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Impact of genomic damage and ageing on stem cell function

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Abstract

Impairment of stem cell function contributes to the progressive deterioration of tissue maintenance and repair with ageing. Evidence is mounting that age-dependent accumulation of DNA damage in both stem cells and cells that comprise the stem cell microenvironment are partly responsible for stem cell dysfunction with ageing. Here, we review the impact of the various types of DNA damage that accumulate with ageing on stem cell functionality, as well as the development of cancer. We discuss DNA-damage-induced cell intrinsic and extrinsic alterations that influence these processes, and review recent advances in understanding systemic adjustments to DNA damage and how they affect stem cells.

Even some of the most primitive forms of metazoan life rely on the regenerative capacities of stem cells. In higher animals, multiple tissues require a tissue-specific stem and progenitor cell pool for active replenishment during the lifespan of the organism. Stem cells have the unique capacity of long-term self-renewal, but this capacity also carries an intrinsic challenge: as stem cells are the most long-lived cells of the organism, the risk of acquiring genomic damage is increased. Several factors can contribute to the accumulation of DNA damage in stem cells of the adult organism, including telomere shortening, DNA replication

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stress and the failure of repair systems. Further, there is emerging evidence that aneuploidy contributes to the accumulation of genome instability in lineage-primed progenitor cells during ageing[1,2].

Mechanisms of DNA damage induction have already been reviewed in many publications (see, for example, the recent review by Zeman and Cimprich[3] on DNA replication stress). Our review focuses instead on the recent advances in the understanding of the outcome of genome instability in stem cells. There are two distinct consequences of DNA damage on the fate of cells. First, when DNA damage alters gene function through mutations or chromosomal rearrangements, the result can be aberrations in gene expression and activity, such as the dysregulation of genes that control stem cell differentiation and self-renewal, the inactivation of tumour suppressors or the activation of oncogenes[4,5]. Such changes can lead to cancerous growth, and tumorigenic alterations in stem cells can be particularly dangerous given the high inherent regenerative potential of these cells. To prevent such alterations, DNA damage checkpoints evolved as *bona fide* tumour suppressor mechanisms to limit the growth of damaged cells by inducing cell cycle arrest, cellular senescence or apoptosis[6]. As a side effect, the DNA damage response could compromise stem cell function and tissue renewal during ageing. DNA damage accumulation throughout life might underlie the declining regenerative potential of tissues and organs with ageing. Interestingly, the maintenance of stem cells does not rely solely on DNA damage responses that are cell autonomous. Recent evidence suggests that systemic adjustments to DNA damage could alter the regeneration of stem cell pools and influence clonal selection of subpopulations of stem cells with distinct functions[7,8]. As knowledge about the organismal consequences of DNA damage is only starting to emerge, we will provide an outlook on what to expect from integrated and organismal studies of responses to genome instability.

Consequences of DNA damage checkpoint activation in stem cells

Cellular DNA damage checkpoints determine the fate of cells that carry genomic damage (Fig. 1). DNA lesions trigger activation of signalling pathways, in particular of the protein kinase ATM (ataxia telangiectasia mutated) and the related kinase ATR (ataxia telangiectasia and Rad3-related), which mediates a cascade of post-translational modifications to chromatin and to proteins recruited to damaged DNA[9]. Stem cells that are deficient in either of these kinases are dysfunctional and are frequently exhausted prematurely, resulting in early ageing phenotypes[10–14]. The outputs of DNA damage checkpoint activation include cell cycle arrest, apoptosis and senescence — decisions that ATM and ATR coordinate with repair. Although ATM activation is central to the double-strand break response[15], and ATR activation responds primarily to replication stress and exposure of single-stranded DNA[16], in some cases the kinases cooperate, either in series or in parallel[17–20]. In addition to these classical checkpoint responses, there is emerging evidence that DNA-damage-induced differentiation eliminates damaged stem cells by inhibiting self-renewal and by pushing the damaged stem cells into the short-lived progenitor cell compartment[8,11].

