

Stem cells: balancing resistance and sensitivity to DNA damage

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Embryonic stem cells (ESCs) are known to be very sensitive to DNA damage and undergo rapid apoptosis even after low-damage doses. By contrast, adult stem cells show variable sensitivity to damage. Here we describe the multiple pathways that have been proposed to affect the sensitivity of stem cells to damage, including proximity to the apoptotic threshold (mitochondrial priming) and the p53 signaling pathway, through activation of transcription or direct interaction with proapoptotic proteins in the cytoplasm. We also discuss which cellular factors might connect mitochondrial priming with pluripotency and the potential therapeutic advances that can be achieved by better understanding of the molecular mechanisms leading to sensitivity or resistance of embryonic or adult stem cells from different tissues.

Stem cells must respond appropriately to DNA damage

A major component of cellular aging is the detrimental accumulation of mutations in the cell's DNA. Mutations that facilitate deregulated proliferation or predispose cells to acquire further mutations are often associated with the development of cancer [1]. Cellular stress, such as DNA damage, contributes to the accrual of such deleterious mutations and therefore cells mount responses to guard against genomic instability [2]. Different cell types favor different responses to cellular stress, ranging from cell cycle arrest and DNA repair to senescence or apoptosis [3]. ESCs, both human (hESCs) and mouse, are known to be acutely sensitive to DNA damage [4–8]. As an important cell population that gives rise to all of the tissues in the body, ESCs are expected to undergo apoptosis after damage to prevent damaged cells from compromising the genomic integrity of the population. Conversely, adult stem cells are more resistant to cell death after DNA damage, possibly to prevent uncontrolled apoptosis that might compromise tissue and organ structure. However, this resistance may lead to genomic instability if damage-induced mutations are not properly repaired [9]. Hence, stem cells are caught between two opposing needs. On the one hand,

they must block the propagation of mutations to their progeny cells, because fixed mutations can have long-term functional consequences including predisposition to malignancy. On the other hand, stem cells have a responsibility to maintain existing tissue organization. Here we describe the current understanding of the DNA damage response in hESCs, in particular how their sensitivity is maintained close to the apoptotic threshold. We also discuss the variability in sensitivity of adult stem cells to DNA damage and propose multiple regulatory networks that may affect the sensitivity of adult stem cells in different tissues.

Multiple distinct mechanisms sensitize hESCs to DNA damage

Many studies have shown that hESCs have higher rates of apoptosis after DNA damage than differentiated cells [4–6], yet this phenomenon remains incompletely understood. Several distinct mechanisms have been proposed to explain how the regulatory networks that control apoptosis might have unique functions in hESCs (Figure 1). Among these mediators is the tumor suppressor protein p53. Well known as a regulator of cell fate decisions in somatic cells [10], p53 is also induced after various types of DNA damage in hESCs. Different classes of DNA lesions, including DNA double-strand breaks (DSBs) and single-stranded DNA (ssDNA), can be generated by various damaging agents, such as γ -ray irradiation and UV irradiation, respectively [11]. Apoptosis of hESCs in response to both DSBs and ssDNA was shown to be dependent on p53 [4,5]. However, multiple mechanisms have been proposed to explain how p53 induces the intrinsic (mitochondrial) apoptotic pathway (Box 1) in hESCs, including p53 transcriptional activation of proapoptotic target genes, direct interaction of p53 with mitochondrial proteins, and p53-mediated translocation of the proapoptotic protein Bax.

p53 activates the transcription of multiple genes involved in apoptosis, such as *BAX*, *PUMA*, *NOXA*, and *APAF1* [12]. On treatment with the DNA-damaging drug etoposide [13], p53 was found to translocate to the nucleus of hESCs and to upregulate *PUMA* [5]. Independently of its transcriptional activity, p53 can promote apoptosis by directly interacting with mitochondrial proteins [14]. It was shown in hESCs treated with UV irradiation that p53-dependent apoptosis occurred without transcription of p53 target genes; instead, p53 associated with the mitochondria [4]. It is currently unclear why in some cases p53 acts as a transcription factor whereas in others it

