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Genome instability, cancer and aging

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Abstract

DNA damage-driven genome instability underlies the diversity of life forms generated by the evolutionary process but is detrimental to the somatic cells of individual organisms. The cellular response to DNA damage can be roughly divided in two parts. First, when damage is severe, programmed cell death may occur or, alternatively, temporary or permanent cell cycle arrest. This protects against cancer but can have negative effects on the long term, e.g., by depleting stem cell reservoirs. Second, damage can be repaired through one or more of the many sophisticated genome maintenance pathways. However, erroneous DNA repair and incomplete restoration of chromatin after damage is resolved, produce mutations and epimutations, respectively, both of which have been shown to accumulate with age. An increased burden of mutations and/or epimutations in aged tissues increases cancer risk and adversely affects gene transcriptional regulation, leading to progressive decline in organ function. Cellular degeneration and uncontrolled cell proliferation are both major hallmarks of aging. Despite the fact that one seems to exclude the other, they both may be driven by a common mechanism. Here, we review age related changes in the mammalian genome and their possible functional consequences, with special emphasis on genome instability in stem/progenitor cells.

Keywords

Aging; Cancer; DNA damage; Genome instability; Stem cell; Epimutation; Senescence; Apoptosis

1. Introduction

The genome, the repository of our genetic information, is walking a tightrope between stability and change. Too much change drives a species towards extinction, while too little change compromises its evolvability, i.e., the capacity to mutate away from environmental challenges through natural selection. This balance between stability and plasticity is accomplished through a refined system of genome maintenance, which allows – through spontaneous errors arising during imperfect damage processing – the DNA sequence variation that is the substrate of evolution. However, first and foremost, genome maintenance defends nucleic acids against rapid breakdown and is probably our most ancient longevity system. Indeed, without some form of genome maintenance the first

replicating nucleic acids in a world exposed to high fluxes of damaging ultraviolet radiation would not have been able to survive long enough to multiply [1].

Initially, in unicellular organisms, longevity and evolvability had to be balanced in the same cell. The appearance of multicellular organisms led to a separation of germ cells from somatic cells. While germ line genomes must retain some plasticity, somatic genomes could in theory now be equipped with near perfect maintenance systems. However, further improvement of genome maintenance is costly, which is evident from the multitude of proteins involved only in genome maintenance. In some cases an entire protein molecule is sacrificed for the removal of a single lesion [2]. Moreover, in the wild animals sooner or later fall prey to accidents, famine or predation and often do not age beyond the period of first reproduction. Hence, selection for further perfecting somatic genome repair and maintenance systems is limited [3]. This realization led Tom Kirkwood to his disposable soma theory of aging [4]. This theory states that organisms in the wild face a trade-off between investment in somatic maintenance and reproductive effort. This predicts that high extrinsic mortality would favor investment of limited resources in early reproduction rather than somatic maintenance and repair, which would not be required for a population with a low risk of survival in the wild. This of course implies that age-related somatic degeneration and death is caused by the accumulation of unrepaired somatic damage, a reasonable explanation, which is now supported by a large body of evidence. Damage to DNA is often considered as most critical because of its role as the primary template of structural and functional information and the observation of premature aging in mice and humans harboring defects in genome maintenance [5]. Here, we review age-related changes in the mammalian genome and their possible functional consequences, with special emphasis to genome instability in stem/progenitor cells.

2. DNA damage as a driver of aging

In a broad sense, aging of multicellular organisms is the process of progressive loss of adaptive abilities with the passage of time. Aging is always accompanied by functional decline in most tissues and organs, but the exact pattern of these changes varies from individual to individual and depends on genotype and environmental conditions. It is conceivable that in most cases cellular changes are the ultimate cause of the degenerative phenotypes observed in aged organisms. Hence, genome instability could, directly or indirectly, be a primary cause of aging. Here we will focus on the nuclear genome, but it should be noted that also damage to the mitochondrial genome has been the subject of much interest (for a recent review, see [6]).

