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Evolved Cellular Mechanisms to Respond to Genotoxic Insults: Implications for Radiation-Induced Hematologic Malignancies

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Abstract

Human exposure to ionizing radiation is highly associated with adverse health effects, including reduced hematopoietic cell function and increased risk of carcinogenesis. The hematopoietic deficits manifest across blood cell types and persist for years after radiation exposure, suggesting a long-lived and multi-potent cellular reservoir for radiation-induced effects. As such, research has focused on identifying both the immediate and latent hematopoietic stem cell responses to radiation exposure. Radiation-associated effects on hematopoietic function and malignancy development have generally been attributed to the direct induction of mutations resulting from radiation-induced DNA damage. Other studies have illuminated the role of cellular programs that both limit and enhance radiation-induced tissue phenotypes and carcinogenesis. In this review, distinct but collaborative cellular responses to genotoxic insults are highlighted, with an emphasis on how these programmed responses impact hematopoietic cellular fitness and competition. These radiation-induced cellular programs include apoptosis, senescence and impaired self-renewal within the hematopoietic stem cell (HSC) pool. In the context of sporadic DNA damage to a cell, these cellular responses act in concert to restore tissue function and prevent selection for adaptive oncogenic mutations. But in the contexts of whole-tissue exposure or whole-body exposure to genotoxins, such as radiotherapy or chemotherapy, we propose that these programs can contribute to long-lasting tissue impairment and increased carcinogenesis.

HUMAN EXPOSURE TO GENOTOXINS

Throughout our evolution, humans (and nonhuman ancestors) have been exposed to natural sources of ionizing radiation from the environment, such as air and soil. In the modern era, exposure to radiation has dramatically increased, primarily due to medical applications. Currently, exposure to man-made radiation constitutes about one-half of an individual's yearly radiation dose, of which over 90% occurs through medical procedures (U.S. Nuclear

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Regulatory Commission, www.nrc.gov). Radiation therapy is used to treat approximately 60% of solid tumors and the growing application of computed tomography (CT) scans for diagnostic purposes has increased radiation exposures (*1, 2*). The number of CT scans performed has increased over 20-fold, from nearly 3.3 million in the early 1980s to about 80 million in 2010 (*3*). Concurrently, the average yearly dose for medical radiation exposure increased nearly sixfold between 1982 and 2006, from 0.54 mSV to about 3 mSv (*4–7*). While current occupational exposures have decreased over recent years with the ALARA approach, in some professions such as those involving interventional radiology, exposures can limit time on the job (*8, 9*). Radiation exposure is also a concern for manned space exploration (*10*) and several proposed missions would exceed permissible exposure limits (*11, 12*).

Genotoxic agents, including ionizing radiation, have a considerable impact on the rapidly cycling cell populations of the gastrointestinal and hematopoietic systems (*13, 14*). Consequently, the symptoms exhibited shortly after total-body exposure to genotoxic agents include vomiting, diarrhea and pancytopenia. For acute radiation syndrome, the presentation and severity of symptoms, as well as its medical management, depend largely on the dose received (*15, 16*).

EFFECTS OF GENOTOXIC INSULTS ON THE HEMATOPOIETIC COMPARTMENT

The hierarchical structure, rapid turnover and ability to longitudinally assay bone marrow and peripheral blood of humans make the hematopoietic compartment an ideal system in which to study the long-term effects of radiation exposure. Individuals exposed to radiation present with a wide array of hematopoietic defects that can persist for up to 50 years after exposure. Long-term studies following atomic bomb survivors have documented reduced hemoglobin levels (*17*), reduced helper T cell frequencies and increased inflammatory cytokines in the peripheral blood (*18*). Additional long-term studies on atomic bomb survivors have identified reductions in total peripheral T-cell numbers and function, as well as altered B cell frequencies and antibody production (*19*). Moreover, survivors of childhood leukemias treated with chemo- and radiotherapies have developed long-term impairments in tissue functions, which are particularly problematic for those who received radiotherapy (*20, 21*). The success of modern therapies in improving long-term survival for cancer patients has been accompanied by the growing problem of secondary cancers, particularly myeloid leukemias, resulting from exposures to therapeutic radiation and chemotherapy (*22, 23*). It has been shown that pediatric leukemia patients treated with chemo- and/or radiotherapy are at higher risk for secondary malignancies as adults (*20*). The incidence of these secondary malignancies is expected to continue to rise, since cure rates for common childhood cancers, such as acute lymphoid leukemias (ALL), have increased from a few percent to about 90% in the last 50 years (*24*). Many chemotherapeutics are known genotoxic agents, and a number of the effects described here that apply to radiation exposures would also apply to these chemical exposures. Studies have also shown that irradiated individuals, including atomic bomb survivors, have an increased risk of developing acute myeloid leukemia (AML) (*23, 25–27*).

