iNKT cells. *Nature Communications*, 11(1):2198, 2020.

[82] Esther Peralbo, Olga DelaRosa, Inmaculada Gayoso, Maria L Pita, Raquel Tarazona, and Rafael Solana. Decreased frequency and proliferative response of invariant Vα24Vβ11 natural killer T (iNKT) cells in healthy elderly. *Biogerontology*, 7:483–492, 2006.

[83] JM Holland, TJ Mitchell, LC Gipson, and MS LC. Survival and cause of death in aging germfree athymic nude and normal inbred C3Hf/He mice. *Journal of the National Cancer Institute*, 61(5):1357–1361, 1978.

[84] Ruben Aquino-Martinez, Sundeep Khosla, Joshua N Farr, and David G Monroe. Periodontal disease and senescent cells: new players for an old oral health problem? *International Journal of Molecular Sciences*, 21(20):7441, 2020.

[85] Hanada Miyazaki, N Hanada, MI Andoh, Yo Yamashita, T Saito, A Sogame, K Goto, R Shirahama, and T Takehara. Periodontal disease prevalence in different age groups in Japan as assessed according to the CPITN. *Community Dentistry and Oral Epidemiology*, 17(2):71–74, 1989.

[86] Marta Relvas, Paula López-Jarana, Luis Monteiro, José Júlio Pacheco, Ana Cristina Braga, and Filomena Salazar. Study of prevalence, severity and risk factors of periodontal disease in a Portuguese population. *Journal of Clinical Medicine*, 11(13):3728, 2022.

[87] Maximina H Yun, Hongorzul Davaapil, and Jeremy P Brockes. Recurrent turnover of senescent cells during regeneration of a complex structure. *eLife*, 4, 2015.

[88] Qinghao Yu and Maximina H Yun. Interconnection between cellular senescence, regeneration and ageing in salamanders. In *Senolytics in Disease, Ageing and Longevity*, pages 43–62. Springer, 2020.

[89] Hélène Martini and João F Passos. Cellular senescence: all roads lead to mitochondria. *The FEBS Journal*, 290(5):1186–1202, 2023.

[90] Yu Sun, Xia Wang, Tianwei Liu, Xiaoyan Zhu, and Xudong Pan. The multifaceted role of the SASP in atherosclerosis: from mechanisms to therapeutic opportunities. *Cell & Bioscience*, 12(1):1–20, 2022.

[91] Philip R Coryell, Brian O Diekman, and Richard F Loeser. Mechanisms and therapeutic implications of cellular senescence in osteoarthritis. *Nature Reviews Rheumatology*, 17(1):47–57, 2021.

[92] Natalia Loaiza and Marco Demaria. Cellular senescence and tumor promotion: is aging the key? *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*, 1865(2):155–167, 2016.

[93] Kyra Ungerleider, Jessica Beck, Delphine Lissa, Casmir Turnquist, Izumi Horikawa, Brent T Harris, and Curtis C Harris. Astrocyte senescence and SASP in neurodegeneration: Tau joins the loop. *Cell Cycle*, 20(8):752–764, 2021.

[94] Alexandra E Butler, Juliette Janson, Susan Bonner-Weir, Robert Ritzel, Robert A Rizza, and Peter C Butler. β-cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. *Diabetes*, 52(1):102–110, 2003.

[95] Lichao Wang, Binsheng Wang, Nathan S Gasek, Yueying Zhou, Rachel L Cohn, Dominique E Martin, Wulin Zuo, William F Flynn, Chun Guo, Evan R Jellison, Kim Taewan, Larissa G P Langhi Prata, Allyson K Palmer, et al. Targeting p21Cip1 highly expressing cells in adipose tissue alleviates insulin resistance in obesity. *Cell Metabolism*, 34(1):75–89, 2022.

[96] Elisa Carrasco, Manuel M Gómez de las Heras, Enrique Gabandé-Rodríguez, Gabriela Desdín-Micó, Juan Francisco Aranda, and Maria Mittelbrunn. The role of T cells in age-related diseases. *Nature Reviews Immunology*, 22(2):97–111, 2022.

[97] Graham Pawelec, Evelyna Derhovanessian, and Anis Larbi. Immunosenescence and cancer. *Critical Reviews in Oncology/Hematology*, 75(2):165–172, 2010.

[98] Jingyao Lian, Ying Yue, Weina Yu, and Yi Zhang. Immunosenescence: a key player in cancer development. *Journal of Hematology & Oncology*, 13(1):1–18, 2020.

[99] Fuquan Wang, Haifa Xia, and Shanglong Yao. Regulatory T cells are a double-edged sword in pulmonary fibrosis. *International Immunopharmacology*, 84:106443, 2020.

[100] Angelica Varesi, Salvatore Chirumbolo, Lucrezia Irene Maria Campagnoli, Elisa Pierella, Gaia Bavestrello Piccini, Adelaide Carrara, Giovanni Ricevuti, Catia Scassellati, Cristian Bonvicini, and Alessia Pascale. The role of antioxidants in the interplay between oxidative stress and senescence. *Antioxidants*, 11(7):1224, 2022.

[101] Clara Correia-Melo and João F Passos. Mitochondria: are they causal players in cellular senescence? *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1847(11):1373–1379, 2015.

[102] Y Ruzankina and EJ Brown. Relationships between stem cell exhaustion, tumour suppression and ageing. *British Journal of Cancer*, 97(9):1189–1193, 2007.

[103] Shu-Min Chou, Yu-Hsin Yen, Fang Yuan, Su-Chun Zhang, and Cheong-Meng Chong. Neuronal senescence in the aged brain. *Aging and Disease*, 14(5):1618, 2023.

[104] Britta Engelhardt. Molecular mechanisms involved in T cell migration across the blood–brain barrier. *Journal of Neural Transmission*, 113:477–485, 2006.

[105] Britta E Jones, Megan D Maerz, and Jane H Buckner. IL-6: a cytokine at the crossroads of autoimmunity. *Current Opinion in Immunology*, 55:9–14, 2018.

[106] Kai K Kummer, Maximilian Zeidler, Theodora Kalpachidou, and Michaela Kress. Role of IL-6 in the regulation of neuronal development, survival and function. *Cytokine*, 144:155582, 2021.

[107] Shinichi Takahashi. Neuroprotective function of high glycolytic activity in astrocytes: common roles in stroke and neurodegenerative diseases. *International Journal of Molecular Sciences*, 22(12):6568, 2021.

[108] George A Brooks. The science and translation of lactate shuttle theory. *Cell Metabolism*, 27(4):757–785, 2018.

[109] Ellen A Silva, Ana P Dalla Costa, Juliana S Ruas, Edilene S Siqueira-Santos, Annelise Francisco, and Roger F Castilho. Proliferating astrocytes in primary culture do not depend upon mitochondrial respiratory complex I activity or oxidative phosphorylation. *Cells*, 12(5):683, 2023.

[110] Justin Cohen and Claudio Torres. Astrocyte senescence: evidence and significance. *Aging Cell*, 18(3):e12937, 2019.

[111] Pia März, Klaus Heese, Beatrice Dimitriades-Schmutz, Stefan Rose-John, and Uwe Otten. Role of interleukin-6 and soluble IL-6 receptor in region-specific induction of astrocytic differentiation and neurotrophin expression. *Glia*, 26(3):191–200, 1999.

[112] S Jay Olshansky, Bruce A Carnes, and Christine Cassel. In search of Methuselah: estimating the upper limits to human longevity. *Science*, 250(4981):634–640, 1990.

[113] Carlos López-Otín, Maria A Blasco, Linda Partridge, Manuel Serrano, and Guido Kroemer. The hallmarks of aging. *Cell*, 153(6):1194–1217, 2013.

[114] Laurence J Walsh. Safety issues relating to the use of hydrogen peroxide in dentistry. *Australian Dental Journal*, 45(4):257–269, 2000.

[115] M Segasothy and Paddy A Phillips. Vegetarian diet: panacea for modern lifestyle diseases? *Qjm*, 92(9):531–544, 1999.

[116] Lixia Zhang and Xin Lu. Amphibians live longer at higher altitudes but not at higher latitudes. *Biological Journal of the Linnean Society*, 106(3):623–632, 2012.

[117] Gustavo R Zubieta-Calleja and Natalia A Zubieta-DeUrioste. Extended longevity at high altitude: benefits of exposure to chronic hypoxia. *BLDE University Journal of Health Sciences*, 2(2):80, 2017.

[118] Catherine M Hill, Dagmara Dimitriou, Ana Baya, Rebecca Webster, Johanna Gavlak-Dingle, Veline Lesperance, Kate Heathcote, and Romola S Bucks. Cognitive performance in high-altitude Andean residents compared with low-altitude populations: from childhood to older age. *Neuropsychology*, 28(5):752, 2014.

[119] Dennis P Clifford and John E Repine. Hydrogen peroxide mediated killing of bacteria. *Molecular and Cellular Biochemistry*, 49:143–149, 1982.

[120] Abiodun O Arigbede, B Osagbemiro Babatope, and M Kolude Bamidele. Periodontitis and systemic diseases: a literature review. *Journal of Indian Society of Periodontology*, 16(4):487, 2012.

[121] Frank DeStefano, Robert F Anda, Henry S Kahn, David F Williamson, and Carl M Russell. Dental disease and risk of coronary heart disease and mortality. *British Medical Journal*, 306(6879):688–691, 1993.

[122] Cavin K Ward-Caviness, Jamaji C Nwanaji-Enwerem, Kathrin Wolf, Simone Wahl, Elena Colicino, Letizia Trevisi, Itai Kloog, Allan C Just, Pantel Vokonas, Josef Cyrys, Christian Gieger, Joel Schwartz, Andreaa A Baccarelli, et al. Long-term exposure to air pollution is associated with biological aging. *Oncotarget*, 7(46):74510, 2016.

[123] Karl Fagerström. The epidemiology of smoking: health consequences and benefits of cessation. *Drugs*, 62:1–9, 2002.

[124] Do-Yeon Cho, Wei Le, Dawn T Bravo, Peter H Hwang, Beate Illek, Horst Fischer, and Jayakar V Nayak. Air pollutants cause release of hydrogen peroxide and interleukin-8 in a human primary nasal tissue culture model. In *International Forum of Allergy & Rhinology*, volume 4, pages 966–971. Wiley Online Library, 2014.

