OPINION

# DNA damage response in adult stem cells: pathways and consequences

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Abstract | In contrast to postmitotic or short-lived somatic cells, tissue-specific stem cells must persist and function throughout life to ensure tissue homeostasis and repair. The enormous functional demands and longevity of stem cells raises the possibility that stem cells might be uniquely equipped to maintain genomic integrity in ways different than somatic cells. Indeed, evidence suggests that stem cell compartments possess unique properties that combine to either limit or, in some instances, accelerate DNA damage accrual.

Throughout life, DNA damage constantly arises from DNA replication, spontaneous chemical reactions and assaults by external or metabolism-derived agents[1]. This induces an evolutionarily conserved signalling pathway (referred to as the DNA damage response (DDR)) that ensures that DNA damage is repaired. The DDR involves sensors (the MRE11–RAD50–NBS1 (MRN) complex, ataxia-telangiectasia mutated (ATM), ataxia-telangiectasia- and RAD3-related (ATR) and DNA-dependent protein kinase (DNA-PK)), mediators (mediator of DNA damage checkpoint 1 (MDC1), p53-binding protein 1 (53BP1), claspin and breast cancer type 1 susceptibility (BRCA1)) and effectors (checkpoint kinase 1 (CHK1) and CHK2)[2]. The action of these proteins culminates in either transient cell cycle arrest and DNA repair or elimination of damaged cells by apoptosis and/or senescence (FIG. 1). Although stringent, the repair machinery is not infallible and can lead to the accumulation of misrepaired lesions or genomic instability[3].

The fact that adult stem cells (SCs) persist throughout life is thought to increase their risk of accumulating deleterious mutations. Moreover, misrepaired or unrepaired lesions arising in SCs can be passed down and amplified in daughter SCs and downstream progeny through the processes of self-renewal and differentiation, respectively[3,4]. Thus, mutations arising in SCs can be propagated throughout a substantial part of the tissue.

Although considerable evidence has shown that DNA damage compromises SC function[5], surprisingly little is known about how adult SCs respond to DNA damage and whether the response of adult SCs to DNA damage differs from downstream progenitor and effector cells. In this Opinion article, we describe recent evidence regarding the mechanisms by which SCs maintain genomic integrity. We argue that DNA damage accumulation and the mechanisms by which adult SCs respond to DNA damage have the potential to impart deleterious consequences to the long-term function of tissues and may promote tumorigenesis.

> “... DNA damage constantly arises from DNA replication, spontaneous chemical reactions and assaults by external or metabolism-derived agents.”

## Stem cell physiology and DNA damage

Along with embryonic SCs, tissue-specific adult SCs are functionally defined by their ability to both self-perpetuate, through a process known as self-renewal, and give rise to effector cell types, through differentiation. Long-term maintenance of these essential SC properties relies, to a large extent, on interaction with supporting cells that comprise the SC niche. Although the properties and behaviour of adult SCs markedly differ across tissues, certain aspects of SC physiology are shared by many adult SCs; most significant are those that have a role in maintaining ‘stemness’. Among these, the quiescent nature of many adult SCs (but not all — for example, intestinal SCs), combined with their low metabolic activity and decreased production of reactive oxygen species (ROS), provides near-term benefits with regard to DNA damage acquisition. However, we think that these characteristics may lead to long-term adverse consequences, as DDR pathways may be attenuated in quiescent SCs[6].

***Proliferation and cell cycle dynamics.*** Over the past decade, much has been learned about DNA damage-induced signalling pathways and their consequences in somatic cells. Different pathways operate to repair DNA damage at various stages of the cell cycle (FIG. 1). However, little is known about the cellular and molecular consequences of DNA damage in adult SCs. This is in part because of the complicated nature of the DDR in SCs (see below).