The decision whether to arrest the cell cycle temporarily, to allow time to repair the damage, or to undergo apoptosis or differentiation to remove the damaged stem cell from the

organism, depends not only on the type of damage encountered but also on the cell type and the developmental context. In addition, species differences may exist. Murine adult haematopoietic stem cells (HSCs) respond to low-level irradiation by initiating repair and remaining quiescent, although this reduces their long-term repopulating ability and may increase the risk of tumorigenesis resulting from gross chromosomal rearrangements[21]. A recent study reported that although irradiation of adult murine HSCs induced symmetric divisions to increase the stem cell capacity in the short term, long-term self-renewal of the HSCs was reduced after ionizing radiation[22]. Quiescent human umbilical cord blood cells, in contrast, tend to undergo p53-dependent apoptosis in response to similar doses of ionizing radiation[23]. Stem cell characteristics in response to DNA damage seem to also be important for cancer therapies. Cancer stem cells represent a subpopulation of cells in a tumour that are more resistant to DNA damaging agents than the bulk of the other tumour cells. In the context of chronic myelocytic leukaemia, the population of quiescent leukaemia-initiating cells is resistant to chemotherapy and must be forced into cycling to undergo apoptosis, for example by deletion of the c-Myc-destabilizing ubiquitin ligase component Fbxw7 (ref. 24).

Some stem cell populations, such as HSCs, mainly reside in a non-cycling state under homeostatic conditions. It is thought that the quiescent state protects stem cells from the harmful effects of elevated metabolic activity during the active phases of the cell cycle and from mutational hazards that can occur during DNA replication[25]. The importance of quiescence for HSC maintenance is seen in serial transplantation experiments, where HSCs completely exhaust after five to six rounds of transplantation. It was shown that proliferative stress leads to an accumulation of oxidative stress in transplanted HSCs, which restricts self-renewal capacity[26]. Other stem cells, such as $LGR5^+$ (leucine-rich repeat-containing G-protein coupled receptor 5) stem cells of the intestinal epithelium, proliferate at a high rate. Quiescent and highly cycling stem cells seem to employ different pathways to repair DNA damage. Whereas actively cycling $LGR5^+$ intestinal stem cells are able to use the highly accurate homologous recombination pathway[27], this pathway cannot function in quiescent HSCs, as the homologous DNA sequence only becomes available during S phase of the cell cycle. Quiescent stem cells, such as HSCs and hair follicle bulge stem cells, instead rely on non-homologous end joining (NHEJ) to rapidly join the DNA ends[21,28] — a process prone to error because of local end resection. Thus, although quiescence protects against replication-induced damage, it may indirectly lead to deletions or translocations arising from error-prone repair.

Telomere damage and aneuploidy in stem cell ageing

Excessively short, uncapped or dysfunctional telomeres are recognized as DNA damage by the checkpoint and repair machinery, and may result in loss or translocation of genetic material (Fig. 1). Stem cells in adult tissues exhibit some level of telomerase activity but still show significant shortening of telomeres during ageing[29]. Heterozygous mutations in telomere binding proteins — for example, TINF2 (TERF1-interacting nuclear factor 2) or POT1 (protection of telomeres 1) — lead to premature defects in tissue maintenance and increased rates of cancer in humans[30,31]. These defects predominantly affect the haematopoietic system, indicating that, in humans, HSCs are most sensitive to telomere capping defects caused by mutations in telomere-binding proteins. Recent work in mouse

embryonic stem cells revealed that short telomeres lead to unstable differentiation[32], a phenotype that could contribute to ageing-associated defects in tissue maintenance if similar perturbations occur in adult stem cells. Supporting this idea, telomere shortening in adult intestinal stem cells provokes marked increases in genome instability and defective differentiation in mice lacking the tumour suppressor p53 (ref. 33).