Molecular characterization of malignancies associated with radiation exposure has provided valuable information on their initiation and progression. In a study of atomic bomb survivors, mutations in the *AML1* (previously known as *RUNX1*) gene were found in nearly 50% of individuals examined who developed myelodysplastic syndrome (MDS) (*28*), while genomic alterations in *AML1* have been identified in less than 5% of all AML patient samples (*29–32*). *AML1* regulates the expression of the C/EBPα transcription factor, and leukemia-associated translocations and mutations involving either the *AML1* or *CEBPA* genes have been shown to result in the inhibition of C/EBPα activity (*33–39*). C/EBPa is a critical regulator of granulocyte differentiation (*40, 41*) and reducing this differentiative activity may be key for leukemia development. In addition, it has been shown that the incidence of TP53 mutations is higher in therapy-related rather than *de novo* AML (*42–44*). Interestingly, in two cases tumor cells with *TP53* mutations expanded from *TP53* mutant cells present prior to cytotoxic therapy, leading to selection of TP53 mutant cells in the context of therapy, rather than the direct generation of mutant cells by the therapy (*45*).

In addition to an increased risk for AML, studies following irradiated individuals have shown an increased risk for other leukemias, including ALL and chronic myeloid leukemia (*25*). Additionally, patients receiving chemo- and radiotherapy have been shown to develop secondary cancers in the form of solid tumors, including breast, bladder and lung, among others (*25, 46–49*).

MOUSE MODELS OF RADIATION-INDUCED LEUKEMIA

Studies using inbred mouse strains that are predisposed to myeloid leukemia after whole-body irradiation have provided valuable insight into radiation-induced myeloid leukemias. Rivina *et al.* published a comprehensive review of these mouse models (*50*). After irradiation, RF, SJL/J, C3H and CBA mice develop myeloid leukemias that are morphologically similar to human AML and also have similar dose-response kinetics. These strains differ in their susceptibility to spontaneous leukemia, incidence of radiation-induced myeloid leukemia and induction of other radiation-induced cancers, including lymphoma. Notably, hemizygous deletions in chromosome 2 have been identified in the radiation-induced myeloid leukemias of these strains. *Sfpi1*, which encodes the PU.1 protein, maps within the deleted region of chromosome 2, and the nondeleted copy of the gene is generally mutated in radiation-induced AMLs, leading to biallelic inactivation. PU.1 is a lineage-specific transcription factor and its inhibition results in a blockade of hematopoietic differentiation, similar to that observed with C/EBPa inhibition. Interestingly, mice with genetic impairment of C/EBPα or PU.1 develop AML disease within months after irradiation (*51, 52*).

In contrast to the strains above, C57BL/6 mice are resistant to radiogenic myeloid leukemias, and instead develop a disease similar to human T-cell ALL (T-ALL) after irradiation (*53, 54*). Over 60% of human T-ALL patient samples possess an activating mutation in Notch (*55*), an important hematopoietic stem cell (HSC) self-renewal-promoting transcription factor (*56–61*). Moreover, Notch1-activating mutations are found in about half of radiation-induced thymic lymphomas in mice (*54*).

Susceptibilities to radiogenic hematopoietic malignancies are genetically determined in mice, with some inbred strains being predisposed to T-cell lymphomas and others to myeloid leukemias. The initial radiation-induced damage to the target cells should be the same regardless of strain. This raises the question of what accounts for the interstrain susceptibility differences. One possibility is that different strains experience different frequencies of pre-leukemic mutations in Notch or *Sfpi1* in HSCs and primitive progenitor cells on which radiation-induced selection will act, biasing leukemia development towards lymphoid or myeloid lineage. In addition, we argue that key effects of radiation exposure within the HSC pool include not only induction of oncogenic mutations, but also alterations in selective pressures: the radiation-perturbed HSC pool and microenvironment will engender selection for oncogenic events (that might not be advantageous under nonperturbed condition conditions). For example, exposure to radiation of strains predisposed to myeloid leukemias may result in preferential selection for cells carrying genomic alterations that result in PU.1 or C/EBPα inhibition, whereas selective pressure in strains predisposed to T-lymphoid leukemias may be preferentially exerted on cells carrying activating mutations in Notch1. Although both PU.1/C/EBPα inhibition and Notch activation would enhance self-renewal and inhibit the differentiation of HSCs, these oncogenic events promote myeloid and T-cell lineage bias, respectively. As humans mostly develop myeloid leukemias after radiation therapy, we suggest that there may be increased selection for inactivation of myeloid differentiation programs after irradiation in human HSCs.