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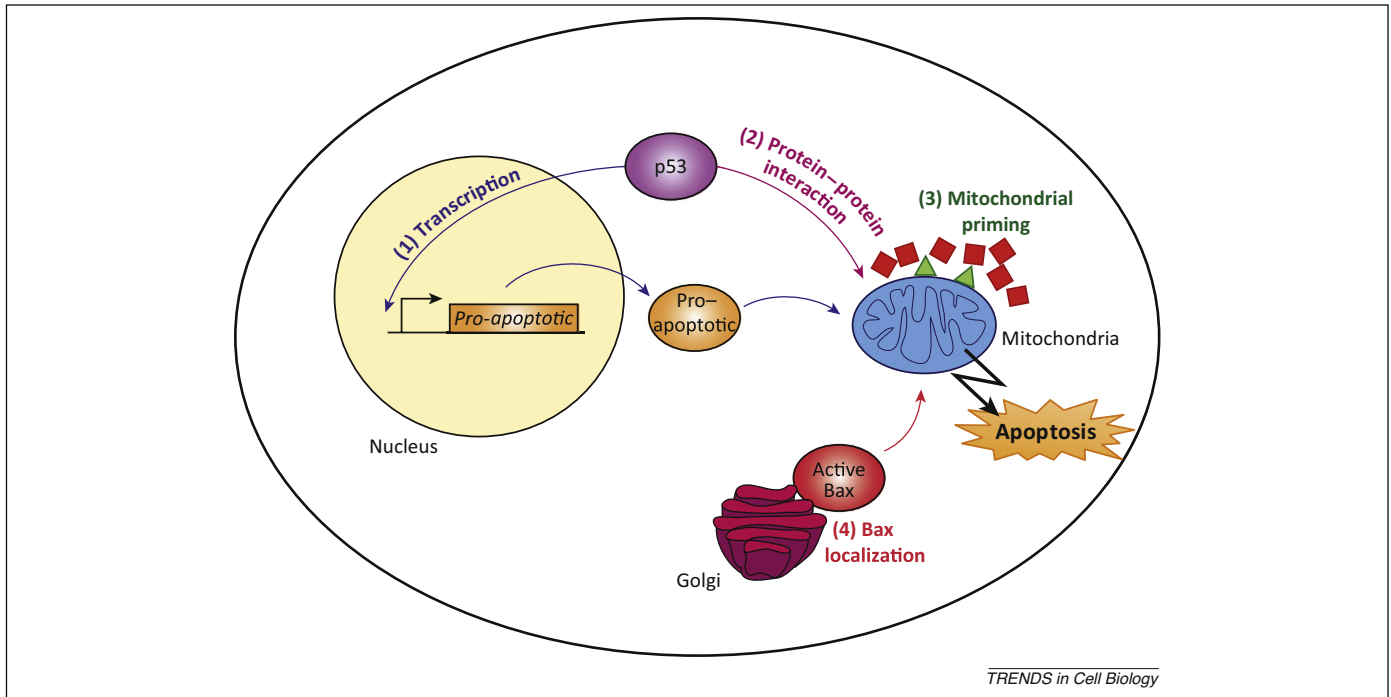


Figure 1. Multiple pathways contribute to human embryonic stem cell (hESC) sensitivity to DNA damage. (1) The tumor suppressor protein p53 acts as a transcription factor, upregulating the expression of proapoptotic target genes. The proapoptotic protein products proceed to induce apoptosis via the mitochondrial pathway. (2) p53 can act independently of transcription to directly interact with cytoplasmic proapoptotic and antiapoptotic proteins, thereby promoting apoptosis. (3) High mitochondrial priming, implying a high ratio of proapoptotic to antiapoptotic proteins, maintains hESCs close to the apoptotic threshold. (4) The apoptotic protein Bax is localized in its active conformation at the Golgi apparatus in some hESCs, poised to induce rapid apoptosis after damage-induced translocation to the mitochondria.