Many (but not all) adult SCs are largely quiescent, residing primarily in the G0 phase of cell cycle. The quiescent state is maintained by both extrinsic and intrinsic mechanisms and has been postulated to be a way to preserve long-term proliferative potential and genomic integrity[7]. However, by adopting a quiescent state, adult SCs may be faced with a different problem in maintaining genomic integrity, as DNA damage checkpoints and several repair pathways are cell cycle dependent[8–11] (FIG. 1). Indeed, the quiescent state of haematopoietic SCs (HSCs) has been suggested to underlie the propensity of these cells to accumulate DNA damage during ageing, ultimately leading to an attenuation of regenerative capacity[6,12]. Moreover, damaged SCs entering the cell cycle confront G1 phase, during which DNA damage is primarily repaired by the non-homologous end joining (NHEJ) pathway. NHEJ is error-prone and is thus thought to contribute to the acquisition of mutations during repair (see below)[13]. It is important to note that NHEJ has a key role in DNA repair

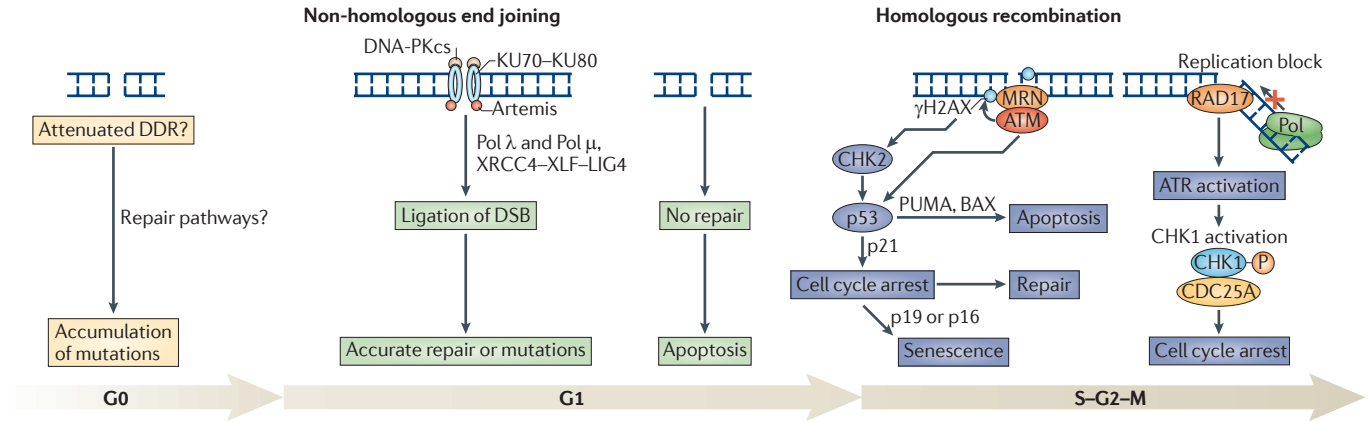


Figure 1 | **DSB repair during the cell cycle.** DNA double-strand breaks (DSBs) caused by extrinsic and intrinsic agents are repaired by two main pathways that operate during different phases of the cell cycle[2,49]. During G1, the non-homologous end joining (NHEJ) pathway is the predominant DSB repair pathway. DSBs are recognized by the KU dimer (KU70–KU80), which provides a scaffold and recruits the proteins that carry out DNA repair. Specifically, KU70–KU80 recruits the DNA-dependent protein kinase (DNA-PK) catalytic subunit (DNA-PKcs), leading to assembly and activation of DNA-PK, which, together with the nuclease Artemis, regulates end processing and resection. This is followed by DNA polymerase-λ (Pol λ)- and Pol μ-mediated gap-filling and ligation of the DNA ends by a ligase complex that comprises X-ray repair cross-complementing 4 (XRCC4), XRCC4-like factor (XLF) and DNA ligase 4 (LIG4), which ligates the two strands. However, NHEJ is error-prone and can lead to *de novo* generation of mutations (deletions, insertions, mismatches and translocations). Unrepaired lesions can lead to apoptosis. Cycling cells, and particularly cells in the S and G2 phases, tend to use homologous recombination (HR), which is a higher-fidelity repair pathway than NHEJ, to repair DSBs. For lesions repaired by HR, DSBs are detected and processed by the MRE11–RAD50–NBS1 (MRN) complex. This leads to recruitment and activation of ataxia-telangiectasia mutated (ATM) by autophosphorylation. ATM, in turn, phosphorylates many substrates, including histone variant H2AX (known as γH2AX when phosphorylated), which flanks the break site. Activation of downstream ATM targets, including checkpoint kinase 2 (CHK2) and p53, leads to transient cell cycle arrest. Replication blocks during S phase activate ataxia-telangiectasia- and RAD3-related (ATR) and subsequently CHK1, leading to cell cycle arrest. Unrepaired DNA damage can lead to permanent cell cycle arrest (senescence) or apoptosis. Many adult SCs reside in G0 phase of the cell cycle, and details regarding DNA repair are poorly understood. DDR, DNA damage response.

in SCs, as HSCs from mice deficient in components of the NHEJ pathway, including DNA ligase 4 (LIG4) and KU80, show impaired repopulating potential[12,14].