In addition to telomere-related damage at chromosome ends, increases in losses and gains of chromosomes and chromosomal regions also contribute to the accumulation of genome instability with age[1,34] (Fig. 1). Although the underlying mechanisms remain largely unexplored, one contributing factor may be age-related telomere shortening[33] as well as decreases in the expression of BUBR1 (also known as BUB1B; BUB1 mitotic checkpoint serine/threonine kinase B), a core component of a mitotic checkpoint that ensures proper attachment of duplicated chromosomes to the mitotic spindle before anaphase onset[35]. Mutant mice in which the decline of BubR1 is accelerated develop premature ageing phenotypes owing to early senescence of certain progenitor cell populations and loss of regenerative potential[2]. Furthermore, transgenic mice with sustained high levels of BubR1 throughout life are less susceptible to age-related aneuploidization and show a marked extension in healthy lifespan[1]. A recent study showing that induction of aneuploidy in neural stem cells leads to microcephaly underscores the importance of chromosomal integrity for tissue development and maintenance[36]. However, the analysis of the role of euploidy-controlling genes in ageing clearly needs to be extended to other aneuploidy models that have so far mostly been used for short-term cancer studies in early life[37].

Alterations of the stem cell environment with ageing

How age-related accumulation of DNA damage affects the functionality of stem cells in tissue maintenance has primarily been studied from a stem-cell-intrinsic perspective. However, evidence is mounting that changes in the stem cell microenvironment (or stem cell niche) and in the systemic circulatory environment also contribute to the ageing-associated decline in stem cell function (Fig. 2). Importantly, studies using telomere-dysfunctional mice show that genotoxic stress provokes cell-extrinsic alterations that impair HSC function, and ageing-associated defects in HSC differentiation, characterized by profound suppression of lymphopoiesis[38].

Given their apparent role in the ageing-related stem cell decline, it is of interest to investigate such stem-cell-extrinsic mechanisms of ageing in greater detail (Fig. 2). One such mechanism of muscle stem cell deterioration involves the Delta-like 1 (DLL1)-ligand–Notch-receptor signalling pathway. Studies on mouse models provide compelling evidence that skeletal muscle ageing is characterized by defects in DLL1-mediated activation of quiescent satellite cells following injury, resulting in impaired tissue repair[39–41]. These defects were rescued by exposure to a young blood circulatory environment (see below). In addition, Wnt signalling pathways play a key role in regulating stem cell fate and self-renewal in different organ compartments and cancer (reviewed in ref. 42). Studies on skeletal muscle ageing revealed that ageing influences muscle stem cell function by affecting Wnt signalling activity. Specifically, studies in mice provided experimental evidence for hyperactivation of canonical Wnt signalling in ageing skeletal muscle, which

perturbs tissue repair and homeostasis through altering stem cell fate, resulting in increased fibrosis[43,44]. The study also showed that ageing-associated alterations in the blood serum contribute to increases in canonical Wnt signalling activity[44]. In contrast to the negative effects of the canonical Wnt signalling pathway, non-canonical Wnt signalling mediated by Wnt7A was reported to enhance muscle stem cell self-renewal and muscle fibre regeneration[45,46]. Interestingly, Wnt7A activation ameliorated muscular dystrophy[47], but whether it can reverse ageing-associated impairments in muscle regeneration remains to be elucidated. Niche-mediated mechanisms were also found to impair muscle regenerative potential during ageing (Fig. 2). FGF2 expression is chronically elevated in old muscles, especially in the stem cell niche[48]. Increased FGF2 signalling in the aged muscle impairs Sprouty1-mediated maintenance of stem cell quiescence[49], thus diminishing the pool of functional satellite cells with age.

Parabiosis experiments — a surgical technique in which two animals are joined to establish a common circulatory system[40] — provided a proof of concept that alterations in the cell-extrinsic system contribute to impairments in stem cell function during ageing. In experiments where young and old mice were joined, cell-extrinsic factors from the young mice were found to restore neural and muscle stem cell function in the old mice[40,50]. As well as underscoring the critical importance of the circulatory environment in age-related stem cell malfunction, these experiments provided a proof of concept that it is possible to rejuvenate aged stem cells and the capacity of tissue regeneration. Identification of the key cell-extrinsic factors involved could provide molecular entry points for the development of therapies aimed at improving human health and lifespan[51].