[125] Zaira Leni, Lisa Künzi, and Marianne Geiser. Air pollution causing oxidative stress. *Current Opinion in Toxicology*, 20:1–8, 2020.

[126] Bing Zhao, Ha Q Vo, Fay H Johnston, and Kazuaki Negishi. Air pollution and telomere length: a systematic review of 12,058 subjects. *Cardiovascular Diagnosis and Therapy*, 8(4):480, 2018.

[127] Yuliana Astuti, Ardyan Wardhana, Johnathan Watkins, and Wahyu Wulaningsih. Cigarette smoking and telomere length: a systematic review of 84 studies and meta-analysis. *Environmental Research*, 158:480–489, 2017.

[128] Martha Ustarroz-Cano, Marisol López-Ángel, Nelly López-Valdez, Isabel García-Peláez, and Teresa I Fortoul. The effect of atmospheric pollution on the thymus. In *Thymus*. IntechOpen, 2019.

[129] Tetsuro Araki, Mizuki Nishino, Wei Gao, Josée Dupuis, Gary M Hunninghake, Takamichi Murakami, George R Washko, George T O'Connor, and Hiroto Hatabu. Normal thymus in adults: appearance on CT and associations with age, sex, BMI and smoking. *European Radiology*, 26:15–24, 2016.

[130] Michael Lynch, Farhan Ali, Tongtong Lin, Yaohai Wang, Jiahao Ni, and Hongan Long. The divergence of mutation rates and spectra across the Tree of Life. *EMBO reports*, 24(10):e57561, 2023.

[131] Motoo Kimura. On the probability of fixation of mutant genes in a population. *Genetics*, 47(6):713, 1962.

[132] Michael M Desai and Daniel S Fisher. Beneficial mutation–selection balance and the effect of linkage on positive selection. *Genetics*, 176(3):1759–1798, 2007.

[133] Julie C Wang and Martin Bennett. Aging and atherosclerosis: mechanisms, functional consequences, and potential therapeutics for cellular senescence. *Circulation Research*, 111(2):245–259, 2012.

[134] Xianliang Dai, Danfeng Zhang, Chaoqun Wang, Zonggui Wu, and Chun Liang. The pivotal role of thymus in atherosclerosis mediated by immune and inflammatory response. *International Journal of Medical Sciences*, 15(13):1555, 2018.

[135] Sarah Heerboth, Genevieve Housman, Meghan Leary, Mckenna Longacre, Shannon Byler, Karolina Lapinska, Amber Willbanks, and Sibaji Sarkar. EMT and tumor metastasis. *Clinical and Translational Medicine*, 4:1–13, 2015.

[136] Lena Lau and Gregory David. Pro-and anti-tumorigenic functions of the senescence-associated secretory phenotype. *Expert Opinion on Therapeutic Targets*, 23(12):1041–1051, 2019.

[137] Eleni Georgakopoulou, Konstantinos Evangelou, and Vassilis G Gorgoulis. Premalignant lesions and cellular senescence. In *Cellular Senescence in Disease*, pages 29–60. Elsevier, 2022.

[138] Xianliang Dai, Li Hua, Hui Chen, Qiheng Li, Wansheng Chen, and Chun Liang. What's the role of thymus in diabetes mellitus? *International Immunopharmacology*, 116:109765, 2023.

[139] Sun-Jae Lee, Kyung-Hyun Kim, and Kwan-Kyu Park. Mechanisms of fibrogenesis in liver cirrhosis: the molecular aspects of epithelial-mesenchymal transition. *World Journal of Hepatology*, 6(4):207, 2014.

[140] Ana S Cruz-Solbes and Keith Youker. Epithelial to mesenchymal transition (EMT) and endothelial to mesenchymal transition (EndMT): role and implications in kidney fibrosis. *Kidney development and disease*, pages 345–372, 2017.

[141] Weijia Cheng, Xiao Li, Dongling Liu, Chaochu Cui, and Xianwei Wang. Endothelial-to-mesenchymal transition: role in cardiac fibrosis. *Journal of Cardiovascular Pharmacology and Therapeutics*, 26(1):3–11, 2021.

[142] Daisuke Iizuka, Megumi Sasatani, Mary Helen Barcellos-Hoff, and Kenji Kamiya. Hydrogen peroxide enhances tgfβ-mediated epithelial-to-mesenchymal transition in human mammary epithelial mcf-10a cells. *Anticancer Research*, 37(3):987–995, 2017.

[143] Myung-Chul Kim, Feng-Ji Cui, and Yongbaek Kim. Hydrogen peroxide promotes epithelial to mesenchymal transition and stemness in human malignant mesothelioma cells. *Asian Pacific Journal of Cancer Prevention*, 14(6):3625–3630, 2013.

[144] Wei Li, Lei Cao, Liang Han, Qinhong Xu, and Qingyong Ma. Superoxide dismutase promotes the epithelial-mesenchymal transition of pancreatic cancer cells via activation of the H2O2/ERK/NF-κB axis. *International Journal of Oncology*, 46(6):2613–2620, 2015.

[145] Yoko Ito, Matthew Hoare, and Masashi Narita. Spatial and temporal control of senescence. *Trends in Cell Biology*, 27(11):820–832, 2017.

[146] Jinmei Xiang, Chunyun Wan, Rui Guo, and Dingzong Guo. Is hydrogen peroxide a suitable apoptosis inducer for all cell types? *BioMed Research International*, 2016, 2016.

[147] Natalia A Belikova, Yury A Vladimirov, Anatoly N Osipov, Alexandr A Kapralov, Vladimir A Tyurin, Maksim V Potapovich, Liana V Basova, Jim Peterson, Igor V Kurnikov, and Valerian E Kagan. Peroxidase activity and structural transitions of cytochrome c bound to cardiolipin-containing membranes. *Biochemistry*, 45(15):4998–5009, 2006.

[148] Giuseppe Petrosillo, Giacoma Casanova, Mariagiuseppa Matera, Francesca Maria Ruggiero, and Giuseppe Paradies. Interaction of peroxidized cardiolipin with rat-heart mitochondrial membranes: induction of permeability transition and cytochrome c release. *FEBS Letters*, 580(27):6311–6316, 2006.

[149] Kambiz N Alavian, Gisela Beutner, Emma Lazrove, Silvio Sacchetti, Han-A Park, Pawel Licznerski, Hongmei Li, Panah Nabili, Kathryn Hockensmith, Morven Graham, George A Porter Jr, and Elizabeth A Jonas. An uncoupling channel within the c-subunit ring of the F1FO ATP synthase is the mitochondrial permeability transition pore. *Proceedings of the National Academy of Sciences*, 111(29):10580–10585, 2014.

[150] Alicia J Kowaltowski, Roger F Castilho, and Anibal E Vercesi. Opening of the mitochondrial permeability transition pore by uncoupling or inorganic phosphate in the presence of Ca2+ is dependent on mitochondrial-generated reactive oxygen species. *FEBS Letters*, 378(2):150–152, 1996.

[151] Martin Ott, John D Robertson, Vladimir Gogvadze, Boris Zhivotovsky, and Sten Orrenius. Cytochrome c release from mitochondria proceeds by a two-step process. *Proceedings of the National Academy of Sciences*, 99(3):1259–1263, 2002.

[152] Liangkun You, Zhanggui Wang, Hongsen Li, Jiawei Shou, Zhao Jing, Jiansheng Xie, Xinbing Sui, Hongming Pan, and Weidong Han. The role of STAT3 in autophagy. *Autophagy*, 11(5):729–739, 2015.

[153] Georgina N Masoud and Wei Li. HIF-1α pathway: role, regulation and intervention for cancer therapy. *Acta Pharmaceutica Sinica B*, 5(5):378–389, 2015.

[154] A Ahluwalia and A S Tarnawski. Critical role of hypoxia sensor-HIF-1α in VEGF gene activation. Implications for angiogenesis and tissue injury healing. *Current Medicinal Chemistry*, 19(1):90–97, 2012.

[155] SJ Kierans and CT Taylor. Regulation of glycolysis by the hypoxia-inducible factor (HIF): implications for cellular physiology. *The Journal of Physiology*, 599(1):23–37, 2021.

[156] Isabel Diebold, Andreas Petry, John Hess, and Agnes Görlach. The NADPH oxidase subunit NOX4 is a new target gene of the hypoxia-inducible factor-1. *Molecular Biology of the Cell*, 21(12):2087–2096, 2010.

[157] Yukio Nisimoto, Becky A Diebold, Daniela Cosentino-Gomes, and J David Lambeth. Nox4: a hydrogen peroxide-generating oxygen sensor. *Biochemistry*, 53(31):5111–5120, 2014.

[158] Maria V Liberti and Jason W Locasale. The Warburg effect: how does it benefit cancer cells? *Trends in Biochemical Sciences*, 41(3):211–218, 2016.

[159] Patrick H Maxwell, Christopher W Pugh, and Peter J Ratcliffe. Activation of the HIF pathway in cancer. *Current Opinion in Genetics & Development*, 11(3):293–299, 2001.

[160] Ildiko Szanto. NADPH Oxidase 4 (NOX4) in cancer: linking redox signals to oncogenic metabolic adaptation. *International Journal of Molecular Sciences*, 23(5):2702, 2022.

[161] Miguel López-Lázaro. Dual role of hydrogen peroxide in cancer: possible relevance to cancer chemoprevention and therapy. *Cancer Letters*, 252(1):1–8, 2007.

[162] Angela Schmid, Silke Krömer, Hans W Heldt, and Roland Benz. Identification of two general diffusion channels in the outer membrane of pea mitochondria. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1112(2):174–180, 1992.

[163] Paul RHJ Timmers, James F Wilson, Peter K Joshi, and Joris Deelen. Multivariate genomic scan implicates novel loci and haem metabolism in human ageing. *Nature Communications*, 11(1):3570, 2020.

[164] Dennis Mangan. Iron: an underrated factor in aging. *Aging (Albany NY)*, 13(19):23407, 2021.

[165] Priyamvada Rai, David E Wemmer, and Stuart Linn. Preferential binding and structural distortion by Fe2+ at RGGG-containing DNA sequences correlates with enhanced oxidative cleavage at such sequences. *Nucleic Acids Research*, 33(2):497–510, 2005.