***ROS-mediated DNA damage.*** In contrast to the metabolic activity of rapidly proliferating embryonic SCs and somatic cells, the metabolic activity of quiescent SCs is relatively modest. However, despite the stark differences in proliferative status of embryonic SCs and adult SCs, both predominantly rely on the glycolytic pathway[15,16] to generate energy, unlike many somatic cell types, which rely more on mitochondrial respiration. This confers a cytoprotective advantage to adult SCs, as mitochondrial respiration generates large amounts of ROS, which are thought to contribute to DNA damage[17]. Although the extent of DNA damage imparted by ROS is unclear, numerous studies have shown that management of ROS levels in SCs is important for their function, including their ability to self-renew.

For example, the forkhead box O (FOXO) subfamily of transcription factors is involved in stress resistance, cell cycle regulation and apoptosis. Mice deficient in all three FOXO family members (FOXO1, FOXO3 and FOXO4) showed decreased HSC numbers and impaired long-term repopulating activity resulting from excessive ROS-mediated oxidative stress[18–20]. ROS levels are also thought to be regulated by ATM, and indeed, loss of *Atm* leads to diminished HSC function resulting from ROS-mediated activation of mitogen-activated protein kinase p38 and cyclin-dependent kinase inhibitor 2A (CDKN2A; also known as p16INK4a), leading to cell cycle arrest[21,22]. Importantly, treatment with the antioxidant *N*-acetyl cysteine (NAC) was able to rescue the defects observed in FOXO-deficient or ATM-deficient mice[19,21], indicating that lower ROS levels and a reduced redox microenvironment are crucial for SC maintenance and function. It was recently shown that, in mice lacking ATM, undifferentiated spermatogonia accumulate DNA damage, leading to cell cycle progression defects mediated by the activation of the p53–p21–p19 pathway[23] (FIG. 1).

In SCs and other cell types, mitochondrial integrity and redox homeostasis are thought to be regulated in part by the polycomb RING-finger oncogene BMI1 (known to be crucial for SC function in numerous settings[24]), which prevents the generation of ROS, thereby minimizing ROS-inflicted DNA damage[25]. Interestingly, overexpression of BMI1 in normal human neural SCs was shown to directly contribute to the DDR by enhancing ATM recruitment to sites of DNA damage, leading to protection from ultraviolet radiation[26]. It therefore seems that BMI1 has a dual role in protecting SC genomic integrity: a direct role, by regulating the DDR through ATM recruitment[26], and an indirect role, by managing ROS levels[25]. Although the extent and biological impact of ROS-mediated DNA damage in SCs remains undetermined, these studies clearly point to an important role for ROS-mediated signalling in normal SC biology.

The maintenance of a hypoxic environment in the SC niche not only preserves the self-renewing potential of adult SCs, by protecting them from exogenous sources of oxidative stress, but also may be involved in instructing lineage commitment[27]. Along these lines, it has been shown that increased oxidative stress itself may prime haematopoietic progenitors to differentiate[28]. By contrast, high levels of ROS seem to promote self-renewal in multipotent neural progenitors[29]. Interestingly, subsets of cancer SCs from human and mouse breast tumours have lower levels of ROS and express higher levels of free radical scavengers than non-tumorigenic cells, although it remains unclear whether such properties affect cancer SC self-renewal or differentiation[30].

### DDR in stem cells

The long-term maintenance and continued function of SC activity has raised the possibility that they are uniquely equipped to handle DNA damage, unlike short-lived effector cells. Emerging evidence suggests that SCs do indeed respond to DNA damage differently from their somatic counterparts, to either limit or, in some cases, contribute to DNA damage accrual (TABLE 1).