activates apoptosis through interacting with mitochondrial proteins. The specific mechanism of activation might be dependent on the type of DNA damage, although further studies are required to better understand the specific relationship between the type of damage and p53 function in hESCs. Recently, the proapoptotic protein Bax was shown to be a major activator of apoptosis in hESCs dependent on p53. Unlike differentiated cells, hESCs were found to maintain Bax in its active conformation at the Golgi apparatus in basal conditions, whereas previously Bax activation was thought to be exclusive to apoptotic cells [15]. In hESCs damaged with the DNA-damaging drug etoposide, Bax rapidly localizes to the mitochondria and initiates apoptosis. Although Bax translocation was shown to be dependent on p53, the exact mechanism generating the translocation remains unclear. Notably, the basal level of active Bax varies substantially among different hESC lines and is not detectable in the H1 cell line. Nonetheless, H1 cells show the typical hESC sensitivity to DNA damage [16], suggesting that basally activated Bax is not required for induction of apoptosis, and hence the other mechanisms presented here can be sufficient to induce apoptosis (Figure 1).

The specific mechanisms leading to DNA damage-induced apoptosis in hESCs were recently investigated by simultaneously probing the DNA damage response in differentiated cells and comparing it with the response in their differentiated progeny [16]. Consistent with previous findings, activation of apoptosis was dependent on p53, and the transcription of apoptotic target genes of p53, including *PUMA*, *APAF1*, and *FAS*, was induced after damage in hESCs. However, apoptosis was still induced

when transcription was inhibited, suggesting that although p53 transcriptional activity is functional, it is not required for apoptosis [16]. Differentiated cells showed similar induction of apoptotic targets, suggesting that p53 transcriptional activity does not distinguish hESCs from differentiated cells. Looking further, p53 was observed in the cytoplasm of hESCs and a cytoplasm-confined p53 mutant confirmed that cytoplasmic p53 alone is able to induce apoptosis. However, cytoplasmic p53 was also present in differentiated cells. These results suggest that, although p53 signaling appears similar in hESCs and differentiated cells, the internal environment of the two cell types may differ, explaining their differential sensitivity to DNA damage. Indeed, DNA damage sensitivity in hESCs was shown to correlate with a cell-intrinsic property termed mitochondrial priming [16]. Mitochondrial priming is determined by the balance between proapoptotic and antiapoptotic Bcl-2 family proteins and can predict a cell's sensitivity to DNA-damaging drugs [17]. It can be measured via Bcl-2 homology domain 3 (BH3) profiling, which quantifies the magnitude of mitochondrial outer membrane permeabilization (MOMP) on exposure to a panel of proapoptotic promiscuously interacting BH3 peptides. BH3 profiling revealed that hESCs exhibited higher mitochondrial priming than their differentiated progeny and hence were closer to the apoptotic threshold [16]. Boosting the priming of differentiated cells using ABT-263, an inhibitor against antiapoptotic Bcl-2 family proteins, led to damage-induced apoptosis in differentiated cells expressing either wild type or the cytoplasm-confined p53 mutant. Therefore, the p53-regulated DNA damage response acts similarly in hESCs and differentiated cells, but due to