RATE-LIMITING STEPS IN RADIATION LEUKEMOGENESIS AND LYMPHOMAGENESIS

Historically, the rate-limiting step in radiation-induced cancer has largely been attributed to the accumulation of oncogenic mutations arising from radiation-induced DNA damage (*18, 62–64*). Indeed, exposure to radiation has been shown to result in a wide array of DNA damage, of which double-strand breaks are considered to be the most pertinent with regards to leukemogenesis and lymphomagenesis (*65, 66*). However, the mutation-centric model ignores a key contributor to cancer evolution, selection and how context can impact the adaptive value of mutations.

When considering tissue integrity and carcinogenesis, the influence of natural competition among cells vying for niche occupation must be considered. Cellular fitness, defined as the ability of a cell to transmit a particular epigenotype/genotype onto the next cell generation, dictates the competitiveness of a particular cell clone with respect to local competitor clones (Fig. 1). Cells with high fitness preferentially expand, clearing out less fit cells and thus promoting tissue integrity. Cell fitness and competition are context dependent, with the relative fitness of one cell being dependent on the status of neighboring competitor cells, as well as on the cell's adaptation to its microenvironment (*67*). Heritable alterations that impact cell function influence that clone's respective fitness and thus its clonal potential. Many oncogenic mutations actually result in reduced HSC self-renewal under physiological conditions in young, relatively unperturbed bone marrow, even while increasing cell cycling (*68*). In the physiological context of competition with fit HSCs, these rare mutant cells

would be typically lost from the HSC pool [e.g., due to differentiation (*68*)] and replaced by surrounding competitor cells.

Phenotypic selection has long been observed at the species level, as changes in environment can result in alterations in the adaptive value of particular traits. The fuel on which phenotypic selection acts lies within the genetic variation of a species. When changes occur in the environment, natural selection acts on this phenotypic variation. This process results in selection for phenotypic traits most suited to the new environment and elimination of traits that have become disadvantageous. Thus, phenotypic selection allows for a species to adapt to changing environments. This same concept can be applied at the cellular level.

Taking into account both cellular competition and phenotypic selection, an alternative model of carcinogenesis has been proposed: adaptive oncogenesis (Fig. 2) (*69*, *70*). In this model, a key rate-limiting step in cancer progression is selection for context-specific adaptive oncogenic mutations. According to this model, cells within a young, healthy, unperturbed HSC pool are functioning near optimally. In this highly fit context, acquisition of a phenotype-altering mutation is not likely to provide a cell with an advantage relative to local competing cells, and cells with such mutations should therefore be maintained at low levels or eliminated from the HSC pool. In contrast, pool-wide exposure to environmental insults or aging results in alterations to tissue function and cellular fitness. The resulting reduction in cellular fitness creates ‘‘room for improvement’’, which increases the likelihood that a particular phenotype-altering mutation will be adaptive. Cells harboring such an advantageous mutation would be selected and preferentially expanded, creating a larger pool in which secondary mutations may be acquired. This selective pressure for only adaptive oncogenic mutations that provide an advantage can thus comprise a rate-limiting step in oncogenesis.

Healthy hematopoiesis can be viewed as a form of tumor suppression, by providing cellular competition to eliminate unfit cells, which would include most cells with phenotypealtering mutations. For example, most radiotherapy treatment regimens do not directly impact all early hematopoietic progenitor cells (although the effects of radiation can extend beyond the irradiated field). The hematopoietic compartment’s capacity for circulation provides an opportunity for migration of and repopulation by nondamaged distal HSCs, which can outcompete the irradiated HSCs. In support, addition of an out-of-field HSC migration parameter (along with HSC inactivation, initiation and proliferation) corrected the previous overestimation of relative leukemia risk after local irradiation to a value more representative of actual epidemiologic data (*71*).

Moreover, in classic experiments by Kaplan and colleagues, the incidence of thymic lymphomas in mice after irradiation is substantially reduced by shielding either the hindlimb or spleen during irradiation, or by transplantation of nonirradiated cells postirradiation (*72*–*74*). Exposure of the shielded extremity to local irradiation within 24 h of the primary radiation exposure event resulted in restored incidence of lymphomas (*75*). These results suggest that residual nonirradiated hematopoietic stem/progenitor cells are able to prevent carcinogenesis, perhaps through restoration of healthy hematopoiesis and thus prevention of oncogenic selection. Nonetheless, it is notable that other experiments appear to show that

shielding of mice partially (but not fully) prevents radiation-induced AML development (*76*) and injection of nonirradiated bone marrow postirradiation also failed to prevent AML inductions (*77*). Currently, it is not clear why radiation-induced thymic lymphomas are effectively suppressed by partial protection of the hematopoietic bone marrow, while those for AML are only variably suppressed (depending on the experiment). Notably, secondary hematopoietic malignancies do occur after focal radiation therapy in humans (*78, 79*), although the incidence of secondary AMLs after localized irradiation does appear low (*80*). These secondary malignancies may be driven more by localized effects (particularly for malignancies initiated in solid tissues) or by radiation-induced bystander effects. More research will be required to determine the molecular, cellular and systemic mechanisms influencing leukemogenesis/lymphomagenesis after focal irradiation.