Instability of the nuclear genome is a broad concept. Distinctions should be made between DNA damage and its consequences – apoptosis, cellular senescence, mutations, and epigenomic alterations. DNA damage is a primary event, caused by both extrinsic and intrinsic factors, and includes chemical alterations in DNA structure, leading to a non-informative template; that is, a structure that can no longer serve as a substrate for faithful replication or transcription. Spontaneous DNA damage in animal cells and tissues is induced with high frequency as a consequence of hydrolysis, oxidation and other physical, chemical and biological processes [7]. However, its steady state level is low due to efficient

mechanisms for repair. While there have been many attempts to quantify spontaneous DNA damage, the potential for artifact has thus far precluded any firm conclusions about a possible age-related increase. What we do know, however, is that under normal conditions the level is extremely low, i.e., not more than one or few lesions per million basepairs [5,8]. There has also been much speculation about the hypothesized decline in DNA repair activities as a possible contributing factor to increased DNA damage during aging. However, DNA repair is very difficult to measure, especially in vivo, and a decline in one particular enzymatic activity with age could simply point towards a shift in utilization of pathways or a reduced need for that activity, for example, because of a decline in cell proliferative activity [5]. One should also distinguish DNA repair activity from accuracy, which makes it even more difficult to interpret the large amount of often conflicting data. Instead of a direct effect of DNA damage, as determined by DNA repair capacity of the tissue or organism, a more likely cause of aging and age-related disease can be found in the consequences of DNA damage processing.

Once inflicted, DNA damage is sensed by check-point control mechanisms triggering a cellular response. Depending on the type and severity of the DNA lesions and the type and physiological state of the cell, this response can be two-fold (Fig. 1). First, the cell initiates a set of DNA repair activities resulting in a restoration of the original chemical structure. This, however, is sometimes accompanied by errors during synthesis of new DNA strands, handling of DNA ends (in case of double-strand breaks) or restoration of proper DNA or histone modification patterns. Second, when damage is severe, the cell may choose to self-eliminate through a process called programmed cell death or apoptosis. Alternatively, in case of mitotically active cells, temporary or permanent cell cycle arrest will prevent mutation accumulation.

For practical reasons most attention has always been focused on the second category of the DNA damage response, since it is much easier to study processes such as apoptosis in cell cultures after relatively high doses of DNA damaging agents than assessing stochastic end points, such as DNA damage and mutations, that can differ from cell to cell. However, how often are cells in vivo exposed to such high doses of DNA damage that massive apoptosis or senescence ensues? The relevance for the in vivo situation of such findings, therefore, is questionable. While under normal conditions cell loss, especially stem cell loss (see below), almost certainly contributes to aging, it is unlikely that normative aging is caused exclusively by loss of cells in response to DNA damage. Since the mutational and epimutational consequences of aging are rarely reviewed we will begin discussing this aspect of genome instability.

3. DNA mutations and epimutations

DNA mutations are heritable changes in genomic DNA sequence, which may be transmitted to daughter cells or to offspring (when they occur in germ cells). Mutations can vary from point mutations, involving single or very few base pairs, to large deletions, insertions, duplications, inversions, and translocations. In organisms with multiple chromosomes, DNA from one chromosome can be joined to another and the actual chromosome number can be affected. Epigenomic alterations (or epimutations) are stable changes in patterns of DNA

modification, such as methylation patterns or the histone code, which can influence patterns of gene expression without altering the sequence of base pairs.

DNA damage at relatively low levels, presumably the norm in wild-type animals, is efficiently repaired. As discussed above, during normative aging the mutations or epimutations that result from the inevitable errors made during the repair of DNA damage or (in dividing cells) during DNA replication are likely to contribute to organ and tissue functional decline and the increased incidence of disease, most notably cancer [5]. Mutations almost always have adverse effects [9] and spontaneous instability of the nuclear genome of somatic cells has been considered as a possible explanation for aging since the 1940s when the biological effects of high-energy radiation were first systematically studied in mice [10]. In the first somatic mutation theories it was reasoned that since radiation was known to induce mutations in DNA, aging could be the result of life-long exposure to low, natural levels of background radiation [11,12]. The main challenge for these theories came from assumptions that errors in the genome were too rare to have phenotypic effects, except by clonal outgrowth, i.e., in cancer [13]. However, as we will see, spontaneous mutation rates are far higher than these early estimates would suggest.