[166] Joshua D Podlevsky, Christopher J Bley, Rebecca V Omana, Xiaodong Qi, and Julian J-L Chen. The telomerase database. *Nucleic Acids Research*, 36(suppl_1):D339–D343, 2007.

[167] Annia Galano and J Raul Alvarez-Idaboy. Guanosine + OH radical reaction in aqueous solution: a reinterpretation of the UV-vis data based on thermodynamic and kinetic calculations. *Organic Letters*, 11(22):5114–5117, 2009.

[168] Wareed Ahmed and Joachim Lingner. PRDX1 counteracts catastrophic telomeric cleavage events that are triggered by DNA repair activities post oxidative damage. *Cell Reports*, 33(5):108347, 2020.

[169] Simone Petersen, Gabriele Saretzki, and Thomas von Zglinicki. Preferential accumulation of single-stranded regions in telomeres of human fibroblasts. *Experimental Cell Research*, 239(1):152–160, 1998.

[170] Torsten Richter, Gabriele Saretzki, Glyn Nelson, Mathias Melcher, Sharon Olijslagers, and Thomas von Zglinicki. TRF2 overexpression diminishes repair of telomeric single-strand breaks and accelerates telomere shortening in human fibroblasts. *Mechanisms of Ageing and Development*, 128(4):340–345, 2007.

[171] Thomas von Zglinicki, Rita Pilger, and Nicolle Sitte. Accumulation of single-strand breaks is the major cause of telomere shortening in human fibroblasts. *Free Radical Biology and Medicine*, 28(1):64–74, 2000.

[172] Stephen Barnard, Simon Bouffler, and Kai Rothkamm. The shape of the radiation dose response for DNA double-strand break induction and repair. *Genome Integrity*, 4(1):1–8, 2013.

[173] M Levitt. How many base-pairs per turn does DNA have in solution and in chromatin? Some theoretical calculations. *Proceedings of the National Academy of Sciences*, 75(2):640–644, 1978.

[174] Keiji Okamoto, Cristina Bartocci, Iliana Ouzounov, Jolene K Diedrich, John R Yates III, and Eros Lazzerini Denchi. A two-step mechanism for TRF2-mediated chromosome-end protection. *Nature*, 494(7438):502–505, 2013.

[175] David Van Ly, Ronnie Ren Jie Low, Sonja Frölich, Tara K Bartolec, Georgia R Kafer, Hilda A Pickett, Katharina Gaus, and Anthony J Cesare. Telomere loop dynamics in chromosome end protection. *Molecular Cell*, 71(4):510–525, 2018.

[176] Zeenia Kaul, Anthony J Cesare, Lily I Huschtscha, Axel A Neumann, and Roger R Reddel. Five dysfunctional telomeres predict onset of senescence in human cells. *EMBO Reports*, 13(1):52–59, 2012.

[177] Marzia Fumagalli, Francesca Rossiello, Michela Clerici, Sara Barozzi, Davide Cittaro, Jessica M Kaplunov, Gabriele Bucci, Miryana Dobreva, Valentina Matti, Christian M Beausejour, Utz Herbig, Maria Pia Longhese, and Fabrizio d'Adda di Fagagna. Telomeric DNA damage is irreparable and causes persistent DNA-damage-response activation. *Nature Cell Biology*, 14(4):355–365, 2012.

[178] Francesca Rossiello, Utz Herbig, Maria Pia Longhese, Marzia Fumagalli, and Fabrizio d'Adda di Fagagna. Irreparable telomeric DNA damage and persistent DDR signalling as a shared causative mechanism of cellular senescence and ageing. *Current Opinion in Genetics & Development*, 26:89–95, 2014.

[179] Graeme Hewitt, Diana Jurk, Francisco DM Marques, Clara Correia-Melo, Timothy Hardy, Agata Gackowska, Rhys Anderson, Morgan Taschuk, Jelena Mann, and João F Passos. Telomeres are favoured targets of a persistent DNA damage response in ageing and stress-induced senescence. *Nature Communications*, 3(1):1–9, 2012.

[180] Nuno MV Gomes, Oliver A Ryder, Marlys L Houck, Suellen J Charter, William Walker, Nicholas R Forsyth, Steven N Austad, Chris Venditti, Mark Pagel, Jerry W Shay, and Woodring E Wright. Comparative biology of mammalian telomeres: hypotheses on ancestral states and the roles of telomeres in longevity determination. *Aging Cell*, 10(5):761–768, 2011.

[181] Toyoki Maeda, Jing-Zhi Guan, Masamichi Koyanagi, and Naoki Makino. Telomerase activity and telomere length distribution in vascular endothelial cells in a short-term culture under the presence of hydrogen peroxide. *Geriatrics & Gerontology International*, 13(3):774–782, 2013.

[182] Violeta Serra, Thomas Von Zglinicki, Mario Lorenz, and Gabriele Saretzki. Extracellular superoxide dismutase is a major antioxidant in human fibroblasts and slows telomere shortening. *Journal of Biological Chemistry*, 278(9):6824–6830, 2003.

[183] Jing Zhao, Lei Zhang, Aiping Lu, Yingchao Han, Debora Colangelo, Christina Bukata, Alex Scibetta, Matthew J Yousefzadeh, Xuesen Li, Aditi U Gurkar, Sara J McGowan, Luise Angelini, Ryan O'Kelly, et al. ATM is a key driver of NF-κB-dependent DNA-damage-induced senescence, stem cell dysfunction and aging. *Aging (Albany NY)*, 12(6):4688, 2020.

[184] Myeong Uk Kuk, Jae Won Kim, Young-Sam Lee, Kyung A Cho, Joon Tae Park, and Sang Chul Park. Alleviation of senescence via ATM inhibition in accelerated aging models. *Molecules and Cells*, 42(3):210, 2019.

[185] Zhaohui Feng, Wenwei Hu, Angelika K Teresky, Eva Hernando, Carlos Cordon-Cardo, and Arnold J Levine. Declining p53 function in the aging process: a possible mechanism for the increased tumor incidence in older populations. *Proceedings of the National Academy of Sciences*, 104(42):16633–16638, 2007.

[186] Minxian Qian, Zuojun Liu, Linyuan Peng, Xiaolong Tang, Fanbiao Meng, Ying Ao, Mingyan Zhou, Ming Wang, Xinyue Cao, Baoming Qin, Zimei Wang, Zhongjun Zhou, Guangming Wang, et al. Boosting ATM activity alleviates aging and extends lifespan in a mouse model of progeria. *eLife*, 7:e34836, 2018.

[187] S Banin, L Moyal, S-Y Shieh, Y Taya, CW Anderson, Luciana Chessa, NI Smorodinsky, C Prives, Y Reiss, Y Shiloh, and Y Ziv. Enhanced phosphorylation of p53 by ATM in response to DNA damage. *Science*, 281(5383):1674–1677, 1998.

[188] Liu-Ya Tang, Adam Thomas, Ming Zhou, and Ying E Zhang. Phosphorylation of SMURF2 by ATM exerts a negative feedback control of DNA damage response. *Journal of Biological Chemistry*, 295(52):18485–18493, 2020.

[189] Yahui Kong, Hang Cui, and Hong Zhang. Smurf2-mediated ubiquitination and degradation of Id1 regulates p16 expression during senescence. *Aging Cell*, 10(6):1038–1046, 2011.

[190] Hyung Min Jeong, Sung Ho Lee, Jinah Yum, Chang-Yeol Yeo, and Kwang Youl Lee. Smurf2 regulates the degradation of YY1. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, 1843(9):2005–2011, 2014.

[191] Hani Rayess, Marilene B Wang, and Eri S Srivatsan. Cellular senescence and tumor suppressor gene p16. *International Journal of Cancer*, 130(8):1715–1725, 2012.

[192] Janakiraman Krishnamurthy, Chad Torrice, Matthew R Ramsey, Grigoriy I Kovalev, Khalid Al-Regaiey, Lishan Su, and Norman E Sharpless. Ink4a/Arf expression is a biomarker of aging. *The Journal of Clinical Investigation*, 114(9):1299–1307, 2004.

[193] Hyman B Muss, Andrew Smitherman, William A Wood, Kirsten Nyrop, Sascha Tuchman, Paramjeet K Randhawa, Amy R Entwistle, Natalia Mitin, and Shlomit S Shachar. p16 a biomarker of aging and tolerance for cancer therapy. *Translational Cancer Research*, 9(9):5732, 2020.

[194] Hasan Safwan-Zaiter, Nicole Wagner, and Kay-Dietrich Wagner. P16INK4A—More than a senescence marker. *Life*, 12(9):1332, 2022.

[195] Dawn E Ouelle, Frédérique Zindy, Richard A Ashmun, and Charles J Sherr. Alternative reading frames of the INK4a tumor suppressor gene encode two unrelated proteins capable of inducing cell cycle arrest. *Cell*, 83(6):993–1000, 1995.

[196] Anshu Agrawal, Jianhui Yang, Richard F Murphy, and Devendra K Agrawal. Regulation of the p14ARF-Mdm2-p53 pathway: an overview in breast cancer. *Experimental and Molecular Pathology*, 81(2):115–122, 2006.

[197] Norman E Sharpless, Matthew R Ramsey, Periasamy Balasubramanian, Diego H Castrillon, and Ronald A DePinho. The differential impact of p16INK4a or p19ARF deficiency on cell growth and tumorigenesis. *Oncogene*, 23(2):379–385, 2004.

[198] Guangchao Sui, El Bachir Affar, Yujiang Shi, Chrystelle Brignone, Nathan R Wall, Peng Yin, Mary Donohoe, Margaret P Luke, Dominica Calvo, Steven R Grossman, and Yang Shi. Yin Yang 1 is a negative regulator of p53. *Cell*, 117(7):859–872, 2004.

[199] Razmik Mirzayans, Bonnie Andrais, April Scott, and David Murray. New insights into p53 signaling and cancer cell response to DNA damage: implications for cancer therapy. *Journal of Biomedicine and Biotechnology*, 2012, 2012.