EVOLVED CELLULAR MECHANISMS TO MAINTAIN FITNESS AFTER GENOTOXIC INSULT

Multiple cellular mechanisms that exploit cell competition and phenotypic selection have evolved to remove damaged cells, preventing their contributions to hematopoiesis long-term (Fig. 3). To some extent, these successive responses may be dependent on the degree of cellular damage (although we note that this is speculative). To prevent fixation of DNA damage, cells with damaged DNA initiate a DNA damage response (DDR) with the goal of repairing damage. The type of DDR that is initiated is dependent on the cellular state. Although no difference in radiosensitivity was identified, cycling HSCs were found to preferentially initiate repair by homologous recombination repair (HRR) whereas quiescent HSCs favor the more error-prone non-homologous end-joining (NHEJ) pathway (*81*). Thus the pool of HSCs that remain after irradiation may contain radiation-induced genomic aberrations.

Cells with extensive DNA damage often undergo apoptosis or programmed cell death. Apoptosis is one of the first cellular programs evident after exposure to a genotoxic insult. It has been shown that radiation-induced apoptosis of thymocytes is dependent on p53 and Puma (*82–84*). Multiple studies have shown that radiation-induced cell competition between HSCs shortly after exposure is dependent on the relative p53 status of neighboring competitor HSCs (*85, 86*). This competition allows for removal of more damaged cells, shown to be those with high p53 levels relative to competitors.

DNA damage response signaling has also been shown to induce senescence, an irreversible cell cycle arrest. Radiation exposure results in the induction of senescence within the HSC population, and senescent HSCs are apparent months after the insult (*87, 88*). Exposure of C57BL/6 mice to 6.5 Gy of ^{137}Csγ rays resulted in sustained decreases in the number of HSCs. A portion of HSCs from previously irradiated but homeostatically restored mice stained positive for SA-β-gal, a commonly used marker for cellular senescence (*89*). The authors concluded that radiation exposure results in increased senescence, and thereby decreased *in vivo* replication potential and *in vivo* clonogenic potential.

More recent studies indicate that long after the genotoxic exposure event the number and function of HSCs is significantly reduced (*87, 88, 90*). Previously irradiated bone marrow

cells compete poorly with nonirradiated bone marrow cells in competitive bone marrow transplant assays, and limiting dilution transplantation assays revealed a nearly tenfold reduction in HSCs repopulating activity present months after a sublethal dose of total-body irradiation (TBI) (*90*). Additionally, previously irradiated HSCs expressed elevated levels of reactive oxygen species and increased expression of senescence markers (*87*, *88*).

An explanation of the reduced HSC numbers and function months after irradiation may lie in radiation-induced skewing in HSCs away from self-renewal towards differentiation. Shortly after irradiation, HSCs exhibit increased lymphoid differentiation in a GCSF/STAT-3/BATF-dependent manner (*91*). In accord, a decrease in HSC numbers was identified at 24 h postirradiation, suggesting that BATF-mediated lymphoid differentiation of HSCs after irradiation leads to a more myeloid-biased HSC pool over time. An additional study identified reduced self-renewal and enhanced differentiation of the HSCs present in mice months postirradiation (*92*). This reduced self-renewal and precocious differentiation (myeloid, at least *in vivo*) was in part due to an increase in C/EBPa activity. Remarkably, this particular defect of irradiated HSCs was reversible *in vivo* upon activation of Notch, a promoter of self-renewal (*56–61*), or inhibition of C/EBPa. The diminished self-renewal and precocious differentiation of HSCs is a process known as ‘‘programmed mediocrity’’. Essentially, damaged cells are ‘‘programmed’’ to become ‘‘mediocre’’, resulting in reduced fitness of the damaged cells and eventually their competitive elimination. Programmed mediocrity allows for removal of damaged HSCs from the HSC pool while still allowing their temporary contribution to the rapidly turned-over differentiated pool. This restoration of pool fitness by gradual replacement of damaged HSCs by healthy competitor HSCs may exert less stress on residual HSCs compared to that which would be exerted by an immediate and large void due to, for example, mass apoptosis.