***Rate and fidelity of repair.*** Cellular outcome following DNA damage depends on the severity of insult and the rate of clearance, which is largely determined by the duration and robustness of the activation of the tumour suppressor p53. One way of alleviating strong and prolonged p53 activation is to repair DNA damage more rapidly. In the skin epidermis, bulge SCs repair DNA lesions faster than other epidermal cells owing to more efficient NHEJ activity. This results from a higher nuclear expression and activity of DNA-PK catalytic subunit (DNA-PKcs), one of the key proteins involved in the initial step of NHEJ repair[31] (FIG. 1). Indeed, decreased DNA-PKcs activity in mice with severe combined immunodeficiency (SCID), which results from a nonsense mutation at Tyr4046 in DNA-PKcs[32], abrogates the resistance of bulge SCs to irradiation. This suggests that the rapidity of DNA repair contributes to the resistance of bulge SCs to apoptosis[31].

The downside of preferential use of the NHEJ repair pathway in SCs is that NHEJ is more error-prone than homologous recombination (HR) and can introduce small deletions or insertions into the repaired region[9,11]. Quiescent SCs called into the cell cycle enter G1, when NHEJ is the predominant double-strand break repair pathway. Consistent with this, irradiation and *in vitro* culture of quiescent HSCs leads to genomic rearrangements, including reciprocal translocations, interstitial deletions and complex chromosomal rearrangements, resulting from the use of NHEJ[13]. By contrast, HSCs driven into the cell cycle before irradiation can access the higher-fidelity HR repair pathway, resulting in a reduced frequency of genomic alterations[13,33]. In agreement with the idea that NHEJ can promote *de novo* mutations arising from DNA damage and thereby induce cancer development, SCID mice have fewer chemically induced skin tumours than wild-type mice, a finding that has been attributed to an increase in apoptosis following carcinogen administration and the elimination of mutated epidermal cells with high DNA damage burden[34]. Taken together, these results suggest that the presence of efficient but error-prone NHEJ DNA repair mechanisms could be a double-edged sword for adult SCs, promoting their short-term survival after DNA damage at the expense of the long-term maintenance of genomic integrity. This is even more important under physiological settings, when sublethal insults to the genome could be commonly encountered. Attenuated DDR and a milder induction of p53 may prevent the elimination of damaged SCs and allow their expansion. Indeed, studies have shown that p53-deficient HSCs have a selective advantage and can outcompete wild-type cells under stress conditions[35,36]. In this way, loss of p53 function may represent a key mechanism underlying the clonal expansion of defective SC subsets, which may contribute to tumorigenesis (BOX 1; FIG. 2).

***DDR in SCs: consequences for ageing and cancer.*** DNA damage is followed by robust activation of DDR pathways in all cells. Depending on the extent of the damage and the duration and strength of the DDR, damage can be repaired, can result in the induction of apoptosis or senescence, or can persist as fixed mutations, which in the SC compartment can lead to significant long-term functional consequences (BOX 1).

It has long been postulated that cell quiescence may contribute to resistance to cell death following irradiation. Indeed, tissues undergoing high turnover, such as embryonic tissue, blood and the gut, tend to be the most sensitive to irradiation[37,38]. By contrast, many tissue-specific SCs and even cancer SCs[30,39] are thought to show increased

Table 1 | **Differential attributes of SCs and somatic cells with respect to DNA damage**