Somatic mutagenesis is difficult to study in higher organisms. Most assays are indirect and based on alterations in phenotypic characteristics, such as the mouse or *Drosophila* spot tests [14,15]. However, recent evidence indicates very high somatic mutation loads under normal conditions. For example, in both mouse and human brain, a significant fraction of cells, including neurons, was found to be aneuploid, with both loss and gain of chromosomes [16]. Results from Martin et al., using the HPRT selectable marker gene in human kidney tubular epithelial cells, indicate mutation frequencies of over 1 per 10,000 loci [17], corresponding to more than 1000 mutations per cell when extrapolated to the genome overall.

In the past, we have generated transgenic mouse models harboring chromosomally integrated lacZ-plasmid constructs that can be recovered in *E. coli* for the subsequent quantification and sequence characterization of a broad range of spontaneous mutations [18]. The results with this system indicate that somatic mutations accumulate in virtually all organs and tissues albeit at different rates [19]. Also, the types of mutations found to accumulate with age proved to be very different among organs. For example, while many mutations in heart and liver were large genome rearrangements, e.g., deletions, inversions or translocations, sometimes involving millions of basepairs, virtually all mutations that had accumulated in the small intestine of old mice were point mutations, i.e., basepair substitutions or very small deletions or insertions [19]. Extrapolation from the size of the transgene locus to the genome overall indicated a load of genome rearrangements in the mouse heart at old age (some deletions are millions of base pairs) of almost 40 per cell [20]. It should be kept in mind that in most selectable systems weak mutations, which could reduce cellular function without abolishing viability, would go undetected. Hence, results obtained with selectable systems are underestimates of the mutation frequency.

Epigenomic alterations are of special concern because once established epigenetic states can drift compared to the more static DNA sequence [21]. Epigenetic changes are increasingly recognized as part of aging and age-related pathology [22]. We as well as others have found

aging to be associated with a general hypomethylation, which is probably a consequence of less faithful maintenance of methylation patterns in repetitive elements [23]. However, hypermethylation, especially of promoter-associated CpG islands has been observed to increase with age in normal colon tissue of patients with colorectal neoplasia [24]. Indeed, increased hypermethylation of tumor suppressor genes in normal, aged tissue likely contributes to the increased cancer risk at old age. The causes of such hypermethylation could be the same as DNA sequence alterations, i.e., errors in restoring normal patterns of methylation after DNA repair or replication [25]. The repair of DNA lesions requires chromatin remodeling to mobilize repair proteins and provide their access to the site of the lesion [26]. The eukaryotic cell transcriptional machinery is organized in higher order structures, or transcription factories [27], where even distant genes together with associated histones form distinct foci in intra-nuclear space. Histones here serve as structural units and also as carriers of epigenetic information that affect and orchestrate gene function. Chromatin disruption due to the temporary removal of histones and DNA methylation challenges epigenomic integrity and may lead to stable chromatin alterations [28]. These changes inevitably affect expression of the structurally bound genes, causing transcriptional deregulation.

It is now clear that the overall mutation/epimutation load of cells in apparently normal tissues is substantial, and increases during aging [5]. The exact types of events, their cell and tissue-specificity and functional impact remain unknown. However, given the magnitude of the random genomic stress now being revealed, it seems unlikely that such (epi)genomic decay has no adverse effect other than increasing cancer risk.

4. Functional consequences of DNA mutations

Random mutations can affect cellular phenotypes by altering protein-coding sequences, but probably much more frequently by affecting gene regulatory patterns [29]. Like mutations, epigenomic alterations can be expected to modify transcriptional activity in a stochastic manner. An increased burden of mutations and/or epimutations in aged tissues could adversely affect regulated gene transcription, for example, through haploinsufficiency after deletions, position effects after translocations or derangements in chromatin looping, which can also result from deletions or from point mutations in nuclear matrix attachment regions. The stochastic nature of these processes implies that aging is associated with increasingly divergent cellular genomes, which can be expected to cause random variation in normal patterns of gene expression (Fig. 2). Indeed, work from our laboratory has shown increased cell-to-cell variation in gene expression levels among cardiomyocytes from old mice as compared to young mice [29]. The same stochastic increase in gene expression variation was observed after treatment of cells in culture with DNA damaging agents [29]. While as yet unclear if increased genome instability is the cause of the increased cell-to-cell variation in gene expression at old age, the role of chance events in aging is increasingly appreciated [30] and increased noise in gene expression has been implicated in reduced organismal fitness [31].