Immune-mediated removal of genetically altered cells in *Drosophila* has also been shown to play a role in promoting somatic cell pool fitness. Meyer and colleagues demonstrated that removal of less fit ‘‘loser’’ wing disc clones required the activation of innate immune mechanisms, particularly of the Toll receptor pathway (*93*). This pathway was shown to impinge on the expression of Myc, potentially a key barometer of cellular fitness. Such immune-mediated clearance of ‘‘less-fit’’ competitors may also contribute to the elimination of genotoxically damaged cells in mammals.

MYC: A POTENTIAL BAROMETER FOR CELL FITNESS

Myc is known to play a role in many cell fate decisions that influence tissue maintenance, including proliferation, apoptosis, senescence and cellular differentiation (*94*). Previously irradiated HSCs, which exhibit reduced self-renewal and precocious differentiation but no apoptotic phenotype, also exhibit a marked and significant decrease in Myc target gene expression, without alterations in the level of Myc itself (*92*). This altered Myc signature correlates with alterations in cellular fitness in the context of prior irradiation. Because of Myc’s pleiotropic biologic activities, we propose that Myc activity may serve as a barometer of cellular fitness, orchestrating cell fate decisions to maintain tissue integrity during homeostasis and in response to genotoxic insults (*95*) (Fig. 4).

It has been shown that relative levels of Myc activity can determine whether a cell is a good or poor competitor during normal tissue development. *Drosophila* wing disc cells that express Myc at ~1.5-fold above the endogenous level outcompete cells expressing wild-type levels of Myc (*95*). Removal of these less-fit cells occurred in a Toll receptor- and NFκB-dependent manner that was shown to impinge upon Myc. Studies utilizing induced random genetic mosaics in mouse epiblast and embryonic stem cell populations corroborate cell competition as being influenced by relative Myc levels (*96*). Modest overexpression (~1.5×) of Myc *in vivo* in a subpopulation of mouse epiblast resulted in the competitive elimination of epiblast expressing wild-type Myc levels. Further, cells expressing wild-type levels of Myc or only one allele of Myc outcompeted cells expressing relatively lower levels of Myc. Again, elimination of the less-fit, low Myc expressers occurred via apoptosis and phagocytosis by their more fit neighbors. While these examples demonstrate cell competition as a mechanism to maintain tissue integrity during normal tissue development, cell competition could also mediate the elimination of stem cells damaged by genotoxic insults such as radiation exposure.

Though modest increases in Myc expression may improve cellular fitness, studies have shown that high levels of Myc overexpression can drive apoptosis, and that this can serve as an intrinsic tumor-suppressive mechanism (*94*). Using mice that were heterozygous or homozygous for conditionally expressed Myc, Murphy and colleagues showed that low levels of deregulated Myc expression drove proliferation, but higher levels drove apoptosis, suggesting that the ability of Myc to drive opposing biological processes may be governed by different thresh-olds of Myc expression (*97*). Thus, if a cell highly expresses Myc, at levels that may be oncogenic, it is eliminated from a tissue via apoptosis. So, while regenerating tissue after genotoxic insult requires cell proliferation, this intrinsic tumor-suppressive mechanism may serve to ensure the appropriate proliferative potential.

In HSCs, Myc levels have been shown to influence self-renewal and differentiation. Conditional inactivation of *Myc* in mouse bone marrow cells results in severe cytopenia, caused by a block in differentiation that results in the accumulation of LT-HSC and decreased proliferation of lineage cells (*98*). Further investigation revealed that while *Myc*-deficient HSCs accumulate in the bone marrow, they are nonfunctional, as assessed by bone marrow transplant and CFU assay (*99*). Moreover, forced Myc expression resulted in loss of long-term self-renewal activity, due to premature differentiation rather than increased apoptosis, ultimately resulting in reduced HSC fitness (*68, 98*). Overall, these studies show that Myc may differentially impact HSC fitness based on its expression levels. Perhaps a certain level of Myc expression makes a cell well adapted to the HSC niche, but deviations from that level result in its elimination from the niche, such as via differentiation.

At either extreme of Myc expression, grossly over- or underexpressed, cells may be directly removed from a tissue by apoptosis, senescence or differentiation. However, more subtle deviations may result in tissue fine-tuning by cell competition, where fitness appears to be dictated not by absolute levels of Myc but rather on levels relative to neighboring competitor cells. We hypothesize that this Myc barometer contributes to radiation-induced elimination of damaged cells [as irradiated HSCs exhibit reduced Myc-dependent transcription (*92*)], and could also prevent the fixation of oncogenic mutations that lead to substantial increases

in Myc activity (Fig. 4). Sequential cellular processes employed to re-equilibrate cell pool fitness back to near optimum –apoptosis, senescence, programmed mediocrity and immune-mediated removal of damaged cells – may progressively alter the overall Myc phenotype of the HSC pool. For instance, after irradiation, clones with relatively lower levels of Myc may be eliminated via programmed mediocrity. Thus, the HSC pool strives to reattain optimum Myc levels. Further studies are required to elucidate how Myc integrates information on cell fitness to maintain tissue integrity in response to genotoxic stresses.