Epigenetic modifications in response to DNA damage lead to the induction of $p16^{Ink4a}$, a key marker of cellular senescence (Fig. 2). An age-related increase in $p16^{Ink4a}$ expression has been observed in various tissue compartments of ageing mice[52]. Deletion of $p16^{Ink4a}$ increases the regenerative capacity of haematopoietic and neuronal stem cells as well as that of pancreatic islets during ageing[53–55]. Thus, senescence of stem cells and differentiated cells may represent an important cell-intrinsic mechanism driving age-related loss of tissue regenerative potential. Furthermore, studies designed to dissect the mechanism of precocious skeletal muscle degeneration in BubR1 progeroid mice uncovered that senescent cells may also limit tissue regeneration by altering the stem-cell niche through the factors they secrete, including pro-inflammatory cytokines and proteases that disrupt tissue architecture, commonly referred to as the senescence-associated secretory phenotype (SASP)[56] (see below: ‘Systemic adjustments to genomic damage’). The key observation supporting this concept was that clearance of senescent cells from these mice attenuated skeletal muscle degeneration[57].

The role of the immune system in senescent cell clearance and tissue ageing is complex and remains to be defined. Senescent cells in tumours and pre-malignant lesions are efficiently cleared by the immune system[58,59], but they accumulate in multiple tissues during ageing[60]. Whether an ageing-associated decline in immune function contributes to this process remains to be investigated. It is possible that ageing-associated impairment in the lymphoid differentiation potential of HSCs as well as the upregulation of senescent checkpoints in

peripheral lymphocytes leads to reductions in the clearance of senescent cells from ageing tissues[61,62].

Systemic adjustments to genomic damage

Beyond the tissue microenvironment, the systemic adjustments of the ageing organism are increasingly being implicated in determining the regenerative capacities of the stem cell compartments (Fig. 1). Whereas young organisms grow and thrive, the endocrine growth environment declines with ageing. However, as ageing cells accumulate DNA damage, the risk for mutation-driven tumorigenesis steadily increases. It is thought that the endocrine shift antagonizes the increased cancer risk by depriving cancer cells of growth factors. The somatic growth (or somatotropic) axis is controlled by growth hormone (GH) and insulin-like growth factor 1 (IGF-1) signalling[63]. GH is secreted from the pituitary and, through GH receptor (GHR) activation, stimulates IGF-1 production in target tissues. IGF-1 in turn regulates cellular growth and survival signalling through IGF-1 receptor (IGF-1R) signalling. In young animals, the somatic growth axis is required for body growth, but GH–IGF-1 levels decline with ageing[63]. Moderate reduction of IGF-1 activity is associated with elevated general stress resistance, prolonged tissue maintenance and extension of lifespan in organisms ranging from nematode worms to mice, whereas high IGF-1 activity can support cancerous growth in mice[64–66]. Intriguingly, mice that age prematurely as a result of mutations in nucleotide excision repair (NER) genes that are defective in the human progeroid (premature ageing-like) Cockayne syndrome and XPF-ERCC1 (XFE) progeria show low expression levels of somatotropic genes in various tissues and reduced IGF-1 levels in their circulation[67,68]. It is likely that the attenuation of the somatotropic axes accounts for the reduced body growth and apparent cancer protection of those types of progeroid mice[69]. Mechanistically, persistence of DNA lesions that lead to blockage of transcription (in Cockayne syndrome and XFE) results in cellular attenuation of the somatotropic mediators GHR and IGF-1R (ref. 70). Somatotropic attenuation in response to unrepaired DNA damage might thus counteract the detrimental consequences of genome instability by enhancing tissue maintenance and systemically reducing proliferation of potentially damaged cells. However, reconstitution of IGF-1 in mouse models for Hutchinson-Gilford Progeria syndrome (HGPS) rescued the severe postnatal growth retardation and thus prolonged the short lifespan of the mice[71]. These findings suggest that although there are detrimental consequences during early life when the animals need to grow and thrive, attenuation of the GH–IGF1-mediated growth axis in response to DNA damage might promote tissue maintenance and suppress cancer development amid accumulating genome instability with ageing (Fig. 1). The extension of tissue functionality by reduced IGF-1 signalling might predominantly benefit differentiated cell types such as the post-mitotic somatic tissues of nematode worms[64] and neurons in mammals[72]. In contrast, studies in mice with increased genome instability owing to a mutation in the Sirtuin gene *Sirt6* suggest that systemically reduced somatotropic signalling negatively impacts regenerative capacity. Full-body *Sirt6* knockout animals show similar features as the above-mentioned NER-deficient progeroid mice, including reduced somatotropic activity and growth, cachexia, kyphosis and leukopenia. Strikingly, *Sirt6* HSCs were proficient in reconstituting lethally irradiated wild-type recipients, indicating that the bone marrow