Once randomly accumulating mutations and epimutations reach a level high enough to cause significant cell-to-cell variation in the transcriptome, they may eventually compromise tissue

function. A fortuitous combination of such informational changes in a mitotically capable cell could affect selected functions in growth and extracellular communication. Such a cell will clonally develop into a pre-neoplastic and neoplastic pathological lesion, a hallmark of the aging process. Here we are especially interested in increased mutation loads in stem or progenitor cells as a possible cause of both functional decline and increased cancer risk at old age.

5. Stem cells and the aging-cancer relationship

Analysis of statistical data on the survival of laboratory animals in the absence of the extrinsic causes of death shows that the mortality rate increases exponentially, remaining relatively low until approximately two thirds of the maximal lifespan and then growing dramatically [32,33]. This observation is consistent with the current understanding that while organisms can tolerate stresses well for extended periods of time, the consequences of these stresses are somehow “memorized” and can accumulate, for example, in the form of mutations and epimutations. This load gradually decreases organismal adaptability and eventually reaches the critical point when even small everyday challenges become lethal. This phenomenon is possible because animals are redundant in structure and function. Redundancy is a key notion for understanding the nature of aging [32,34,35]. The positive effect of redundancy is damage tolerance, which maintains vigor and permits continuous reproduction. However, the same tolerance results in abrupt changes late in life when reserve capacity is exhausted and aging manifests as a series of rapid, degenerative changes that eventually bring life to a close. In vertebrate organisms a major feature of redundancy is based on somatic stem cells found virtually in all tissues [36], providing regenerative capacity to replace old, worn-out cells, thereby guaranteeing much longer life spans than seen in post-mitotic invertebrate organisms which lack dividing cells during their adult life. The price of this extended longevity is cancer.

Cellular degeneration and uncontrolled cell proliferation are both major hallmarks of aging. Despite the fact that one seems to exclude the other, sufficient information has now accumulated to implicate common processes behind both aging and cancer [37-41]. Both tissue renewal, lack of which causes degeneration due to natural loss of damaged or worn-out differentiated cells, and tumorigenesis depend on the presence of cells capable of proliferation. Tissue regeneration is maintained by somatic stem cell systems and most human malignancies are associated with tissues known to contain an active stem cell population [42].

Aging and cancer are linked by DNA damage and its erroneous processing by genome maintenance systems. DNA damage in stem cells will not only reduce the number of these cells, i.e., through apoptosis or cellular senescence when damage levels are high, but also lead to the accumulation of mutations and epimutations in surviving cells (through errors in repair and replication). Accumulating mutations and epimutations increase the risk of cancer, but also affect stem cell function in a stochastic manner, i.e., by increasing variation in the transcriptome. Hence, age-related loss of stem cells, age-related stem cell dysfunction and age-related neoplastic transformation of stem cells could all be adverse consequences of the cellular response to DNA damage (Fig. 3).

Several lines of evidence support the hypothesis stated above. First, it has been shown for some tissues that stem cell populations not only deplete with age, but also accumulate mutations and epimutations. Within the nervous system it was demonstrated through both direct identification of stem cells in situ and through in vitro studies, that the neural stem cell population of the brain sub-ventricular zone is reduced by half or more during aging in the mouse [43]. In parallel to the age-related decline in stem cell number neurogenesis in the sub-ventricular zone of mouse brain was also decreased. Analysis of genome integrity of aged neural stem cells revealed a significant age-related accumulation of genome rearrangements in the form of loss of heterozygosity [44], one of the most frequent forms of genome instability in mammalian cells [45]. Neural stem cells in this study were obtained from young and old B6D2F1 (first generation of C57Bl/6 $\times$ DBA/2 hybrids) mice and analyzed by a SNP-based PCR assay to determine the presence or absence of the strain-specific alleles. While all of 15 young neural stem cells showed the presence of both alleles at 9 analyzed loci residing on different chromosomes, 16 out of 17 old neural stem cells demonstrated loss of one or both alleles in one or more chromosomes [44].