Box 1. Pathways to apoptosis

Comprising multiple stages, apoptosis is a highly stereotyped mode of cell death [46]. It plays an important role not only in defense against damage but also in development, aging, and homeostasis. The intrinsic apoptotic pathway is non-receptor-mediated and relies on intracellular signaling such as the DNA damage response. By contrast, the extrinsic apoptotic pathway involves binding of extracellular death ligands to transmembrane receptors. Both pathways converge on mitochondria, the sites of apoptotic initiation. A hallmark of the intrinsic apoptotic cascade is MOMP, which is regulated by proteins of the Bcl-2 family [47]. These proteins, defined by conserved sequence motifs known as Bcl-2 homology domains (BH1–4), include both pro- and antiapoptotic members [48]. The proapoptotic proteins of this family can be divided into the Bax-like subfamily, including Bax and Bak, and the BH3-only subfamily, which induces Bax and Bak activation, allosteric conformational change, and oligomerization, leading to MOMP. By contrast, Bcl-2 and its close relatives Bcl-xL, Bcl-w, and Mcl-1 perform antiapoptotic functions by binding and sequestering proapoptotic BH3-only proteins as well as blocking Bax and Bak activation and oligomerization. In response to damage, the stabilization, increased transcription, or modification of BH3-only proteins counteracts and overwhelms the reserve of antiapoptotic proteins, leading to Bax and Bak activation and MOMP [49]. Following MOMP, multiple events occur, including the release of cytochrome c from the mitochondrial intermembrane space into the cytoplasm, where it can trigger the formation of a large protein complex called the apoptosome and initiate the cascade of caspase activation leading to apoptosis [50].

At least two major roles for p53 in regulating cell-intrinsic apoptosis have been described. After damage, p53 mediates the transcription of multiple target genes whose protein products are proapoptotic, including Bcl-2 family members such as Puma, Noxa, and Bax [12,51]. In addition, multiple studies show that p53 can promote apoptosis by directly interacting with mitochondrial proteins [14,52]. p53 was shown to bind and antagonize antiapoptotic proteins such as Bcl-2 and Bcl-xL [53,54]. Other studies have demonstrated that p53 can directly activate Bax and release other proapoptotic proteins from antiapoptotic proteins [55–59].

the different cellular environments produces dramatically different cell fates.

Causes of high mitochondrial priming in hESCs

High priming appears to be closely linked to pluripotency. BH3 profiling of hESCs in the process of differentiation reveals that, as cells differentiate, their priming gradually decreases [16]. Although it remains an open question how the highly primed state is created and maintained in hESCs, one potential mechanism could be the regulation of balance between pro- and antiapoptotic proteins. Gene transcripts of proapoptotic Bcl-2 family members, including *NOXA*, *BIK*, *BIM*, *BMF*, and *PUMA*, are found at higher abundance in hESCs than in somatic and cancer cells [18]. At the protein level, hESCs have lower levels of the antiapoptotic protein Bcl-2 and higher levels of the proapoptotic protein Puma than differentiated cells, consistent with the higher priming of hESCs [16]. However, other apoptosis-regulating proteins, including Bid, Bim, and Bcl-xL, show similar levels in undifferentiated and differentiated cells [16]. It is unclear whether specific modifications, localization, or protein interactions might affect how these proteins contribute to priming. As mentioned, some hESC lines maintain basally activated Bax, which provides a quick and direct route to apoptosis [15]. This close proximity to the apoptotic threshold is found

only in undifferentiated cells, because just 2 days of differentiation of these hESCs into embryoid bodies were sufficient for activated Bax to disappear and for the cells to no longer undergo apoptosis after damage [15]. Thus, pluripotency may regulate the state of the cell to keep it highly primed and sensitive to damage, but our understanding of this regulation remains limited.

The relationships between the molecular players in the pluripotency and priming networks could be complex and depend on other pathways (Figure 2). The cell cycle, for instance, appears to be uniquely modified in stem cells relative to differentiated cells. hESCs have a rapid cell cycle and an abbreviated G1 phase [19]. Rapid cell cycle progression in hESCs has been shown to be tied to pluripotency and slowing the cell cycle by lengthening G1 through inhibition of Cdk2 causes hESCs to differentiate [20,21]. Moreover, some of the canonical pluripotency proteins, such as Nanog, have been directly implicated in regulating the cell cycle of hESCs. Nanog overexpression was found to shorten the transition time between G1 and S phase by directly binding to the regulatory regions of Cdk6 and Cdc25A [22]. Given how closely connected the pluripotency and cell cycle networks are, it is possible that priming and pluripotency are linked via cell cycle machinery.