[200] Gretchen H Stein, Linda F Drullinger, Alexandre Soulard, and Vjekoslav Dulić. Differential roles for cyclin-dependent kinase inhibitors p21 and p16 in the mechanisms of senescence and differentiation in human fibroblasts. *Molecular and Cellular Biology*, 19(3):2109–2117, 1999.

[201] Malavika Raman, Svetlana Earnest, Kai Zhang, Yingming Zhao, and Melanie H Cobb. TAO kinases mediate activation of p38 in response to DNA damage. *The EMBO Journal*, 26(8):2005–2014, 2007.

[202] Ariel Bensimon, Ruedi Aebersold, and Yosef Shiloh. Beyond ATM: the protein kinase landscape of the DNA damage response. *FEBS Letters*, 585(11):1625–1639, 2011.

[203] Tina M Thornton and Mercedes Rincon. Non-classical p38 map kinase functions: cell cycle checkpoints and survival. *International Journal of Biological Sciences*, 5(1):44, 2009.

[204] Christophe Frippiat, Janique Dewelle, Jos Remacle, and Olivier Toussaint. Signal transduction in H2O2-induced senescence-like phenotype in human diploid fibroblasts. *Free Radical Biology and Medicine*, 33(10):1334–1346, 2002.

[205] Thierry Pascal, Florence Debacq-Chainiaux, Aline Chrétien, Coralie Bastin, Anne-France Dabée, Vincent Bertholet, José Remacle, and Olivier Toussaint. Comparison of replicative senescence and stress-induced premature senescence combining differential display and low-density DNA arrays. *FEBS Letters*, 579(17):3651–3659, 2005.

[206] Florence Debacq-Chainiaux, Céline Borlon, Thierry Pascal, Véronique Royer, François Eliaers, Noëlle Ninane, Géraldine Carrard, Bertrand Friguet, Françoise de Longueville, Sophie Boffe, José Remacle, and Olivier Toussaint. Repeated exposure of human skin fibroblasts to UVB at subcytotoxic level triggers premature senescence through the TGF-β1 signaling pathway. *Journal of Cell Science*, 118(4):743–758, 2005.

[207] Antero Salminen, Anu Kauppinen, and Kai Kaarniranta. Emerging role of NF-κB signaling in the induction of senescence-associated secretory phenotype (SASP). *Cellular Signalling*, 24(4):835–845, 2012.

[208] Adam Freund, Christopher K Patil, and Judith Campisi. p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. *The EMBO Journal*, 30(8):1536–1548, 2011.

[209] Jess M Cunnick, Jay F Dorsey, Lin Mei, and Jie Wu. Reversible regulation of SHP-1 tyrosine phosphatase activity by oxidation. *IUBMB Life*, 45(5):887–894, 1998.

[210] Tzu-Ting Huang, Jung-Chen Su, Chun-Yu Liu, Chung-Wai Shiau, and Kuen-Feng Chen. Alteration of SHP-1/p-STAT3 signaling: a potential target for anticancer therapy. *International Journal of Molecular Sciences*, 18(6):1234, 2017.

[211] Mirko C Sobotta, Willy Liou, Sarah Stöcker, Deepti Talwar, Michael Oehler, Thomas Ruppert, Annette ND Scharf, and Tobias P Dick. Peroxiredoxin-2 and STAT3 form a redox relay for H2O2 signaling. *Nature Chemical Biology*, 11(1):64–70, 2015.

[212] Robert Z Hopkins. Peroxiredoxins in redox relay. *Reactive Oxygen Species*, 3(9):184–188, 2017.

[213] Elena Butturini, Alessandra Carcereri de Prati, and Sofia Mariotto. Redox regulation of STAT1 and STAT3 signaling. *International Journal of Molecular Sciences*, 21(19):7034, 2020.

[214] Mehrdokht Sadrkhanloo, Maliheh Entezari, Sima Orouei, Marzieh Ghollasi, Shamin Rezaei, Elahe Sadat Hejazi, Amirabbas Kakavand, Hamidreza Saebfar, Mehrdad Hashemi, Mohammad Ali Sheikh Beig Goharrizi, Shokooh Salimimoghadam, Mohsen Rashidi, Afshin Taheriazam, et al. STAT3-EMT axis in tumors: modulation of cancer metastasis, stemness and therapy response. *Pharmacological Research*, 182:106311, 2022.

[215] Jeremy S Duffield. Beyond EMT: epithelial STAT3 as a central regulator of fibrogenesis. *Journal of the American Society of Nephrology*, 27(12):3502–3504, 2016.

[216] Qing-Qing Zhu, Chenhui Ma, Qian Wang, Yong Song, and Tangfeng Lv. The role of TWIST1 in epithelial-mesenchymal transition and cancers. *Tumor Biology*, 37:185–197, 2016.

[217] George Z Cheng, WeiZhou Zhang, Mei Sun, Qi Wang, Domenico Coppola, Mena Mansour, LiMei Xu, Carliann Costanzo, Jin Q Cheng, and Lu-Hai Wang. Twist is transcriptionally induced by activation of STAT3 and mediates STAT3 oncogenic function. *Journal of Biological Chemistry*, 283(21):14665–14673, 2008.

[218] M Saitoh, K Endo, S Furuya, M Minami, A Fukasawa, T Imamura, and K Miyazawa. STAT3 integrates cooperative Ras and TGF-β signals that induce Snail expression. *Oncogene*, 35(8):1049–1057, 2016.

[219] Theresa Vincent, Etienne PA Neve, Jill R Johnson, Alexander Kukalev, Federico Rojo, Joan Albanell, Kristian Pietras, Ismo Virtanen, Lennart Philipson, Philip L Leopold, Ronald G Crystal, Antonio Garcia de Herreros, Aristidis Moustakas, et al. A SNAIL1–SMAD3/4 transcriptional repressor complex promotes TGF-β mediated epithelial–mesenchymal transition. *Nature Cell Biology*, 11(8):943–950, 2009.

[220] Denis Puthier, Régis Bataille, and Martine Amiot. IL-6 up-regulates Mcl-1 in human myeloma cells through JAK/STAT rather than Ras/MAP kinase pathway. *European Journal of Immunology*, 29(12):3945–3950, 1999.

[221] Ralf Buettner, Linda B Mora, and Richard Jove. Activated STAT signaling in human tumors provides novel molecular targets for therapeutic intervention. *Clinical Cancer Research*, 8(4):945–954, 2002.

[222] Stefan Rose-John. IL-6 trans-signaling via the soluble IL-6 receptor: importance for the pro-inflammatory activities of IL-6. *International Journal of Biological Sciences*, 8(9):1237, 2012.

[223] Daniel E Johnson, Rachel A O'Keefe, and Jennifer R Grandis. Targeting the IL-6/JAK/STAT3 signalling axis in cancer. *Nature Reviews Clinical Oncology*, 15(4):234–248, 2018.

[224] Sandeep Robert Datta, Henryk Dudek, Xu Tao, Shane Masters, Haian Fu, Yukiko Gotoh, and Michael E Greenberg. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell*, 91(2):231–241, 1997.

[225] Prashanth T Bhaskar and Nissim Hay. The two TORCs and AKT. *Developmental Cell*, 12(4):487–502, 2007.

[226] Mathieu Laplante and David M Sabatini. Regulation of mTORC1 and its impact on gene expression at a glance. *Journal of Cell Science*, 126(8):1713–1719, 2013.

[227] C Huang, G Yang, T Jiang, G Zhu, H Li, and Z Qiu. The effects and mechanisms of blockage of STAT3 signaling pathway on IL-6 inducing EMT in human pancreatic cancer cells in vitro. *Neoplasma*, 58(5):396, 2011.

[228] Mostafa Karimi Roshan, Arash Soltani, Anvar Soleimani, Kolsoum Rezaie Kahkhaie, Amir R Afshari, and Mohammad Soukhtanloo. Role of AKT and mTOR signaling pathways in the induction of epithelial-mesenchymal transition (EMT) process. *Biochimie*, 165:229–234, 2019.

[229] Ayse Nihan Kilinc, Siyang Han, Lena A Barrett, Niroshan Anandasivam, and Celeste M Nelson. Integrin-linked kinase tunes cell–cell and cell-matrix adhesions to regulate the switch between apoptosis and EMT downstream of TGFβ1. *Molecular Biology of the Cell*, 32(5):402–412, 2021.

[230] Chuanyue Wu and Shoukat Dedhar. Integrin-linked kinase (ILK) and its interactors: a new paradigm for the coupling of extracellular matrix to actin cytoskeleton and signaling complexes. *The Journal of Cell Biology*, 155(4):505–510, 2001.

[231] Paul C McDonald and Shoukat Dedhar. New perspectives on the role of integrin-linked kinase (ILK) signaling in cancer metastasis. *Cancers*, 14(13):3209, 2022.

[232] Amancio Carnero and Jesus M Paramio. The PTEN/PI3K/AKT pathway in vivo, cancer mouse models. *Frontiers in Oncology*, 4:252, 2014.

[233] Kip M Connor, Sita Subbaram, Kevin J Regan, Kristin K Nelson, Joseph E Mazurkiewicz, Peter J Bartholomew, Andrew E Aplin, Yu-Tzu Tai, Julio Aguirre-Ghiso, Sonia C Flores, and J Andres Melendez. Mitochondrial H2O2 regulates the angiogenic phenotype via PTEN oxidation. *Journal of Biological Chemistry*, 280(17):16916–16924, 2005.

[234] Kamran Tariq and Bryan W Luikart. Striking a balance: PIP2 and PIP3 signaling in neuronal health and disease. *Exploration of Neuroprotective Therapy*, 1:86, 2021.

[235] Agata Józefiak, Magdalena Larska, Małgorzata Pomorska-Mól, and Jakub J Ruszkowski. The IGF-1 signaling pathway in viral infections. *Viruses*, 13(8):1488, 2021.

[236] Armando Cevenini, Stefania Orrù, Annamaria Mancini, Andreina Alfieri, Pasqualina Buono, and Esther Imperlini. Molecular signatures of the insulin-like growth factor 1-mediated epithelial-mesenchymal transition in breast, lung and gastric cancers. *International journal of molecular sciences*, 19(8):2411, 2018.

[237] Yue Peng, Fangmei Li, Peihua Zhang, Xiaman Wang, Ying Shen, Yuandong Feng, Yachun Jia, Ru Zhang, Jinsong Hu, and Aili He. IGF-1 promotes multiple myeloma progression through PI3K/Akt-mediated epithelial-mesenchymal transition. *Life Sciences*, 249:117503, 2020.