In contrast to neural stem cells, there is no appreciable age-related decline neither in concentration nor in absolute numbers of hematopoietic stem cells, at least in some mouse strains [46]. Hematopoietic stem cells in mice can outlive their original aged donors, as demonstrated by repeated serial transplantation in lethally irradiated recipients [47]. What is changing in stem cells and most certainly in their differentiated derivatives, is the quality of the function they provide. Indeed, depending on the mouse strain, there is a limit to serial transplantations and functional decline of hematopoietic stem cells has been documented [48]. It is conceivable that accumulation of genomic/epigenomic alterations is a driving mechanism of the observed functional decline. Accumulation of non-resolved DNA damage in aged hematopoietic stem cells has been demonstrated recently by staining for persistent γ -H2AX positive foci [46]. Evidence has been obtained for chromatin dysregulation during hematopoietic stem cell aging in the mouse [49].

Unlike hematopoietic stem cells, primary skeletal muscle stem cells (the satellite cells) are quiescent in adulthood under normal conditions and become active only after damage to the target tissue [50]. Although there is no significant age-related decline in the number of satellite cells [51], regenerative potential of skeletal muscles is impaired in aged animals [52]. Interestingly, this decline can be overcome by placement of aged muscles in the systemic milieu of the young animals, either as a whole-muscle graft [53,54] or by creating parabiotically paired mice [55]. These results suggest that age-related decline in regenerative potential of skeletal muscles is reversible and a result of the aged environment. However, accumulation of age-related alterations in the satellite cells cannot be ruled out as evidenced by changes in expression patterns of human myoblasts of individuals with different ages [56].

As mentioned, senescence is a major outcome of the DNA damage response. The cyclin-dependent kinase inhibitor p16^{Ink4a} is involved in senescence [57] and its expression is markedly up-regulated with age in many tissues [38]. Increased levels of the p16^{Ink4a} were found in aged hematopoietic stem cells [58], neural stem cells [59] and in the pancreatic islets of old mice [60]. Mice lacking p16^{Ink4a} demonstrated higher regenerative capacity

than wild-type animals at older age, as evidenced by increased numbers of hematopoietic and neural stem cells and a more pronounced ability to recover after ablation of insulin producing β -cells. However, mice deficient in p16^{Ink4a} also demonstrated an increased incidence of spontaneous and carcinogen-induced cancers [61]. Hence, the biological sense of p16^{Ink4a} up-regulation in aged stem cells is evident – to restrain potentially tumorigenic cells from further propagation.

A reduction in stem cell numbers during aging can also account for the observed relationship between cancer incidence and age. With a very few exceptions, there is an exponential increase in the incidence of most cancers with a peak at approximately 80 years of age. Beyond this point, a large majority of all cancers show a decline in incidence [62]. If the accumulation of mutations in stem cells were the only contributing factor to cancer incidence, no decline would be expected. However, DNA damage leads to not only accumulation of mutations, but also a decrease in the number of stem cells. The reduced number of stem cells limits the probability of generating the right set of random mutations turning a stem cell into a cancer cell.

Finally, mouse models exhibiting premature aging phenotypes provide some indirect evidence of stem cell involvement as a major target in both aging and cancer. An example of such a model is the p53^{+/-m} mouse generated in the Donehower laboratory [63]. This mutant strain has a constitutively elevated p53 activity and undergoes premature aging. The p53 protein is a typical gatekeeper [64] and in response to a stress signal may induce cell cycle arrest or apoptosis [65], preventing cells from tumorigenic transformation. The p53^{+/-m} mice demonstrated high resistance to spontaneous tumors, but, despite that, had a reduced longevity and early onset of aging symptoms. At least in part this phenotype can be attributed to the enhanced function of p53 in stem cells. Overactive p53 switches the balance from DNA damage repair to apoptosis or cellular senescence, thereby greatly limiting the chance of mutations. Rapid attrition of stem cell populations leads to the exhaustion of self-renewal mechanisms and, consequently, to premature aging. The same depletion decreases the probability of acquiring stem cells with mutations essential for tumor transformation. Thus, the p53 protein has both an anti-tumor and pro-aging function, and may serve as an example of antagonistic pleiotropy [37]. Loss of p53 function may, in theory, postpone the onset of age-related degeneration, but the increased frequency of tumorigenesis then dramatically shortens lifespan [66]. It is conceivable that there is a dynamic balance between quantity and quality of stem cells in vertebrates (Fig. 4) ensuring a natural lifespan free of cancer and aging long enough for maturation and first reproduction.