Mitochondrial morphology itself could be a factor in determining priming. It has long been known that mitochondria can exist in a fused tubular state or a fragmented state and they can convert between these states through fission and fusion processes [23,24]. More recent work has demonstrated that these dynamical changes are coordinated with cellular processes such as cell proliferation and differentiation [25]. For instance, in somatic cells mitochondria reach a fused state during the G1–S transition, which is associated with downregulation of Drp1, a mitochondrial fission-promoting GTPase [26]. Reduced Drp1 leads to elevation of cyclin E, promoting S phase entry and proliferation [26]. However, despite their high proliferative capacity, hESCs are known to have mitochondria that are fragmented, morphologically immature, and deficient at oxidative phosphorylation [27,28], suggesting that hESCs' relatively rapid G1–S transition might be differentially regulated. Indeed, as hESCs differentiate they not only undergo cell cycle elongation but also develop more connected, complex, and metabolically active mitochondria [28,29]. Mitochondrial dynamics may also play a role in reprogramming; blocking fission using a Drp1 inhibitor leads to a decrease in reprogramming efficiency of over 95%, suggesting that mitochondrial fission may be a critical feature of pluripotency [30]. Moreover, it has been suggested that mitochondrial connectivity might have protective effects on cell survival and, conversely, conditions of mitochondrial fragmentation could facilitate apoptosis [31]. Thus, the unique mitochondrial morphology associated with the pluripotent state could have both a direct and an indirect (via the cell cycle) impact on priming. Furthermore, because mitochondrial structure was previously measured only at later time points post-differentiation [28,29], it remains to be determined whether mitochondrial structure can change on the same timescale as the decrease in mitochondrial priming during differentiation [16]. Deciphering the interactions between the various