| Attributes | SCs | Somatic cells |
|---|---|---|
| ***Physiological*** | | |
| Proliferation status and replication error | Quiescent; reduced chance of replication error | • Proliferative cells have greater chance of replication error*<br>• Postmitotic cells do not propagate mutations |
| Metabolic activity | Low; low production of ROS and toxic metabolites | High; high production of ROS* and toxic metabolites* |
| Mutation propagation | • Mutations can be propagated or amplified in the SC pool through self-renewal*<br>• SCs propagate acquired mutations to downstream progenitors* | • Mutations are not heritable in postmitotic cells<br>• Mutations are potentially heritable in proliferative cells* |
| Turnover | Life-long persistence; SCs act as a mutational sink and can predispose to cancer* | High turnover rate, which eliminates damaged cells |
| ABC transporter activity | High; efficient efflux of toxic metabolites and xenobiotics | Low; retention of toxic metabolites* and xenobiotics |
| Niche requirement | Stringent requirement:<br>• Niche is cytoprotective and maintains quiescence<br>• Aberrant proliferation observed in disrupted niche*<br>• Hypoxic niche; lower ROS | Unclear requirement; however, a local microenvironment could be stressful* |
| ***Relating to the DDR pathway*** | | |
| Repair | Efficient but error-prone repair*; attenuated DDR?* | High-fidelity repair owing to robust DDR |
| Pathway used | NHEJ used when quiescent SCs enter the cell cycle* | Homologous recombination primarily used in cycling cells |
| Outcome | • Removal of damaged cells can lead to depletion or exhaustion of SCs and a reduced regenerative response*<br>• *De novo* acquisition of mutations*<br>• If damaged cells escape, they can clonally expand and predispose to cancer* | Damaged cells are removed but can then be replenished by SCs; functional restoration is possible |

ABC, ATP-binding cassette; DDR, DNA damage response; NHEJ, non-homologous end joining; ROS, reactive oxygen species; SCs, stem cells. *Parameters that promote acquisition of DNA damage with potential impairment of cell function.

Box 1 | **SCs as precancerous units**

DNA damage has been shown to specifically accumulate in stem cell (SC) compartments with age. This is a dangerous prospect, given that SCs can propagate heritable mutations both to self-renewing progeny (horizontal transmission) and to downstream progenitors through the process of differentiation (vertical transmission) (FIG. 2). This potentiation of mutations acquired in individual SCs, combined with the longevity of these cells, creates a setting in which the SC pool can serve as a mutation reservoir. As additional mutational events accrue and are transmitted both horizontally and vertically, mutations conferring a selective advantage can further promote the precancerous state through the process of clonal expansion. In this way, the properties of SCs counter-intuitively provide the ideal setting through which tumours can emerge. Interestingly, fully developed tumours usually emerge only in progenitor populations that differentiate from SCs and not in the SCs themselves. Although this is poorly understood, it may be due to the very tight control mechanisms regulating proper homeostasis in SC compartments, which may be absent in downstream populations. It is also possible that the mutational events that ultimately give rise to cancer arise during the increased proliferation that takes place in downstream progenitors following commitment to a specific lineage.

resistance to DNA damage-induced killing because they are quiescent. Interestingly, irradiation-resistant SCs, such as those in the hair follicle bulge and in the blood, exhibit a shorter duration of p53 activation and a more abundant expression of pro-survival proteins of the B cell leukaemia 2 (BCL-2) family compared with the differentiated cells of these tissues[31,38,40–42]. It has therefore been suggested that the relative strength and duration of p53 activation and levels of anti-apoptotic BCL-2 may dictate resistance to irradiation.

The sensitivity to DNA damage and p53-induced apoptosis differs widely across SCs[43]. For example, although p53 expression is rapidly induced following DNA damage to a similar extent in all epidermal cells, the duration of p53 stabilization is much shorter in bulge SCs compared with the other basal epidermal cells[31], thus preventing apoptosis of bulge SCs. Furthermore, SCs from the small intestine are sensitive to DNA damage-induced cell death and undergo massive apoptosis that has been attributed to a robust activation of the p53 pathway[44] and low expression of the anti-apoptotic protein BCL-2 (REF. 45). By contrast, colon SCs are more resistant to irradiation because they express high levels of BCL-2 (REF. 45).

Such different apoptosis sensitivities are thought to contribute to the physiology of these tissues. For example, the low apoptotic threshold and 'altruistic suicide' of SCs of the small intestine decreases the likelihood of accumulating damaged cells with unrepaired DNA lesions and perhaps provides an explanation for why cancers of the small intestine are rare despite the higher turnover of this tissue. By contrast, colonic SCs are more resistant to apoptosis and may therefore be more prone to mutation accumulation, which could underlie the increased incidence of colonic cancers compared with intestinal cancers[45]. Furthermore, differences in apoptosis may affect tissue regeneration, with a low apoptotic threshold leading to a diminished SC compartment that reduces the tissue regenerative capacity during ageing (FIG. 2). Finally, apoptotic resistance following genomic insults may ensure the functionality of the tissue at the expense of genome fidelity, if lesions are not properly repaired (BOX 1).