defects in *Sirt6* knockout animals were non-cell-autonomous[73]. This is reminiscent of the defects in HSC differentiation in telomere-dysfunctional mice that were rescued by HSC transplantation into wild-type mice (see above)[38]. Although it remains unclear if suppression of IGF-1 also contributes to the latter phenotype, these observations suggest that systemic adjustments that arise from persistent DNA damage reduce the growth-supporting environment and result in declining stem-cell-mediated regeneration. Re-establishing a growth-permissive environment might therefore provide the therapeutic means of reconstituting regenerative capacities with ageing. However, it will be important to counterweigh such approaches to avoid the cancer-promoting consequences of growth-factor signalling. It will be of pivotal interest to better understand how adjustments of the endocrine growth environment during ageing affect the balance between maintenance of differentiated cell types and the regenerative capacities of their stem cell niches.

Cellular DNA damage signalling, including activation of the tumour suppressor p53, triggers both innate and adaptive immune responses[58,59] (Fig. 1). Cells that have entered senescence as a consequence of DNA damage acquire a SASP, including secretion of pro-inflammatory cytokines and chemokines that might contribute to the sterile inflammation observed in a variety of tissues[74] and the circulatory environment[75] in response to ageing. The functional consequences of these inflammatory changes have yet to be explored in animal models of ageing. However, there is evidence that the increased secretion of pro-inflammatory signals from cells that carry DNA damage could have multiple effects on tissue maintenance and cancer formation by: impairing the functionality of stem cells in ageing tissues (see above and refs 7,38,76); promoting the immunologic clearance of senescent tumour cells and premalignant cells[58,59]; influencing tissue remodelling in response to injury[77]; and stimulating the growth of cancerous cells[78].

The systemic consequences of innate immune responses to genome instability in regenerative tissues have recently been demonstrated in the *Caenorhabditis elegans* system. In adult *C. elegans,* the only stem cell niche is in the germ line, which contains mitotically active germ cells that differentiate through meiosis into gametes, whereas somatic tissues are post-mitotic. DNA damage specifically in the germ line was shown to trigger an ancestral innate immune response that evokes stress resistance throughout somatic tissues by activating the ubiquitin proteasome system (UPS)[79]. It was suggested that the elevated somatic endurance extends the reproductive lifespan to allow germ cells to restore genome stability before resuming offspring generation[80]. It will be interesting to explore whether the innate immune responses to DNA damage in mammalian stem cells can impact the endurance of differentiated tissues.

Clonal drifts in the stem cell pool and selection of aberrant clones

Ageing of the stem cell compartment is not always associated with a decrease in stem cell number (Fig. 3). In fact, the number of immunophenotypically defined stem cells increases during ageing in the haematopoietic system both in mice and humans[81]. However, the functionality of stem cells on a per-cell basis decreases during ageing[82–84]. Furthermore, in the haematopoietic system the clonal composition of stem cells can change during ageing[85].

Current data indicate that ageing-associated clonal drifts in the HSC compartment are induced by both cell-intrinsic and cell-extrinsic processes[7,83].