[238] Chunfang Wang, Ke Su, Yanyan Zhang, Weiwei Zhang, Qian Zhao, Danxia Chu, and Ruixia Guo. IR-A/IGF-1R-mediated signals promote epithelial-mesenchymal transition of endometrial carcinoma cells by activating PI3K/AKT and ERK pathways. *Cancer Biology & Therapy*, 20(3):295–306, 2019.

[239] EP Kopantzev, MR Kopantseva, EV Grankina, A Mikaelyan, VI Egorov, and ED Sverdlov. Activation of IGF/IGF-IR signaling pathway fails to induce epithelial-mesenchymal transition in pancreatic cancer cells. *Pancreatology*, 19(2):390–396, 2019.

[240] Helen E Gruber, H James Norton, and Edward N Hanley Jr. Anti-apoptotic effects of IGF-1 and PDGF on human intervertebral disc cells in vitro. *Spine*, 25(17):2153–2157, 2000.

[241] Naira Baregamian, Jun Song, Marc G Jeschke, B Mark Evers, and Dai H Chung. IGF-1 protects intestinal epithelial cells from oxidative stress-induced apoptosis. *Journal of Surgical Research*, 136(1):31–37, 2006.

[242] Nazli Khatib, Shilpa Gaidhane, Abhay M Gaidhane, Mahanaaz Khatib, Padam Simkhada, Dilip Gode, and Quazi Syed Zahiruddin. Ghrelin: ghrelin as a regulatory peptide in growth hormone secretion. *Journal of Clinical and Diagnostic Research: JCDR*, 8(8):MC13, 2014.

[243] Yutaka Takahashi. The role of growth hormone and insulin-like growth factor-I in the liver. *International Journal of Molecular Sciences*, 18(7):1447, 2017.

[244] Anuhya S Kotta, Abigail S Kelling, Karen A Corleto, Yuxiang Sun, and Erin D Giles. Ghrelin and cancer: examining the roles of the ghrelin axis in tumor growth and progression. *Biomolecules*, 12(4):483, 2022.

[245] Yun-Hee Youm, Hyunwon Yang, Yuxiang Sun, Roy G Smith, Nancy R Manley, Bolormaa Vandanmagsar, and Vishwa Deep Dixit. Deficient ghrelin receptor-mediated signaling compromises thymic stromal cell microenvironment by accelerating thymic adiposity. *Journal of Biological Chemistry*, 284(11):7068–7077, 2009.

[246] Alison L Brittain, Reetobrata Basu, Yanrong Qian, and John J Kopchick. Growth hormone and the epithelial-to-mesenchymal transition. *The Journal of Clinical Endocrinology & Metabolism*, 102(10):3662–3673, 2017.

[247] Norbert Schuster and Kerstin Krieglstein. Mechanisms of TGF-β-mediated apoptosis. *Cell and Tissue Research*, 307:1–14, 2002.

[248] Sneha Ramesh, Xiao-Jun Qi, Gary M Wildey, Janet Robinson, Jeffery Molkentin, John Letterio, and Philip H Howe. TGFβ-mediated BIM expression and apoptosis are regulated through SMAD3-dependent expression of the MAPK phosphatase MKP2. *EMBO Reports*, 9(10):990–997, 2008.

[249] Kunxin Luo. Signaling cross talk between TGF-β/Smad and other signaling pathways. *Cold Spring Harbor Perspectives in Biology*, 9(1):a022137, 2017.

[250] Kyung Song, Hui Wang, Tracy L Krebs, and David Danielpour. Novel roles of Akt and mTOR in suppressing TGF-β/ALK5-mediated Smad3 activation. *The EMBO Journal*, 25(1):58–69, 2006.

[251] Ingrid Remy, Annie Montmarquette, and Stephen W Michnick. PKB/Akt modulates TGF-β signalling through a direct interaction with Smad3. *Nature Cell Biology*, 6(4):358–365, 2004.

[252] Qi Zhang, Feifei Cui, Lei Fang, Jian Hong, Biao Zheng, and Jingwu Z Zhang. TNF-α impairs differentiation and function of TGF-β-induced Treg cells in autoimmune diseases through Akt and Smad3 signaling pathway. *Journal of Molecular Cell Biology*, 5(2):85–98, 2013.

[253] Kyung Song, Susan C Cornelius, Michael Reiss, and David Danielpour. Insulin-like growth factor-I inhibits transcriptional responses of transforming growth factor-β by phosphatidylinositol 3-kinase/Akt-dependent suppression of the activation of Smad3 but not Smad2. *Journal of Biological Chemistry*, 278(40):38342–38351, 2003.

[254] Pasithorn A Suwanabol, Stephen M Seedial, Fan Zhang, Xudong Shi, Yi Si, Bo Liu, and K Craig Kent. TGF-β and Smad3 modulate PI3K/Akt signaling pathway in vascular smooth muscle cells. *American Journal of Physiology-Heart and Circulatory Physiology*, 302(11):H2211–H2219, 2012.

[255] Hong-Hao Zhou, Lin Chen, Hui-Fang Liang, Guang-Zhen Li, Bi-Xiang Zhang, and Xiao-Ping Chen. Smad3 sensitizes hepatocelluar carcinoma cells to cisplatin by repressing phosphorylation of AKT. *International Journal of Molecular Sciences*, 17(4):610, 2016.

[256] Baokun He, Yuying Liu, Thomas K Hoang, Xiangjun Tian, Christopher M Taylor, Meng Luo, Dat Q Tran, Nina Tatevian, and J Marc Rhoads. Antibiotic-modulated microbiome suppresses lethal inflammation and prolongs lifespan in Treg-deficient mice. *Microbiome*, 7:1–12, 2019.

[257] Hao Wang, Hong-Sheng Wang, Bin-Hua Zhou, Cui-Lin Li, Fan Zhang, Xian-Feng Wang, Ge Zhang, Xian-Zhang Bu, Shao-Hui Cai, and Jun Du. Epithelial–mesenchymal transition (EMT) induced by TNF-α requires AKT/GSK-3β-mediated stabilization of snail in colorectal cancer. *PLOS One*, 8(2):e56664, 2013.

[258] Clara Sciorati, Riccardo Gamberale, Antonella Monno, Lorena Citterio, Chiara Lanzani, Rebecca De Lorenzo, Giuseppe A Ramirez, Antonio Esposito, Paolo Manunta, Angelo A Manfredi, and Patrizia Rovere-Querini. Pharmacological blockade of tnfα prevents sarcopenia and prolongs survival in aging mice. *Aging (Albany NY)*, 12(23):23497, 2020.

[259] Heidi Glosli, Hans Prydz, and Borghild Roald. Involution of thymus and lymphoid depletion in mice expressing the hTNF transgene. *Apmis*, 112(1):63–73, 2004.

[260] AV Kulikov, LV Arkhipova, PA Kulikova, AA Glazkov, E Yu Mndlyan, VB Gavrilyuk, and DA Kulikov. Effects of birth season and thymus transplantation on experimental animal longevity. *Biology Bulletin*, 46:33–37, 2019.

[261] Ming Li, Nader G Abraham, Luca Vanella, Yuming Zhang, Muneo Inaba, Naoki Hosaka, Sho-Ichi Hoshino, Ming Shi, Yoko Miyamoto Ambrosini, M Eric Gershwin, and Susumu Ikehara.

Successful modulation of type 2 diabetes in db/db mice with intra-bone marrow–bone marrow transplantation plus concurrent thymic transplantation. *Journal of Autoimmunity*, 35(4):414–423, 2010.

[262] Gregory M Fahy, Robert T Brooke, James P Watson, Zinaida Good, Shreyas S Vasanawala, Holden Maecker, Michael D Leipold, David TS Lin, Michael S Kobor, and Steve Horvath. Reversal of epigenetic aging and immunosenescent trends in humans. *Aging Cell*, 18(6):e13028, 2019.

[263] F Waldhauser, J Ková, and E Reiter. Age-related changes in melatonin levels in humans and its potential consequences for sleep disorders. *Experimental Gerontology*, 33(7-8):759–772, 1998.

[264] MC Naranjo, JM Guerrero, A Rubio, PJ Lardone, A Carrillo-Vico, MP Carrascosa-Salmoral, S Jiménez-Jorge, MV Arellano, SR Leal-Noval, M Leal, E Lissen, and P Molinero. Melatonin biosynthesis in the thymus of humans and rats. *Cellular and Molecular Life Sciences*, 64:781–790, 2007.

[265] Reza Sharafati-Chaleshtori, Hedayatollah Shirzad, Mahmoud Rafieian-Kopaei, and Amin Soltani. Melatonin and human mitochondrial diseases. *Journal of Research in Medical Sciences: The Official Journal of Isfahan University of Medical Sciences*, 22, 2017.

[266] Radomir M Slominski, Russel J Reiter, Natalia Schlabritz-Loutsevitch, Rennolds S Ostrom, and Andrzej T Slominski. Melatonin membrane receptors in peripheral tissues: distribution and functions. *Molecular and Cellular Endocrinology*, 351(2):152–166, 2012.

[267] Vladimir N Anisimov, Irina G Popovich, Mark A Zabezhinski, Sergey V Anisimov, Georgy M Vesnushkin, and Irina A Vinogradova. Melatonin as antioxidant, geroprotector and anticarcinogen. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1757(5-6):573–589, 2006.

[268] Olga Junemann, Inna Bukreeva, Dmitry A Otlyga, Alessia Cedola, Michela Fratini, and Sergei V Saveliev. Human pineal gland involutionary process: new findings. *The Journals of Gerontology: Series A*, page glad091, 2023.

[269] Maki Goto, Itsuki Oshima, Takeshi Tomita, and Shizufumi Ebihara. Melatonin content of the pineal gland in different mouse strains. *Journal of Pineal Research*, 7(2):195–204, 1989.

[270] G Oxenkrug, P Requintina, and S Bachurin. Antioxidant and antiaging activity of N-acetylserotonin and melatonin in the in vivo models. *Annals of the New York Academy of Sciences*, 939(1):190–199, 2001.