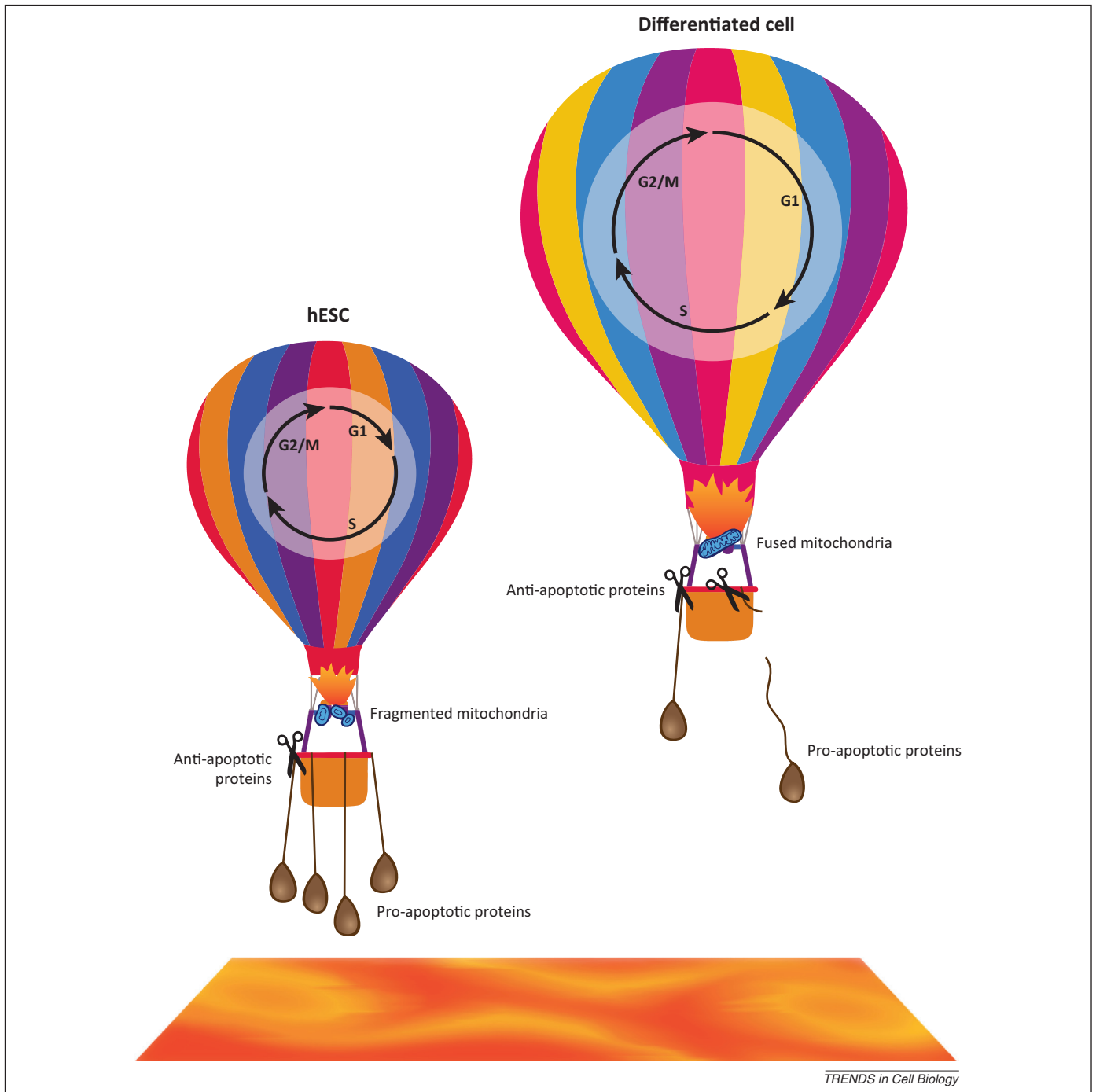


Figure 2. Proposed model of the apoptotic threshold between human embryonic stem cells (hESCs) and differentiated cells. hESCs (left) and differentiated cells (right) are represented as hot-air balloons floating over lava (death). In this analogy, the size of the balloon envelope is the length of the cell cycle; a longer G1 phase in differentiated cells allows them to float higher, conferring protection from death. The burners that generate hot air for the balloons are represented by the mitochondria; fused mitochondria in differentiated cells provide better propulsion (shown as a larger flame) than fragmented mitochondria associated with hESCs. In hESCs, proapoptotic proteins (sandbags weighing down the balloon) outnumber antiapoptotic proteins (scissors), whereas in differentiated cells antiapoptotic proteins prevent the activation of proapoptotic proteins. Overall, the hESC balloon is in greater danger of sinking toward death than the differentiated cell balloon.

players in the pluripotency, cell cycle, and priming networks continues to be a challenge and most likely will require further studies looking at multiple systems and the interconnections between them in the same cell.

Adult stem cells vary in their sensitivity to DNA damage

Adult tissues have reserves of multipotent stem cells that perform important roles in homeostasis and recovery after injury. In general, adult stem cells tend to be more

resistant to cell death following damage than ESCs [9], although the exact mechanisms are not fully understood. Because direct comparisons are difficult to make in human tissues, many adult stem cell experiments have been performed in mice. In several mouse tissues, the most primitive stem cells are relatively more resistant to damage. For instance, compared with more committed progenitors, purified hematopoietic stem cells (HSCs) are resistant to ionizing radiation [32]. Similarly, mammary stem cells