In the haematopoietic system, ageing is characterized by a decrease in lymphopoiesis and an increase in myelopoiesis, and clonal drifts in the composition of HSCs seem to contribute to these alterations[84,85]. The pool of HSCs consists of different subpopulations, including lymphoid-biased HSCs and myeloid-biased HSCs. During ageing, the population of lymphoid-biased HSCs decreases while the population of myeloid-biased cells is maintained, although the latter population exhibits reduced functionality on a per-cell basis[84]. The drifts in the clonal composition of HSCs are thought to contribute to the decline of immune function and to the increased risk of myeloid leukaemia with age[61]. There is emerging evidence that drifts in the clonal composition of stem cells occur also in other organ systems, such as skeletal muscle[86]. It is tempting to speculate that these drifts in clonality at the stem cell level lead to changes in the composition and function of various tissues. However, studies on intestinal stem cells (ISCs) have shown that clonal drifts in stem cell compartments can also be neutral[87,88]. In addition, these studies revealed evidence that oncogenic mutations can lead to clonal selection of ISCs (ref. 89). The clonal advantage of mutant ISCs can be condition-dependent; for example, the selection of p53-deficient ISCs over wild-type ISCs was found to be dependent on the context of chronic inflammation[90]. Studies on human colon crypts revealed an increase in chromosomal imbalances with increasing age, suggesting that clonal selection may favour mutant stem cells during ageing[34].

Molecular mechanisms that lead to the evolution of clonal drifts in ageing stem cell compartments remain to be defined. Interestingly, it was shown that the accumulation of DNA damage leads to the evolution of clonal drifts in HSCs by inducing a BATF (basic leucine zipper transcription factor, ATF-like)-dependent differentiation checkpoint that results in preferential depletion of lymphoid-biased HSCs (ref. 8). It is possible that DNA damage, which occurs in HSCs during physiological ageing in humans[91], could also contribute to ageing-associated drifts in clonality in human HSCs. In agreement with this idea, patients with myelodysplastic syndrome (MDS) — an ageing-associated bone marrow failure syndrome characterized by increased myelopoiesis and leukaemia risk — display telomere shortening and BATF induction in $CD34^+$ HSCs (ref. 8).

Clonal evolutions in stem cells may not only contribute to tissue ageing but also to the development of cancer (Fig. 3). Work on HSCs revealed that leukaemia-initiating cells (LICs) show an increase in clonal selection and an elevated malignant potential when the proliferative competition of non-transformed stem and progenitor cells declines[92,93]. These mechanisms could be highly relevant for the age-dependent increase in malignancies originating from stem and progenitor cells. A series of recent studies identified an age-dependent accumulation of mutations in human HSCs (refs 94,95). Interestingly, these mutations occur frequently in human leukaemia[94,96]. These data suggest that mutations at the stem cell level can occur before the development of disease symptoms or full-blown leukaemia. Many of these ageing-associated, pre-leukaemic mutations in HSCs affect genes involved in the control of the epigenome, supporting the concept that ageing selects for alterations at the epigenetic level that lead to clonal expansion of aberrant stem cells (Fig. 2),

thus setting the stage for the evolution of malignancies. Mechanisms that enhance clonal selection of aberrant stem cells in ageing tissues remain to be delineated. In addition to cell-intrinsic processes and loss of proliferative competition, it is possible that alterations in the systemic blood circulatory environment contribute to this process. In support of this assumption, the accumulation of DNA damage in ageing telomere-dysfunctional mice leads to premature evolution of clonal drifts of HSCs. Interestingly, these changes were associated with a loss of HSC quiescence[76], and loss of quiescence was shown to impair the function of HSCs by inducing alterations in the DNA methylation landscape[97] (Fig. 2).

Outlook

A variety of mechanisms contribute to the accumulation of genomic damage in ageing stem cells. The relative contribution of these sources of genome instability to human stem cell ageing remains to be delineated. The downstream signals responding to genomic damage determine the consequences for stem cell functionality, but remain incompletely understood. These signals function primarily to protect ageing tissues from cancer formation. However, the same pathways can promote tissue dysfunction and selection of malignant clones during ageing, involving both cell-intrinsic and cell-extrinsic alterations that are activated in response to genome damage. It will be of tremendous interest whether interference with the molecular DNA damage response systems could improve tissue function during ageing and slow the age-dependent increase in cancer. In principle, such approaches would aim to impair age-dependent accumulation of genomic damage and/or alleviate toxic ageing-promoting responses to such genomic insults. Experimental evidence indicates that both approaches work in mouse models of ageing[1,98,99]. Future research will determine if these approaches can be translated to increase human health-span during ageing.