[271] Vladimir N Anisimov, Natalia Y Zavarzina, Mark A Zabezhinski, Irina G Popovich, Olga A Zimina, Anastasia V Shtylick, Alexander V Arutjunyan, Tatiana I Oparina, Valentina M Prokopenko, Anatoli I Mikhalski, and Anatoli I Yashin. Melatonin increases both life span and tumor incidence in female CBA mice. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 56(7):B311–B323, 2001.

[272] Steven M Reppert and David R Weaver. Melatonin madness. *Cell*, 83(7):1059–1062, 1995.

[273] Patrick H Roseboom, MA Aryan Namboodiri, Drazen B Zimonjic, Nicholas C Popescu, Ignacio R Rodriguez, Jonathan A Gastel, and David C Klein. Natural melatonin 'knockdown' in C57BL/6J mice: rare mechanism truncates serotonin N-acetyltransferase. *Molecular Brain Research*, 63(1):189–197, 1998.

[274] Hakan Oner, Ilter Kus, Jale Oner, Murat Ogeturk, Enver Ozan, and Ahmet Ayar. Possible effects of melatonin on thymus gland after pinealectomy in rats. *Neuroendocrinology Letters*, 25(1/2):115–118, 2004.

[275] Mats I Nilsson, Jacqueline M Bourgeois, Joshua P Nederveen, Marlon R Leite, Bart P Hettinga, Adam L Bujak, Linda May, Ethan Lin, Michael Crozier, Daniel R Rusiecki, CHris Moffatt, Paul Azzopardi, Jacob Young, et al. Lifelong aerobic exercise protects against inflammaging and cancer. *PLOS One*, 14(1):e0210863, 2019.

[276] Naichun Ji, Jing Luan, Fengrui Hu, Yirong Zhao, Bosen Lv, Wen Wang, Meng Xia, Xin Zhao, and Kejing Lao. Aerobic exercise-stimulated Klotho upregulation extends life span by attenuating the excess production of reactive oxygen species in the brain and kidney. *Experimental and Therapeutic Medicine*, 16(4):3511–3517, 2018.

[277] Hiroko P Indo, Mercy Davidson, Hsiu-Chuan Yen, Shigeaki Suenaga, Kazuo Tomita, Takeshi Nishii, Masahiro Higuchi, Yasutoshi Koga, Toshihiko Ozawa, and Hideyuki J Majima. Evidence of ROS generation by mitochondria in cells with impaired electron transport chain and mitochondrial DNA damage. *Mitochondrion*, 7(1-2):106–118, 2007.

[278] Diana R Gutsaeva, Martha Sue Carraway, Hagir B Suliman, Ivan T Demchenko, Hiroshi Shitara, Hiromichi Yonekawa, and Claude A Piantadosi. Transient hypoxia stimulates mitochondrial biogenesis in brain subcortex by a neuronal nitric oxide synthase-dependent mechanism. *Journal of Neuroscience*, 28(9):2015–2024, 2008.

[279] Lingyun Zhu, Qiang Wang, Lin Zhang, Zhixiang Fang, Fang Zhao, Zhiyuan Lv, Zuguang Gu, Junfeng Zhang, Jin Wang, Ke Zen, Yang Xiang, Dongjin Wang, and Chen-Yu Zhang. Hypoxia induces PGC-1α expression and mitochondrial biogenesis in the myocardium of TOF patients. *Cell Research*, 20(6):676–687, 2010.

[280] Robert S Rogers, Hong Wang, Timothy J Durham, Jonathan A Stefely, Norah A Owiti, Andrew L Markhard, Lev Sandler, Tsz-Leung To, and Vamsi K Mootha. Hypoxia extends lifespan and neurological function in a mouse model of aging. *PLOS Biology*, 21(5):e3002117, 2023.

[281] Jennifer L Steiner, E Angela Murphy, Jamie L McClellan, Martin D Carmichael, and J Mark Davis. Exercise training increases mitochondrial biogenesis in the brain. *Journal of Applied Physiology*, 111:1066–1071, 2011.

[282] So-ichiro Fukada, Yuran Ma, and Akiyoshi Uezumi. Adult stem cell and mesenchymal progenitor theories of aging. *Frontiers in Cell and Developmental Biology*, 2:10, 2014.

[283] Yu Pan, ZhenZhen Gu, Yansi Lyu, Yi Yang, Manhon Chung, Xiaohua Pan, and Sa Cai. Link between senescence and cell fate: senescence-associated secretory phenotype and its effects on stem cell fate transition. *Rejuvenation Research*, 25(4):160–172, 2022.

[284] Marta Annunziata, Riccarda Granata, and Ezio Ghigo. The IGF system. *Acta Diabetologica*, 48:1–9, 2011.

[285] Cynthia J Kenyon. The genetics of ageing. *Nature*, 464(7288):504–512, 2010.

[286] Koutarou D Kimura, Heidi A Tissenbaum, Yanxia Liu, and Gary Ruvkun. daf-2, an insulin receptor-like gene that regulates longevity and diapause in Caenorhabditis elegans. *Science*, 277(5328):942–946, 1997.

[287] David W Walker, Gawain McColl, Nicole L Jenkins, Jennifer Harris, and Gordon J Lithgow. Evolution of lifespan in C. elegans. *Nature*, 405(6784):296–297, 2000.

[288] David Gems and Ryan Doonan. Antioxidant defense and aging in C. elegans: is the oxidative damage theory of aging wrong? *Cell Cycle*, 8(11):1681–1687, 2009.

[289] Paul Davis, Magdalena Zarowiecki, Valerio Arnaboldi, Andrés Becerra, Scott Cain, Juancarlos Chan, Wen J Chen, Jaehyoung Cho, Eduardo da Veiga Beltrame, Stavros Diamantakis, Sibyl Gao, Dionysis Grigoriadis, Christian A Grove, et al. WormBase in 2022—data, processes, and tools for analyzing Caenorhabditis elegans. *Genetics*, 220(4):iyac003, 2022.

[290] Arwen W Gao, Reuben L Smith, Michel Van Weeghel, Rashmi Kamble, Georges E Janssens, and Riekelt H Houtkooper. Identification of key pathways and metabolic fingerprints of longevity in C. elegans. *Experimental Gerontology*, 113:128–140, 2018.

[291] Thomas Heimbucher, Zheng Liu, Carine Bossard, Richard McCloskey, Andrea C Carrano, Christian G Riedel, Bogdan Tanasa, Christian Klammt, Bryan R Fonslow, Celine E Riera, Bjorn F Lillemeier, Kenneth Kemphues, John R Yates 3rd, et al. The deubiquitylase MATH-33 controls DAF-16 stability and function in metabolism and longevity. *Cell Metabolism*, 22(1):151–163, 2015.

[292] Neeraj Kumar, Vaibhav Jain, Anupama Singh, Urmila Jagtap, Sonia Verma, and Arnab Mukhopadhyay. Genome-wide endogenous DAF-16/FOXO recruitment dynamics during lowered insulin signalling in C. elegans. *Oncotarget*, 6(39):41418, 2015.

[293] Yu-Waye Chu, Sabrina Schmitz, Baishakhi Choudhury, William Telford, Veena Kapoor, Susan Garfield, David Howe, and Ronald E Gress. Exogenous insulin-like growth factor 1 enhances thymopoiesis predominantly through thymic epithelial cell expansion. *Blood, The Journal of the American Society of Hematology*, 112(7):2836–2846, 2008.

[294] Seogang Hyun. Body size regulation and insulin-like growth factor signaling. *Cellular and Molecular Life Sciences*, 70:2351–2365, 2013.

[295] Jacob A Panici, James M Harper, Richard A Miller, Andrzej Bartke, Adam Spong, and Michal M Masternak. Early life growth hormone treatment shortens longevity and decreases cellular stress resistance in long-lived mutant mice. *The FASEB Journal*, 24(12):5073, 2010.

[296] Holly M Brown-Borg, Sharlene G Rakoczy, Mark A Romanick, and Melissa A Kennedy. Effects of growth hormone and insulin-like growth factor-1 on hepatocyte antioxidative enzymes. *Experimental Biology and Medicine*, 227(2):94–104, 2002.

[297] Jaime Guevara-Aguirre, Gabriela Peña, William Acosta, Gabriel Pazmiño, Jannette Saavedra, Lina Soto, Daniela Lescano, Alexandra Guevara, and Antonio WD Gavilanes. Cancer in growth hormone excess and growth hormone deficit. *Endocrine-related Cancer*, 30(10), 2023.

[298] Lindsay Goffinet, Marie Mottet, Hamid Kermani, Chantal Renard-Charlet, Vincent Geenen, and Henri J Martens. Impact of the somatotrope growth hormone (GH)/insulin-like growth factor 1 (IGF-1) axis upon thymus function: pharmacological implications in regeneration of immune functions. *Immunology, Endocrine & Metabolic Agents in Medicinal Chemistry*, 11(1):10–20, 2011.

[299] Daniela Frasca, Bonnie B Blomberg, and Roberto Paganelli. Aging, obesity, and inflammatory age-related diseases. *Frontiers in Immunology*, 8:1745, 2017.

[300] Giusi Taormina and Mario G Mirisola. Calorie restriction in mammals and simple model organisms. *BioMed Research International*, 2014, 2014.

[301] Wei Peng, Rui Zhou, Ze-Fang Sun, Jia-Wei Long, and Yong-Qiang Gong. Novel insights into the roles and mechanisms of GLP-1 receptor agonists against aging-related diseases. *Aging and Disease*, 13(2):468, 2022.

[302] Vivek P Chavda, Pankti C Balar, Dixa A Vaghela, and Payal Dodiya. Unlocking longevity with GLP-1: A key to turn back the clock? *Maturitas*, page 108028, 2024.

[303] Hyunwon Yang, Yun-Hee Youm, Chiaki Nakata, and Vishwa Deep Dixit. Chronic caloric restriction induces forestomach hypertrophy with enhanced ghrelin levels during aging. *Peptides*, 28(10):1931–1936, 2007.

[304] Hyunwon Yang, Yun-Hee Youm, Bolormaa Vandanmagsar, Jennifer Rood, K Ganesh Kumar, Andrew A Butler, and Vishwa Deep Dixit. Obesity accelerates thymic aging. *Blood, The Journal of the American Society of Hematology*, 114(18):3803–3812, 2009.