An extreme example of how acute loss of somatic stem cells may lead to aging-like phenotypic changes is provided by work of Ruzankina et al. [67]. This study showed that conditional deletion of the *ATR* gene in adult animals leads to the rapid (within 6 months) onset of premature aging. This phenotype correlated with loss of cells in self-renewable tissues and depletion of the thymic progenitor cells and follicle bulge stem cells. The check-point kinase ATR is one of the major regulators of the DNA-damage response and involved in control of cell cycle progression and DNA replication [68]. Since ATR is essential for the viability of replicating cells [69,70] and complete disruption of this gene leads to early embryonic lethality [71,72], the deletion of ATR caused acute loss of stem/progenitor cells,

most likely due to cell cycle arrest. The attrition of stem cell populations may lead to the loss of tissue homeostasis and degeneration, mimicking age-related changes. Of note, no tumors were observed in any mice with a deleted ATR gene. This observation confirms the notion that tumors are originating from stem/progenitor cells.

In summary, aging is associated with either direct loss of stem cells or a loss of their ability to proliferate by entering the senescent state. The stem cells, which are still active in old age, are functionally impaired. The age-related functional decline could well be due to a significant load of mutation/epimutation, inevitable errors of genome maintenance that are irreversible and accumulate throughout lifespan. In some circumstances a fortuitous combination of mutations will drive the mitotically highly capable stem cell into tumorigenesis. Tighter control of the DNA damage response leads to attrition of stem cell populations and a virtually cancer-free life, but at the cost of an earlier onset of non-cancer, degenerative aging symptoms presumably due to exhaustion of regenerative capacity. Vice versa, less tight control, allowing stem cells to survive high loads of DNA damage, shortens organismal lifespan due to cancer.

6. Testing the theory

To test for the possibility that aging is caused by a gradual loss of genome integrity it is not enough to demonstrate an increasing load of genome alterations in aging cells and tissues and correlate that with aging-related functional loss and disease. Further exploration of increased genome instability in various tissues of mice or humans will contribute useful new data sets and provide further insight into how increased mutation loads could be causally related to aging, but will not allow any definite conclusions regarding a causal relationship. While the study of human and mouse mutants displaying premature aging symptoms is important for understanding the relationship between genome maintenance defects and their physiological consequences, it is unlikely that this approach will provide definitive evidence that genomic instability is a key mechanism of aging. In all of these models only a subset of aging phenotypes occur in an accelerated manner and none show a concerted increase in aging symptoms at the correct level of severity as a function of their life span [40].

The strategy of modeling genome maintenance defects in the mouse to test the hypothesis that aging is due to genomic instability has at least two inherent limitations. First, manipulating single genes almost always leads to unwanted side effects due to the multiple roles most genes play in the physiology of an organism. Second, due to the several hundred genes directly involved only in DNA repair it is not realistic to assume that one single gene change is sufficient to create an accelerated copy of the normal aging process. One-way to overcome these limitations is to generate a mouse model allowing a controlled gradual increase in genome instability in different organs and tissues at different ages. To accomplish this we are in the process of generating a genetic system capable of introducing controlled amounts of DNA double-strand breaks into the genome. This system is based on inducible expression of restriction endonucleases, which cleaves the genomic DNA virtually randomly, possibly mimicking processes ongoing during normal aging. Unlike existing models of genome instability, which are based on DNA repair defects, this approach will allow the creation of etiological cell and animal models of aging.

From the above discussion and other researchers' conclusions [50,73] it is clear that somatic stem cells may play an important, if not key, role in onset and progression of aging and age-related disorders. This makes a somatic stem cell pool a perfect target for interventions aiming to address fundamental questions of biology in aging and/or to affect the aging process per se. For example, the controlled DNA damage inducible system mentioned above can be targeted exclusively to stem cells. This highly specific model for a proximate molecular cause of aging will not only give us a major tool for unraveling the mechanistic basis of aging, but will also provide us with the means to test a host of novel stem cell specific interventions in a short-term, highly specific, and predictive pre-clinical model for aging-related ailments and functional decline.