CELLULAR RESPONSES TO GENOTOXINS: CONTEXT-DEPENDENT TUMOR SUPPRESSION AND PROMOTION

We propose that responses to genotoxic insults can be tumor suppressive or tumor promoting depending on the proportion of the HSC population exposed. Humans have evolved to deal with stochastic damage to the occasional cell, and in this context these processes should suppress tumorigenesis (Fig. 5A). By eliciting the removal of the rare damaged HSC, tumor suppression is achieved by, 1. eliminating cells with potentially oncogenic genetic changes and 2. restoring and maintaining HSC pool fitness, thus limiting selection for adaptive oncogenic events.

In contrast, cellular responses to genotoxic insults become tumor promoting in the more modern context of system-wide DNA damage, whereby the function of a critical number of HSCs are altered (Fig. 5B). Such a decrease in mean-population fitness creates ‘‘room for improvement’’ and thereby increases selective pressure for acquisition of adaptive oncogenic mutations that repair or circumvent the fitness defect. For example, TBI results in expansion of more radioresistant HSC clones that possess reduced p53 activity (*85*, *86*). Possession of a loss-of-function mutation in *TP53* (encoding p53) at the time of irradiation confers an immediate survival advantage to hematopoietic cells, promoting clonal expansion of the mutant cells after irradiation and thus increasing the risk of lymphomagenesis. In contrast, experimental inhibition of p53 function months after irradiation did not confer a selective advantage to HSCs (*90*). Furthermore, the selective advantage of p53-deficient cells postirradiation was found to be dependent on the relative p53 status of neighboring competitor cells (*85*). Both studies observed no selection for p53 inhibition under homeostatic conditions. Notably, the increased prevalence of *TP53* mutations in t-AML (relative to spontaneous AMLs) in humans has been suggested to result from cytotoxic therapy-induced selective expansion of *TP53* clones that were present prior to induction of therapy (*45*). In all, these studies indicate that reduced p53 function is advantageous at the time of the genotoxic insult, when apoptosis and DNA damage are prevalent, but not at later time points when these processes have subsided.

Recent studies have also demonstrated that selection for and leukemogenesis driven by the activated Notch1 mutant ICN1 is enhanced long after the initial genotoxic exposure event (*90*). In the context of adaptive oncogenesis, this increased selection for ICN1 suggests that radiation exposure results in persistent defects within the HSC compartment that may be reversed by activation of Notch. In fact, the decreased self-renewal and increased differentiation of previously irradiated HSCs was reversible *in vivo* by activation of Notch or inhibition of C/EBPa (*92*). Interestingly, the activation of Notch restored the Myc gene

expression signature, providing at least one potential explanation for adaptation in the context of prior irradiation. As described above, *CEBPA* loss-of-function and *NOTCH1* gain-of-function mutations have been associated with AML and T-ALL, respectively (*33–39*, *55*), including, as discussed above, radiation-associated leukemias. Collectively, these studies indicate that exposure to radiation results in persistent changes in the function and fitness of individual HSCs, which thereby alters selection for particular oncogenes. In these studies, the preferential expansion of HSCs with *TP53* loss-of-function mutations shortly after irradiation as well as of HSCs with activating mutations in *NOTCH1* at later time points after irradiation were abrogated by the presence of nonirradiated, healthy competitor cells. Thus, a highly fit hematopoietic stem and progenitor cell pool is inherently tumor suppressive.

CONCLUSIONS

Human exposure to radiation continues to increase, in a large part due to increased use and repeated exposure in medical procedures. Exposure to radiation results in both acute and long-term health effects in the hematopoietic compartment, including an increased risk of carcinogenesis. The effects of exposure to radiation on HSCs and early progenitor cell populations have been of particular interest, as these cells are long-lived and multipotent. Radiation-associated leukemogenesis has largely been attributed to radiation-induced DNA damage resulting in the direct generation of oncogenic mutations. However, cell fitness and competition for niche space are also key determinants of carcinogenesis. Numerous studies have identified cellular responses that have evolved to remove damaged HSCs from contributing to hematopoiesis, including increased apoptosis, senescence and differentiation (*85–88*, *90–92*).