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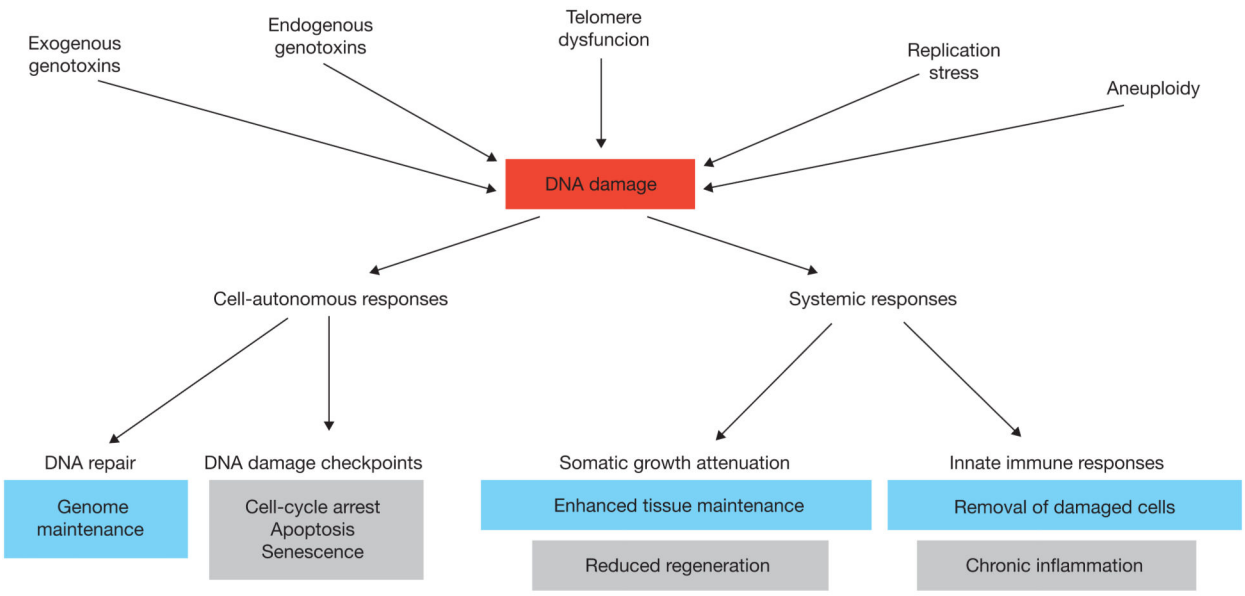

Figure 1.

Cell-autonomous and systemic responses to DNA damage. Various sources of genotoxic stress induce DNA damage that can be removed by specialized DNA repair systems. Cell-autonomous DNA damage checkpoints halt the cell cycle to allow time for repair or, amid severe genome damage, trigger programmed cell death or cellular senescence. Although DNA damage checkpoint mechanisms protect against cancer, the associated removal of cells can contribute to ageing through declining regenerative stem cell pools (grey). Systemic DNA damage responses include attenuation of the somatic growth axis and triggering of innate immune responses, which might support longevity assurance (blue) by enhancing maintenance of tissue functionality and removal of damaged cells, but also contribute to ageing (grey) by damaging tissues and impairing regeneration.