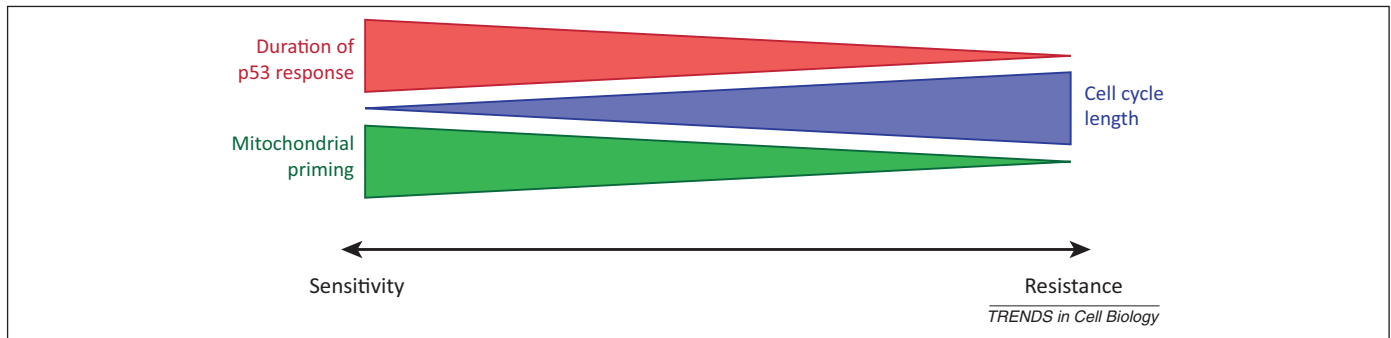


Figure 3. Factors involved in resistance and sensitivity of adult stem cells to DNA damage. Various factors, including the duration of the damage-induced p53 response, cell cycle length, and mitochondrial priming, may contribute to the variability in overall sensitivity of adult stem cells across tissue types. Similar factors can also lead to the higher resistance observed in adult stem cells compared with embryonic stem cells.

(MaSCs) show resistance to X-ray-induced apoptosis, in contrast to progenitor cells from mammospheres [33]. Skin is very sensitive to ionizing radiation, but in human epidermis slow-cycling keratinocyte stem cells (KSCs) undergo post-damage cell death to a lesser extent than their direct progeny, the keratinocyte progenitors [34]. Conversely, candidate stem cells toward the base of the small intestinal crypt are more sensitive to irradiation than small intestine progenitors located higher in the crypt [35].

Variation in sensitivity to DNA damage among adult stem cells can be attributed to at least three underlying causes (Figure 3). Among the three causes, the duration of p53 activation was recently shown to affect cellular outcomes in cancer cells [36]. Differential p53 dynamics might also play a role in cell fate decisions of stem cells in tissues in which p53 is induced. Hair follicle bulge stem cells exhibit transient p53 activation after ionizing radiation and are more resistant to cell death compared with other cells of the epidermis [37]. Long-term reconstituting HSCs are resistant to X-ray irradiation and show a greatly attenuated p53 response [33]. Although short-term reconstituting HSCs upregulate p53, they are also radioresistant, perhaps because p53 is activated to a lesser extent than in more sensitive, committed progenitor cells [33].

A second potential cause for the variation in sensitivity is differential mitochondrial priming as discussed in the previous section, including disparities in the expression of proapoptotic or antiapoptotic proteins. For instance, due to their higher levels of the antiapoptotic protein Bcl-2, colon stem cells tend to be more resistant to irradiation than small intestine stem cells [38]. This observation suggests that colon stem cells sustain more mutations, which may lead to malignancy, potentially explaining the higher frequency of colon cancers compared with cancers of the small intestine [38]. Differential mitochondrial priming can conceivably also arise from differences in mitochondrial structure and activity among various tissue-specific stem cell types, a possibility that remains to be investigated.

A third possibility is cell cycle duration. An important difference between hESCs and their adult counterparts is their progression through the cell cycle. Under normal conditions, many adult stem cells are largely quiescent, resting in the G₀ phase of the cell cycle [39], whereas both hESCs and mouse ESCs cycle rapidly [19,40]. Hence, whereas embryonic stem cells exhibit rapid cell cycle progression and have high sensitivity to DNA damage, slower

cycling in tissue-specific stem cells may contribute to increased resistance to damage. Variation in sensitivity among stem cells from different tissues correlates with this trend. As mentioned above, slow-cycling HSCs and KSCs are relatively resistant after irradiation, whereas actively cycling stem cells from the small intestine undergo apoptosis after low doses of irradiation. Thus, quiescence appears to have a protective effect on stem cell survival.