[305] Hyunwon Yang, Yun-Hee Youm, and Vishwa Deep Dixit. Inhibition of thymic adipogenesis by caloric restriction is coupled with reduction in age-related thymic involution. *The Journal of Immunology*, 183(5):3040–3052, 2009.

[306] Thomas Weichhart. mTOR as regulator of lifespan, aging, and cellular senescence: a minireview. *Gerontology*, 64(2):127–134, 2018.

[307] Nicholas D Bonawitz, Marc Chatenay-Lapointe, Yong Pan, and Gerald S Shadel. Reduced TOR signaling extends chronological life span via increased respiration and upregulation of mitochondrial gene expression. *Cell Metabolism*, 5(4):265–277, 2007.

[308] Xuemin Wang and Christopher G Proud. The mTOR pathway in the control of protein synthesis. *Physiology*, 21(5):362–369, 2006.

[309] Javier Apfeld, Greg O'Connor, Tom McDonagh, Peter S DiStefano, and Rory Curtis. The AMP-activated protein kinase AAK-2 links energy levels and insulin-like signals to lifespan in C. elegans. *Genes & Development*, 18(24):3004–3009, 2004.

[310] David Carling. AMPK signalling in health and disease. *Current Opinion in Cell Biology*, 45:31–37, 2017.

[311] D Grahame Hardie, Fiona A Ross, and Simon A Hawley. AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nature Reviews Molecular Cell Biology*, 13(4):251–262, 2012.

[312] Sang-Min Jeon. Regulation and function of AMPK in physiology and diseases. *Experimental & Molecular Medicine*, 48(7):e245–e245, 2016.

[313] Gregory N Ruegsegger, Ana L Creo, Tiffany M Cortes, Surendra Dasari, and K Sreekumaran Nair. Altered mitochondrial function in insulin-deficient and insulin-resistant states. *The Journal of Clinical Investigation*, 128(9):3671–3681, 2018.

[314] Lijun Zhao, Jianzhong Cao, Kexin Hu, Xiaodong He, Dou Yun, Tanjun Tong, and Limin Han. Sirtuins and their biological relevance in aging and age-related diseases. *Aging and Disease*, 11(4):927, 2020.

[315] Andrei Seluanov, Zhuoxun Chen, Christopher Hine, Tais HC Sasahara, Antonio ACM Ribeiro, Kenneth C Catania, Daven C Presgraves, and Vera Gorbunova. Telomerase activity coevolves with body mass not lifespan. *Aging Cell*, 6(1):45–52, 2007.

[316] Jose A Palacios, Daniel Herranz, Maria Luigia De Bonis, Susana Velasco, Manuel Serrano, and Maria A Blasco. SIRT1 contributes to telomere maintenance and augments global homologous recombination. *Journal of Cell Biology*, 191(7):1299–1313, 2010.

[317] Sangkyu Kim, Xiuhua Bi, Malwina Czarny-Ratajczak, Jianliang Dai, David A Welsh, Leann Myers, Michael A Welsch, Katie E Cherry, Jonathan Arnold, Leonard W Poon, and S Michal Jazwinski. Telomere maintenance genes SIRT1 and XRCC6 impact age-related decline in telomere length but only SIRT1 is associated with human longevity. *Biogerontology*, 13(2):119–131, 2012.

[318] Anna Chuprin, Ayelet Avin, Yael Goldfarb, Yonatan Herzig, Ben Levi, Adi Jacob, Asaf Sela, Shir Katz, Moran Grossman, Clotilde Guyon, Moran Rathaus, Haim Y Cohen, Irit Sagi, et al. The deacetylase Sirt1 is an essential regulator of Aire-mediated induction of central immunological tolerance. *Nature Immunology*, 16(7):737–745, 2015.

[319] Eriko Michishita, Ronald A McCord, Elisabeth Berber, Mitomu Kioi, Hesed Padilla-Nash, Mara Damian, Peggie Cheung, Rika Kusumoto, Tiara LA Kawahara, J Carl Barrett, Howard Y Chang, Vilhelm A Bohr, Thomas Ried, et al. SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin. *Nature*, 452(7186):492–496, 2008.

[320] Junko Oshima. Werner syndrome. *Chromosomal Instability and Aging*, pages 185–204, 2003.

[321] Ruth I Tennen and Katrin F Chua. Chromatin regulation and genome maintenance by mammalian SIRT6. *Trends in Biochemical Sciences*, 36(1):39–46, 2011.

[322] Xiao Tian, Denis Firsanov, Zhihui Zhang, Yang Cheng, Lingfeng Luo, Gregory Tombline, Ruiyue Tan, Matthew Simon, Steven Henderson, Janine Steffan, Audrey Goldfarb, Jonathan Tam, Kitty Zheng, et al. SIRT6 is responsible for more efficient DNA double-strand break repair in long-lived species. *Cell*, 177(3):622–638, 2019.

[323] Tiara LA Kawahara, Eriko Michishita, Adam S Adler, Mara Damian, Elisabeth Berber, Meihong Lin, Ron A McCord, Kristine CL Ongaigui, Lisa D Boxer, Howard Y Chang, and Katrin F Chua. SIRT6 links histone H3 lysine 9 deacetylation to NF-κB-dependent gene expression and organismal life span. *Cell*, 136(1):62–74, 2009.

[324] Qian Zhang, Zhanfeng Liang, Jiayu Zhang, Tong Lei, Xue Dong, Huiting Su, Yifang Chen, Zhaoqi Zhang, Liang Tan, and Yong Zhao. Sirt6 regulates the development of medullary thymic epithelial cells and contributes to the establishment of central immune tolerance. *Frontiers in Cell and Developmental Biology*, 9:655552, 2021.

[325] Andrew M Pickering, Marcus Lehr, Christi M Gendron, Scott D Pletcher, and Richard A Miller. Mitochondrial thioredoxin reductase 2 is elevated in long-lived primate as well as rodent species and extends fly mean lifespan. *Aging Cell*, 16(4):683–692, 2017.

[326] Abdelrahman AlOkda and Jeremy M Van Raamsdonk. Evolutionarily conserved role of thioredoxin systems in determining longevity. *Antioxidants*, 12(4):944, 2023.

[327] Irene Martínez de Toda, Carmen Vida, Antonio Garrido, and Mónica De la Fuente. Redox parameters as markers of the rate of aging and predictors of life span. *The Journals of Gerontology: Series A*, 75(4):613–620, 2020.

[328] Yuhong Wang and Zhongjie Sun. Current understanding of klotho. *Ageing Research Reviews*, 8(1):43–51, 2009.

[329] Ido Wolf, S Levanon-Cohen, S Bose, H Ligumsky, B Sredni, H Kanety, M Kuro-o, B Karlan, B Kaufman, HP Koeffler, and T Rubinek. Klotho: a tumor suppressor and a modulator of the IGF-1 and FGF pathways in human breast cancer. *Oncogene*, 27(56):7094–7105, 2008.

[330] Nguyen Thi Xuan, Nguyen Huy Hoang, Vu Phuong Nhung, Nguyen Thuy Duong, Nguyen Hai Ha, and Nong Van Hai. Regulation of dendritic cell function by insulin/IGF-1/PI3K/Akt signaling through klotho expression. *Journal of Receptors and Signal Transduction*, 37(3):297–303, 2017.

[331] Yuechi Xu and Zhongjie Sun. Molecular basis of Klotho: from gene to function in aging. *Endocrine Reviews*, 36(2):174–193, 2015.

[332] Geert JPL Kops, Tobias B Dansen, Paulien E Polderman, Ingrid Saarloos, Karel WA Wirtz, Paul J Coffer, Ting-T Huang, Johannes L Bos, René H Medema, and Boudewijn MT Burgering. Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. *Nature*, 419(6904):316–321, 2002.

[333] Kyung-Jin Min, Cheol-Koo Lee, and Han-Nam Park. The lifespan of Korean eunuchs. *Current Biology*, 22(18):R792–R793, 2012.

[334] William H Parker, Michael S Broder, Eunice Chang, Diane Feskanich, Cindy Farquhar, Zhimae Liu, Donna Shoupe, Jonathan S Berek, Susan Hankinson, and JoAnn E Manson. Ovarian conservation at the time of hysterectomy and long-term health outcomes in the nurses' health study. *Obstetrics and Gynecology*, 113(5):1027, 2009.

[335] Yuehua Wei and Cynthia Kenyon. Roles for ROS and hydrogen sulfide in the longevity response to germline loss in Caenorhabditis elegans. *Proceedings of the National Academy of Sciences*, 113(20):E2832–E2841, 2016.

[336] James Henderson. On the relationship of the thymus to the sexual organs: I. The influence of castration on the thymus. *The Journal of Physiology*, 31(3-4):222, 1904.

[337] KF Windmill and VWK Lee. Influences of surgical castration on the thymus of male rats. *Journal of Reproductive Immunology*, 44(1-2):29–39, 1999.

[338] Shuhei Nakamura and Tamotsu Yoshimori. Autophagy and longevity. *Molecules and Cells*, 41(1):65, 2018.

[339] Jong-Ok Pyo, Seung-Min Yoo, Hye-Hyun Ahn, Jihoon Nah, Se-Hoon Hong, Tae-In Kam, Sunmin Jung, and Yong-Keun Jung. Overexpression of Atg5 in mice activates autophagy and extends lifespan. *Nature Communications*, 4(1):1–9, 2013.

[340] Bohan Zhang, David E Lee, Alexandre Trapp, Alexander Tyshkovskiy, Ake T Lu, Akshay Bareja, Csaba Kerepesi, Lauren K McKay, Anastasia V Shindyapina, Sergey E Dmitriev, Gurpreet S Baht, Steve Horvath, Vadim N Gladyshev, et al. Multi-omic rejuvenation and lifespan extension on exposure to youthful circulation. *Nature Aging*, 3(8):948–964, 2023.

[341] Tatiana Yankova, Tatiana Dubiley, Dmytro Shytikov, and Iryna Pishel. Three-month heterochronic parabiosis has a deleterious effect on the lifespan of young animals, without a positive effect for old animals. *Rejuvenation Research*, 25(4):191–199, 2022.