7. Conclusions

Aging of complex multicellular organisms is a two-stage struggle toward off death. Due to a full repertoire of stem cells, the first stage is benign resulting in progressive loss of redundancy but limited functional decline or cancer. Organisms clearly have the ability to withstand, to some extent, the relentless infliction of DNA damage and can maintain viability for prolonged periods of time, sacrificing redundancy for extended longevity. Eventually, however, once redundancy is exhausted, the adverse effects of the DNA damage response hit the organism in full force. Paraphrasing Friedrich Engels [74], life merely provides DNA with the means to exist. The only function of everything beyond this level, from proteins to the whole organism, is to preserve integrity and ensure propagation of the DNA of the genome. Hence, it is conceivable that DNA damage is at the very beginning in the chain of events leading to aging and eventually death. In organisms with renewable tissues, a plausible scenario is that mutations/epimutations accumulate in stem cells over time thereby exhausting regenerative capacity.

DNA damage and its consequences in stem cells, unlike in differentiated cells, do not affect fitness immediately, since stem cells are not involved in carrying out functions specific for target tissues. The biological role of the stem cell system is to maintain the cellular redundancy of tissues and organs by replacement of lost or damaged differentiated cells. In the early postnatal period and until puberty this system is excessive and ensures organismal growth and maturation; later on, the loss of differentiated cells is balanced by cell replacements. Eventually the loss of differentiated cells can no longer be properly compensated. Although there is some evidence that total deterioration of stem cell populations leads to premature aging [67], stem cells are normally never lost completely. More likely, mutations/epimutations accumulated in the stem cells as a result of genome maintenance errors are transmitted to daughter cells that become the newly differentiated cells and this impacts tissue functionality by adversely affecting the transcriptome as discussed above. The increasing load of mutations/epimutations in the proliferation-capable stem cells increases the risk of neoplastic transformation, the price for extended longevity.

Thus, the cellular basis of the aging process is an increasing functional divergence of differentiated cells due to stochastic changes in their genome and epigenome caused by DNA damage. This growing cell-to-cell variability eventually leads to a decline in tissue functionality which may not be pronounced at early stages, but becomes evident after a

functional challenge. The stem cell system was adopted by complex metazoans to postpone the onset of aging, but is the subject of age-related changes as well. In the short story “Protection” by Robert Sheckley [75] the main hero acquires a ghost-like friend who warns him about oncoming dangers. Unfortunately, avoidance of one danger opened the path to others, not ever seen before. Similarly, acquiring regenerative systems increased the lifespan of vertebrates, but opened the path to cancer.

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Fig. 1.

Of the two major branches of genome maintenance, DNA repair aims to restore the original situation by removing the lesion, while the complex of DNA-damage signaling pathways assists in these repair activities or initiates cellular responses that kill or terminate mitotic activity of a cell when it is beyond repair.

Fig. 2.

Age-related, cell functional divergence as a consequence of stochastic effects. Functional decline is indicated by increasing darkness of shading. Even in a young tissue, the function of highly differentiated cells in an organ or tissue is never maximized. The old tissue is different in the sense that there are many more cells that have suffered functional decline, with some of them dying (†) or eliminated (open space). Others have grown into a hyperplastic or neoplastic lesions (hatched) or have been replaced by fibrosis (not shown).

Fig. 3.

Hypothetical relationship between aging, cancer and stem cells (SC). Various endogenous and exogenous factors (e.g., ROS, replication errors, environmental hazards) cause DNA damage, also in stem cells. Among the cellular responses to DNA damage, apoptosis and senescence lead to attrition of stem cell populations, while DNA repair may lead to errors increasing both cancer risk and adversely affect function. Hence, cancer and non-cancer, degenerative dysfunction can both be consequences of stem cells' responses to DNA damage.

Fig. 4.

The optimal balance between maintaining the number of stem cells (“quantity”) and eliminating severely damaged stem cells (“quality”) ensures maximal longevity free of cancer and degenerative changes. Shifts towards one or the other extreme will impair longevity by development of degenerative dysfunction due to exhaustion of regenerative capacity or cancer due to accumulation of proliferation competent cells with significant mutation/epimutation load.