We propose that these processes can be either tumor suppressive or promoting, depending on the extent of radiation-induced damage in the HSC and progenitor populations and the proportion of cells affected. In the context of sporadic DNA damage, a limited number of cells will be affected, and healthy, less damaged cells can outcompete the more damaged cells for niche occupancy. The more damaged cells will be progressively removed from the pool by apoptosis, senescence or differentiation, depending on the degree of damage received. These evolved cellular responses to genotoxic damage maintain tissue integrity and restore cell pool fitness after insult, which in turn reduce selective pressures that could promote fixation of adaptive oncogenic mutations.

In the context of systemic DNA damage, such as after TBI, essentially all HSCs and progenitor cells will be affected. Although cellular competition will still lead to removal of the most damaged cells from the pool, all remaining cells will still have undergone damage above normal endogenous levels, resulting in a reduction in the mean fitness of the cell pool. Consequently, selective pressure will be increased for cells that possess adaptive oncogenic mutations that provide an advantage relative to neighboring cells. Thus in the context of systemic damage, these cellular responses become tumor promoting.

Expanding our knowledge of how cells respond to sporadic and wide-spread genotoxic insults, how these responses shape the evolutionary landscape of the HSC compartment, and

the influence of such changes on selection for adaptive oncogenic events and subsequent leukemogenesis could allow for identification of candidate molecules that may be therapeutically employed to mitigate undesired consequences of radiation exposure. Such studies will be crucial to the development of treatment strategies and therapeutics to prevent, restore or even reverse radiation-induced hematopoietic disease.

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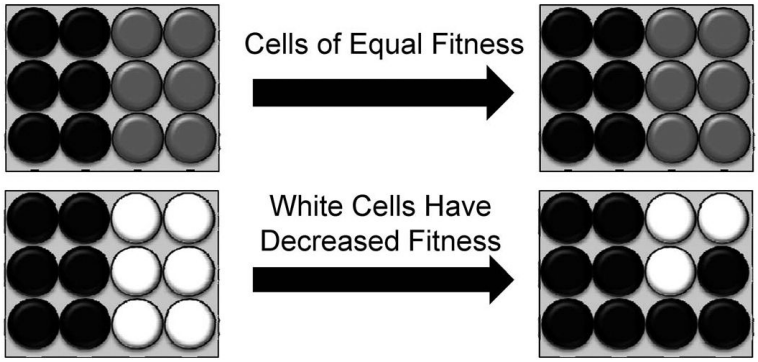

FIG. 1.

Relative cell fitness. A measure of the ability of a stem/ progenitor cell of a certain epigenotype/genotype to pass this type on to subsequent cell generations. Cells with equivalent cell fitness (top panel) contribute equally to future generations. Cells with lower fitness (bottom panel, white cells) relative to a competitor cell population (black cells) will compose a smaller proportion of future cell generations.

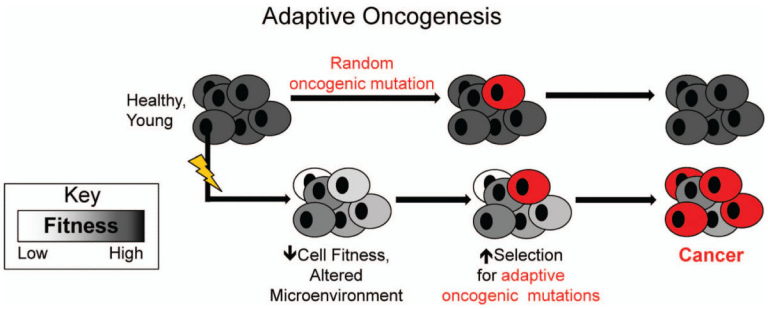

FIG. 2.

Adaptive oncogenesis. In this model, young, healthy cells are well adapted to their microenvironment and have high fitness. Acquisition of an oncogenic mutation in a young, healthy population is thus unlikely to provide any benefit, and is therefore maintained at low levels or, if detrimental, removed from the HSC population. Environmental insults, such as TBI, reduce HSC function and thus pool fitness. Particular oncogenic mutations are now more likely to provide a cell with an advantage. Upon acquisition of an adaptive oncogenic mutation, the mutant cell will outcompete less-fit neighbor cells, clonally expand and promote leukemogenesis.