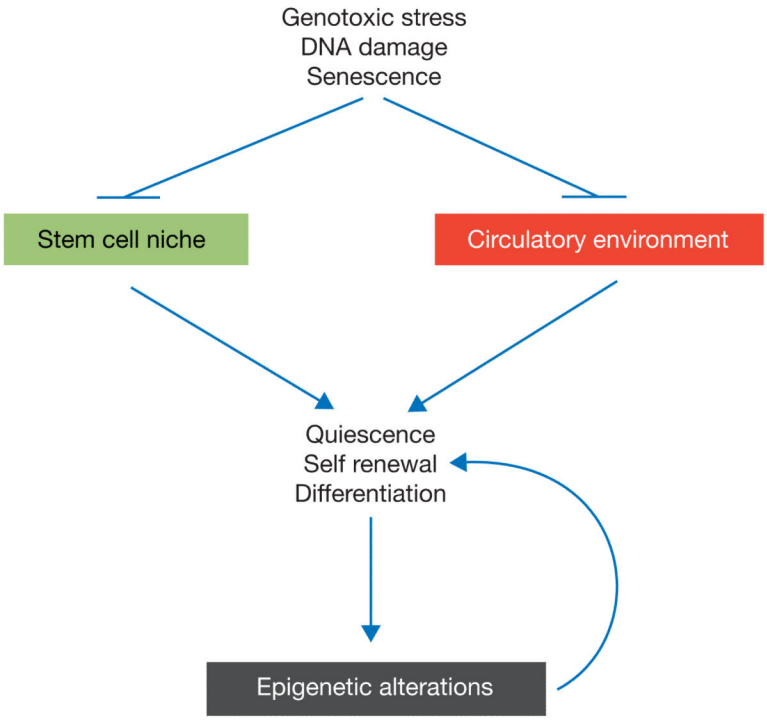

Figure 2.

Impact of DNA damage on the stem cell environment. The accumulation of DNA damage and senescent cells during ageing leads to alterations in the stem cell niche and the systemic circulatory environment. Both processes can interfere with signalling pathways (such as Notch, Wnt and Sprouty1) that are required for the maintenance of stem cell quiescence, self-renewal and differentiation. Disturbances in these basic stem cell parameters lead to alterations in the epigenetic landscape of the DNA of ageing stem cells, further aggravating alterations in stem cell quiescence, self-renewal and differentiation.

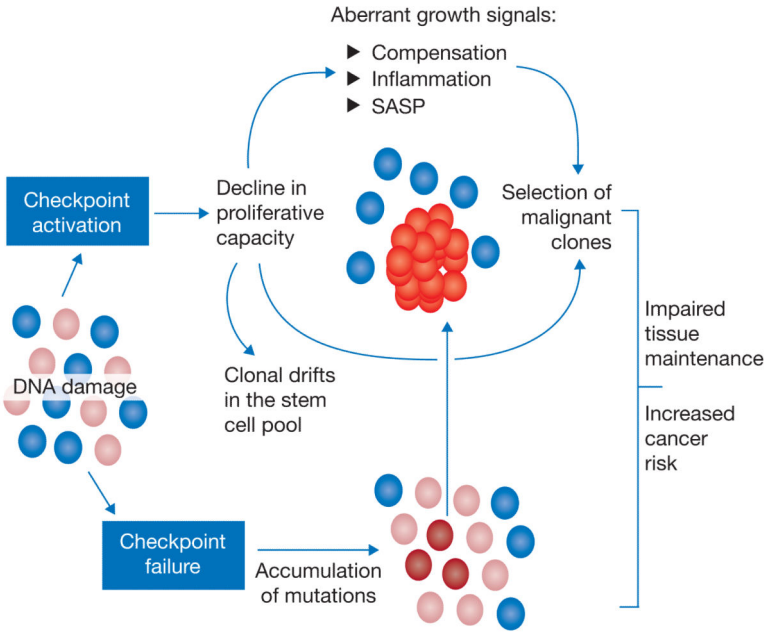

Figure 3.

Consequences of DNA damage on clonal selection in tissue stem cells during ageing. Stem cells in an organ consist of different subpopulations. Ageing-associated accumulation of DNA damage activates checkpoints that remove damaged stem cells by inducing apoptosis, cell cycle arrest or differentiation. This can lead to clonal drifts or imbalances in the pool of remaining stem cells. Checkpoint activation (top) in a growing number of stem cells in ageing tissues impairs the proliferative capacity of these stem cells, which will in turn increase the selective pressure for the outgrowth of mutant cell clones. This process is further accelerated by aberrant growth signals originating from compensatory feedback loops to maintain tissue homeostasis, the accumulation of senescent cells exhibiting a secretory phenotype (SASP), or inflammation as a consequence of immune reactions targeting damaged cells. It is also possible that some stem cells will escape the induction of checkpoints in response to DNA damage (bottom). Checkpoint-deficient stem cells have an increased risk of acquiring mutations that will lead to a selective growth advantage in the context of damage accumulation, checkpoint induction and growth inhibition in the pool of ageing stem cells (top).