Premature differentiation and senescence are alternative outcomes of DNA damage repair that are thought to have a beneficial effect by restricting the accumulation of defective cells in SC compartments. For example, upon DNA damage, melanocyte SCs undergo premature differentiation, inducing depletion of the melanocyte SC pool, which results in hair greying[46]. By contrast, loss of the p53 family member tumour protein 63 (TP63) in dermal precursors leads to skin ulcerations and defects in wound healing response owing to genomic instability and induction of senescence[47]. Similarly, overexpression of the wingless-type mouse mammary tumour virus integration site family member *Wnt1* in the skin leads to rapid growth of the hair follicles (which could lead to tumours) followed by mammalian target of rapamycin (mTOR)-dependent senescence and exhaustion of bulge SCs[48]. In both cases, senescence of the epidermal SCs not only had the beneficial effect of preventing tumour formation but also led to a premature ageing phenotype as the SC pools became exhausted.

## Conclusions and future directions

SCs have numerous properties that uniquely influence the way in which DNA damage is acquired and how it is dealt with. We argue that, although adult SCs are equipped with metabolic and proliferative properties that minimize insults to genomic integrity, residence in the G0 phase of the cell cycle and concomitant reliance on the error-prone NHEJ pathway for repair puts adult SCs at risk of acquiring mutations that could lead to cancer. At the same time, robust activation of the DDR or activation of tumour suppressor pathways in SC compartments can lead to apoptosis or senescence, and this may compromise SC function in the long term and contribute to ageing.

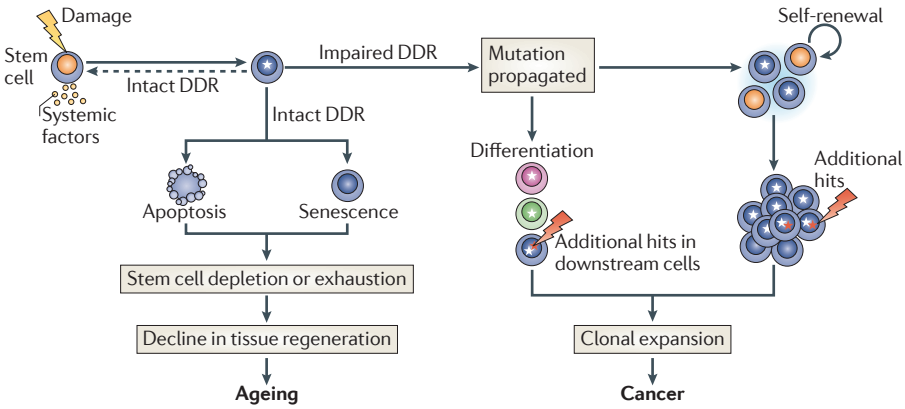


Figure 2 | **Impact of DNA damage on SCs.** DNA damage in the stem cell (SC) compartment is followed by the induction of a robust DNA damage response (DDR), which, when properly executed, leads to DNA repair or elimination of damaged SCs by apoptosis or senescence. If elimination and senescence prevail over a lifetime, this can ultimately lead to SC exhaustion and ageing. If DNA damage escapes the DDR or is misrepaired, the SC pool may accumulate mutations. These can be propagated and amplified horizontally within the SC compartment by self-renewal and vertically to downstream progeny by differentiation. Mutations conferring a selective advantage in SCs or progenitor cells have the possibility of being amplified further through the process of clonal expansion, thereby providing a large pool of cells through which additional mutagenic events can arise, eventually leading to tumorigenesis. Damaged DNA is represented by a star in the nucleus.

Future work will be needed to determine whether *ex vivo* expansion and/or differentiation of adult SCs leads to acquisition of DNA damage and genomic instability that might limit the clinical utility of such cells by predisposing them to cancer or accelerated ageing in transplanted recipients. It will also be interesting to determine whether cancer SCs retain the properties of the SCs of their tissue of origin, or whether stemness is acquired progressively through a selective pressure during cancer progression.

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**Competing interests statement**
The authors declare no competing financial interests.

**FURTHER INFORMATION**
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