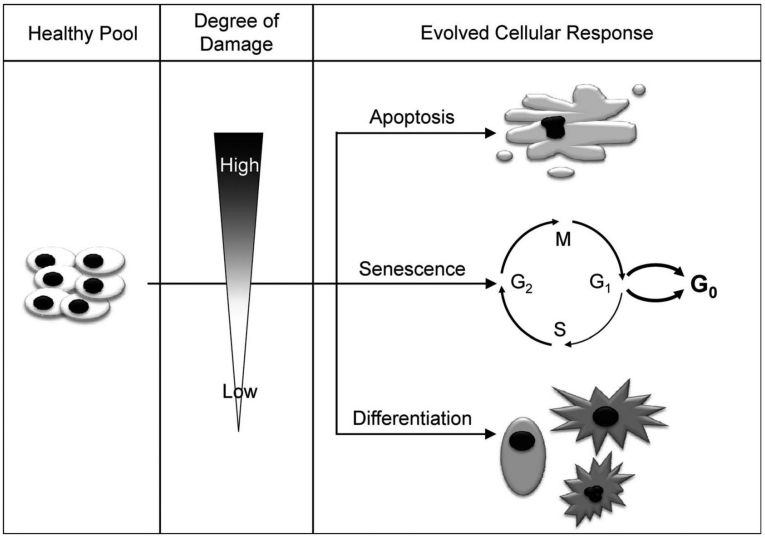

FIG. 3.

Evolved mechanisms for removal of damaged cells. Radiation-damaged cells are removed by apoptosis, senescence and programmed mediocrity (increased differentiation, reduced self-renewal), and we propose that these processes could act sequentially with time after radiation exposure and/or be determined based on the amount of damage received.

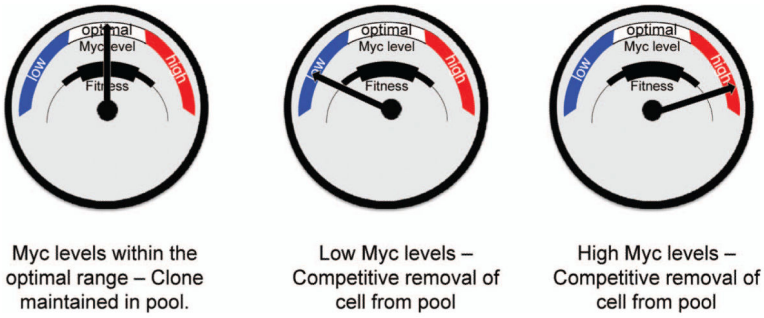

FIG. 4.

Myc, a barometer of relative cell fitness. Optimal Myc levels may dictate optimal cell fitness. Cells with low fitness express Myc levels below or above that of the Myc level found in cells with optimal fitness.

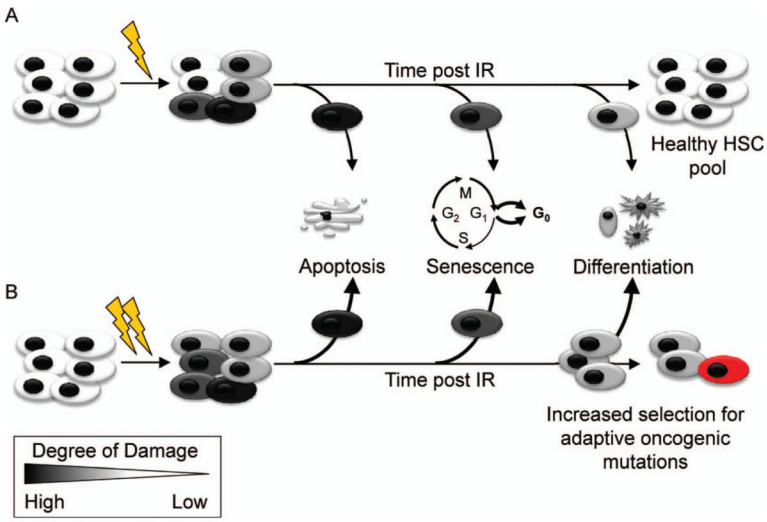

FIG. 5.

Tumor-suppressive and -promoting roles of cellular responses to radiation exposure. Panel A: In the context of sporadic or local genotoxic insults, as with most radio- and chemotherapy treatments, a small fraction of the HSC population is damaged. Damaged HSCs are removed by evolved cellular responses: apoptosis, senescence and programmed mediocrity (increased differentiation, reduced self-renewal). The mean pool fitness is restored upon repopulation of the HSC compartment by healthy, nondamaged HSCs that circulate, proliferate and self-renew. Panel B: In the context of population-wide damage, such as TBI, all HSCs are damaged. The most damaged HSCs are removed by apoptosis, followed by lesser damaged HSCs being removed by senescence; residual HSCs exhibit programmed mediocrity and thus reduced fitness. These processes, from apoptosis to senescence to increased differentiation potential, will engender strong selective pressure for HSCs with oncogenic mutations that repair or circumvent these programs.