Damage sensitivity also depends on the balances between competing signaling pathways, such as DNA repair versus apoptosis. However, which mode of damage response is dominant in various tissues is not thoroughly understood and could vary with organism and developmental stage [32,41]. Moreover, different cell types employ diverse DNA repair pathways, complicating our ability to understand and predict how each system will respond to DNA damage [42]. For instance, quiescent HSCs and hair follicle bulge stem cells enter the cell cycle in G₁ after damage and therefore use the error-prone nonhomologous end joining pathway to repair DNA [32,37]. Consistently, studies have shown that quiescent stem cells accumulate DNA damage with age and their function becomes progressively limited [43]. Loss of DNA repair pathways further threatens the survival, longevity, and genomic stability of the stem cell pool [44]. Together, quiescence and DNA repair interact to affect stem cell sensitivity and resistance, with subsequent effects compounded during aging.

Understanding stem cell sensitivity or resistance can inform therapy design

The ability of stem cells to self-renew is mirrored in the aberrant proliferation of cancer cells. Cancer cells tend to grow faster than normal cells; therefore, if a relationship between faster proliferation and greater radiosensitivity is reliable, it could be exploited for therapeutic purposes. In a recent study, BH3 profiling was applied to predict the effects of chemotherapy in patients with acute myeloid leukemia (AML) [45]. Mitochondrial priming was a determinant of the response to chemotherapy: the leukemia cells that were more primed were the cells more likely to respond. Because apoptosis was measured 24 h or more after treatment, it remains unclear whether the dying leukemia cells undergo apoptosis as rapidly as hESCs. It is also unknown whether the level of priming and the mechanisms leading to apoptosis in sensitive leukemia cells are similar to those in hESCs. In patients cured by

Box 2. Outstanding questions

- What regulates the level of mitochondrial priming in cells?
- What functions does p53 have in the cytoplasm of hESCs? For instance, how does p53 mediate the translocation of activated Bax from the Golgi to the mitochondria?
- What mechanisms link mitochondrial priming to pluripotency, either directly or indirectly?
- How do p53 dynamics interact with mitochondrial priming in different tissue-specific cell types and how might this interaction affect the DNA damage response?
- How can p53 dynamics and/or mitochondrial priming be manipulated in different tissues to achieve a better therapeutic window?

chemotherapy, normal HSCs were less primed than the AML myeloblasts [45]. By contrast, the myeloblasts of patients who had responded poorly to chemotherapy were even less primed than their HSCs, showing that the relative priming of HSCs and leukemia cells characterized a boundary in clinical outcome. Moreover, BH3 profiling demonstrated that the AML myeloblasts tended to depend on Bcl-2 for survival, whereas the HSCs were selectively more dependent on Mcl-1. Hence, combining chemotherapy with the addition of Bcl-2-inhibiting drugs could potentially increase the priming of leukemia cells, improving the efficacy of the treatment.

Concluding remarks

Understanding how damage response pathways and intrinsic mitochondrial priming interact is important for predicting the sensitivity of different tissues and for the overall function and health of the organism. Regulated by interconnected networks including p53 signaling, the cell cycle, and mitochondrial priming, stem cells must navigate the delicate balance between resistance and sensitivity. Resistance puts stem cells at risk of accumulating mutations that might lead to cancer, whereas sensitivity is important for genetic stability but may cause stem cell depletion, compromising function and contributing to aging. The more we learn about how hESCs and adult stem cells from different tissues walk this fine line (Box 2), the better able we will be to predict cellular responses to stress and damage and potentially manipulate them for therapeutic purposes.

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