[342] Matthew J Yousefzadeh, John E Wilkinson, Brian Hughes, Namrata Gadela, Warren C Ladiges, Nam Vo, Laura J Niedernhofer, Derek M Huffman, and Paul D Robbins. Heterochronic parabiosis regulates the extent of cellular senescence in multiple tissues. *Geroscience*, 42(3):951–961, 2020.

[343] Dimitris A Papanicolaou. Interleukin-6: the endocrine cytokine. *The Journal of Clinical Endocrinology & Metabolism*, 85(3):1331–1333, 2000.

[344] Barbara Sherry and Anthony Cerami. Cachectin/tumor necrosis factor exerts endocrine, paracrine, and autocrine control of inflammatory responses. *The Journal of Cell Biology*, 107(4):1269–1277, 1988.

[345] James Flory and Kasia Lipska. Metformin in 2019. *JAMA*, 321(19):1926–1927, 2019.

[346] Jacek Kasznicki, Agnieszka Sliwinska, and Józef Drzewoski. Metformin in cancer prevention and therapy. *Annals of Translational Medicine*, 2(6), 2014.

[347] Marta G Novelle, Ahmed Ali, Carlos Diéguez, Michel Bernier, and Rafael de Cabo. Metformin: a hopeful promise in aging research. *Cold Spring Harbor Perspectives in Medicine*, 6(3):a025932, 2016.

[348] Nir Barzilai, Jill P Crandall, Stephen B Kritchevsky, and Mark A Espeland. Metformin as a tool to target aging. *Cell Metabolism*, 23(6):1060–1065, 2016.

[349] Mark R Owen, Elena Doran, and Andrew P Halestrap. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. *Biochemical Journal*, 348(3):607–614, 2000.

[350] Cécile Batandier, Bruno Guigas, Dominique Detaille, M El-Mir, Eric Fontaine, Michel Rigoulet, and Xavier M Leverve. The ROS production induced by a reverse-electron flux at respiratory-chain complex 1 is hampered by metformin. *Journal of Bioenergetics and Biomembranes*, 38(1):33–42, 2006.

[351] Gaochao Zhou, Robert Myers, Ying Li, Yuli Chen, Xiaolan Shen, Judy Fenyk-Melody, Margaret Wu, John Ventre, Thomas Doebber, Nobuharu Fujii, Nicolas Musi, Michael F Hirshman, Laurie J Goodyear, et al. Role of AMP-activated protein kinase in mechanism of metformin action. *The Journal of clinical Investigation*, 108(8):1167–1174, 2001.

[352] D Bonnefont-Rousselot, B Raji, S Walrand, M Gardes-Albert, D Jore, A Legrand, J Peynet, and MP Vasson. An intracellular modulation of free radical production could contribute to the beneficial effects of metformin towards oxidative stress. *Metabolism*, 52(5):586–589, 2003.

[353] Hyun-Soo Shin, Jiyeon Ko, Dal-Ah Kim, Eun-Sun Ryu, Hye-Myung Ryu, Sun-Hee Park, Yong-Lim Kim, Eok-Soo Oh, and Duk-Hee Kang. Metformin ameliorates the phenotype transition of peritoneal mesothelial cells and peritoneal fibrosis via a modulation of oxidative stress. *Scientific Reports*, 7(1):1–13, 2017.

[354] Carolyn Algire, Olga Moiseeva, Xavier Deschênes-Simard, Lilian Amrein, Luca Petruccelli, Elena Birman, Benoit Viollet, Gerardo Ferbeyre, and Michael N Pollak. Metformin reduces endogenous reactive oxygen species and associated DNA damage. *Cancer Prevention Research*, 5(4):536–543, 2012.

[355] Shu-ping Yang, Qing Su, Ya-ru Zhang, Yun Sun, and Yu-rong Chai. Metformin ameliorates thymus degeneration of mice by regulating mitochondrial function. *International Immunopharmacology*, 108:108744, 2022.

[356] Alice E Kane and David A Sinclair. Epigenetic changes during aging and their reprogramming potential. *Critical Reviews in Biochemistry and Molecular Biology*, 54(1):61–83, 2019.

[357] Philipp Oberdoerffer, Shaday Michan, Michael McVay, Raul Mostoslavsky, James Vann, Sang-Kyu Park, Andrea Hartlerode, Judith Stegmuller, Angela Hafner, Patrick Loerch, Sarah M Wright, Kevin D Mills, Azad Bonni, et al. SIRT1 redistribution on chromatin promotes genomic stability but alters gene expression during aging. *Cell*, 135(5):907–918, 2008.

[358] Aswath Balakrishnan, Kanive Parashiva Guruprasad, Kapaettu Satyamoorthy, and Manjunath B Joshi. Interleukin-6 determines protein stabilization of DNA methyltransferases and alters DNA promoter methylation of genes associated with insulin signaling and angiogenesis. *Laboratory Investigation*, 98(9):1143–1158, 2018.

[359] Yi Li, Jasper Deuring, Maikel P Peppelenbosch, Ernst J Kuipers, Colin de Haar, and C Janneke van der Woude. IL-6-induced DNMT1 activity mediates SOCS3 promoter hypermethylation in ulcerative colitis-related colorectal cancer. *Carcinogenesis*, 33(10):1889–1896, 2012.

[360] Jacqueline A Gasche, Jürgen Hoffmann, C Richard Boland, and Ajay Goel. Interleukin-6 promotes tumorigenesis by altering DNA methylation in oral cancer cells. *International Journal of Cancer*, 129(5):1053–1063, 2011.

[361] Hania Wehbe, Roger Henson, Fanyin Meng, Janna Mize-Berge, and Tushar Patel. Interleukin-6 contributes to growth in cholangiocarcinoma cells by aberrant promoter methylation and gene expression. *Cancer Research*, 66(21):10517–10524, 2006.

[362] Soizic Garaud, Christelle Le Dantec, Sandrine Jousse-Joulin, Catherine Hanrotel-Saliou, Alain Saraux, Rizgar A Mageed, Pierre Youinou, and Yves Renaudineau. IL-6 modulates CD5 expression in B cells from patients with lupus by regulating DNA methylation. *The Journal of Immunology*, 182(9):5623–5632, 2009.

[363] Steve Horvath and Kenneth Raj. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nature Reviews Genetics*, 19(6):371–384, 2018.

[364] Anna J Stevenson, Danni A Gadd, Robert F Hillary, Daniel L McCartney, Archie Campbell, Rosie M Walker, Kathryn L Evans, Sarah E Harris, Tara L Spires-Jones, Allan F McRae, Peter M Visscher, Andrew M Mcintosh, Ian J Deary, et al. Creating and validating a DNA methylation-based proxy for interleukin-6. *The Journals of Gerontology: Series A*, 76(12):2284–2292, 2021.

[365] Aleksandra Trifunovic, Anna Hansson, Anna Wredenberg, Anja T Rovio, Eric Dufour, Ivan Khvorostov, Johannes N Spelbrink, Rolf Wibom, Howard T Jacobs, and Nils-Göran Larsson.

Somatic mtDNA mutations cause aging phenotypes without affecting reactive oxygen species production. *Proceedings of the National Academy of Sciences*, 102(50):17993–17998, 2005.

[366] Laura C Greaves, Nina E Beadle, Geoffrey A Taylor, Daniel Commane, John C Mathers, Konstantin Khrapko, and Doug M Turnbull. Quantification of mitochondrial DNA mutation load. *Aging Cell*, 8(5):566–572, 2009.

[367] Angela Logan, Irina G Shabalina, Tracy A Prime, Sebastian Rogatti, Anastasia V Kalinovich, Richard C Hartley, Ralph C Budd, Barbara Cannon, and Michael P Murphy. In vivo levels of mitochondrial hydrogen peroxide increase with age in mtDNA mutator mice. *Aging Cell*, 13(4):765–768, 2014.

[368] Dao-Fu Dai, Tony Chen, Jonathan Wanagat, Michael Laflamme, David J Marcinek, Mary J Emond, Calvin P Ngo, Tomas A Prolla, and Peter S Rabinovitch. Age-dependent cardiomyopathy in mitochondrial mutator mice is attenuated by overexpression of catalase targeted to mitochondria. *Aging Cell*, 9(4):536–544, 2010.

[369] Viviana I Pérez, Holly Van Remmen, Alex Bokov, Charles J Epstein, Jan Vijg, and Arlan Richardson. The overexpression of major antioxidant enzymes does not extend the lifespan of mice. *Aging Cell*, 8(1):73–75, 2009.

[370] Colin Selman, Jane S McLaren, Andrew R Collins, Garry G Duthie, and John R Speakman. Deleterious consequences of antioxidant supplementation on lifespan in a wild-derived mammal. *Biology Letters*, 9(4):20130432, 2013.

[371] Bronwyn A Mogck, Samantha T Jezak, and Christopher D Wiley. Mitochondria-targeted catalase does not suppress development of cellular senescence during aging. *Biomedicines*, 12(2):414, 2024.

[372] Christopher D Wiley, Michael C Velarde, Pacome Lecot, SU Liu, Ethan A Sarnoski, Adam Freund, Kotaro Shirakawa, Hyung W Lim, Sonnet S Davis, Arvind Ramanathan, Akos A Gerencser, Eric Verdin, and Judith Campisi. Mitochondrial dysfunction induces senescence with a distinct secretory phenotype. *Cell Metabolism*, 23(2):303–314, 2016.

[373] Samuel E Schriner, Nancy J Linford, George M Martin, Piper Treuting, Charles E Ogburn, Mary Emond, Pinar E Coskun, Warren Ladiges, Norman Wolf, Holly Van Remmen, Douglas C Wallace, and Peter S Rabinovitch. Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science*, 308(5730):1909–1911, 2005.

[374] Samuel E Schriner and Nancy J Linford. Extension of mouse lifespan by overexpression of catalase. *Age*, 28:209–218, 2006.

[375] Daan MK van Soest, Paulien E Polderman, Wytze TF den Toom, Janneke P Keijer, Markus J van Roosmalen, Tim MF Leyten, Johannes Lehmann, Susan Zwakenberg, Sasha De Henau, Ruben van Boxtel, BBoudewijn MT Burgering, and Tobias B Dansen. Mitochondrial H2O2 release does not directly cause damage to chromosomal DNA. *Nature Communications*, 15(1):2725, 2024.