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Wiley Interdiscip Rev Dev Biol. Author manuscript; available in PMC 2013 October 30.

Published in final edited form as:
Wiley Interdiscip Rev Dev Biol. 2012 ; 1(6): . doi:10.1002/wdev.56.

Stress and stem cells

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Abstract

The unique properties and functions of stem cells make them particularly susceptible to stresses and also lead to their regulation by stress. Stem cell division must respond to the demand to replenish cells during normal tissue turnover as well as in response to damage. Oxidative stress, mechanical stress, growth factors, and cytokines signal stem cell division and differentiation. Many of the conserved pathways regulating stem cell self-renewal and differentiation are also stress-response pathways. The long life span and division potential of stem cells create a propensity for transformation (cancer) and specific stress responses such as apoptosis and senescence act as antitumor mechanisms. Quiescence regulated by CDK inhibitors and a hypoxic niche regulated by FOXO transcription factor function to reduce stress for several types of stem cells to facilitate long-term maintenance. Aging is a particularly relevant stress for stem cells, because repeated demands on stem cell function over the life span can have cumulative cell-autonomous effects including epigenetic dysregulation, mutations, and telomere erosion. In addition, aging of the organism impairs function of the stem cell niche and systemic signals, including chronic inflammation and oxidative stress.

INTRODUCTION

Stem cells are functionally defined by the capacity for self-renewal and the ability to differentiate into multiple cell types. Embryonic stem cells (ESCs) are pluripotent, meaning they can differentiate into all of the >200 cell types of the body, including the germ line. ESCs in culture can go through hundreds of passages, apparently without limit (immortal).[1] Adult stem cells are found in variety of tissues, including intestine, brain, bone marrow, pancreas, liver, skin, skeletal muscle, and kidney, and can differentiate into a limited range of adult cells (multi-potent), typically the adult tissue in which they are found. Adult stem cells support extensive and sustained tissue renewal through adult life span and have an extensive, but ultimately limited, replication potential (mortal).

Stress can take several forms at the level of the cell and the organism (Table 1). Extrinsic stress is defined as an environmental factor that causes a change in a biological system that is potentially injurious.[2] Intrinsic stresses include specific metabolic challenges such as accumulation of waste products and the generation of reactive metabolites during normal metabolism [e.g. reactive oxygen species (ROS)] as well as accumulated damage and stresses imposed by repeated cell division.

Aging can be interpreted as a stress with particular relevance to stem cells, involving characteristic extrinsic and intrinsic stresses. Aging in biological systems (senescence) is defined as a deteriorative change that causes increased mortality[3] and is thought to arise

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from the presence of gene alleles with deleterious effects that are manifested as deleterious phenotypes at late ages.[4] Effects of such deleterious alleles may be autonomous to the stem cells, as well as non-cell-autonomous, such as altered systemic signaling and cell contacts. For example, aging is characterized by mitochondrial malfunction,[5,6] oxidative stress,[7] proteotoxic stress[8,9], and inflammation,[10] each of which may inhibit normal stem cell function.

Stress reduction in stem cells is critical due to their essential role and because of the risk for malignant transformation (cancer). Mammalian stem cells have several features in common that can be interpreted as antistress and antitransformation protective mechanisms. Stem cells typically reside in protected locations within the tissue and organism, for example, the intestinal crypt and the bone marrow, thereby helping to shield them from extrinsic stresses[11] (Figure 1). The stem cell niche is the specialized microenvironment that confers upon the stem cells the ability to self-renew.[12] For certain stem cell types, the niche has been found to be a hypoxic environment, which is expected to reduce oxidative stress in the stem cells.[13,14] Stem cells often have upregulated stress response and repair pathways, for example, increased chaperone expression[15] and homologous recombination[16] in ESCs, and increased transporter expression in adult stem cells that may facilitate removal of toxins.[17] Finally, relative to their differentiating progeny, the stem cells are generally small in number and divide slowly, thereby reducing the target size and rate for transforming mutations. Apoptosis and cellular senescence are responses to stress that serve as anticancer mechanisms by preventing further cell division, and replicative stress and cellular senescence pathways are implicated in limiting long-term stem cell maintenance and function.

TYPES OF STEM CELL STRESS

Cell division rates must be balanced with rates of cell turnover and apoptosis so that tissues do not hypertrophy or atrophy. Notably, the signals controlling stem cell division in response to tissue needs often include the same stresses that, when chronic or present outside the homeostatic range, are potentially or actually injurious.

Intrinsic Stress to Stem Cells

Because stem cell function involves repeated cell division to replenish tissues, stress due to cell replication is particularly relevant. Replicative senescence (or the ‘Hayflick limit’) was first characterized in mammalian fibroblasts and refers to the limited number of cell divisions that normal cells can undergo *in vitro* before entering an irreversible cell cycle arrest.[18,19] The cells undergo characteristic changes in gene expression and cell morphology and are resistant to apoptosis. This phenotype is linked to telomere shortening and activation of p53 and pRb, and the consequent induction of cell cycle inhibitors such as p16 and p21. In addition to cell division, additional stresses can cause a senescent phenotype, sometimes called stress-induced premature senescence,[20] and together, these senescent phenotypes are called cellular senescence. These stresses include telomere shortening, oxidative stress, DNA damage, and expression of activated oncogenes such as RAS, HER-2, and PTEN. These genotoxic stresses also activate the tumor suppressor proteins p53 and pRb and result in accumulation of cell cycle inhibitors such as p16 and p21. Telomere erosion is accelerated by oxidative stress, and the telomere appears to function as an organelle that integrates several stress signals to control senescence. Cellular senescence is also associated with disruptions in normal patterns of chromatin modifications and gene expression. It appears clear that cellular senescence pathways normally function as potent antitumor mechanisms, because mutations in the tumor suppressor genes *p53* and *Rb* cause increased cancer incidence.[19] In addition, in certain instances, cellular senescence may play a beneficial role in limiting damage to tissues under stress.[21] As discussed below, increasing evidence

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implicates replicative stress and cellular senescence pathways in limiting the function of specific stem cell types.

Oxidative stress in the form of ROS is produced as a product of normal metabolism as well as through other mechanisms. Oxidative stress is particularly relevant to stem cells because it damages all cellular macromolecules, including DNA, and in mammalian cells, oxidative stress promotes both apoptosis and cellular senescence.[22] In addition to hypoxic environments, stem cells protect themselves autonomously through oxidative stress response pathways including FOXO and hypoxia-inducible factor (HIF),[23] as discussed further below. ESCs and adult stem cells including hematopoietic stem cells (HSCs) also preferentially use glycolysis to generate ATP, as opposed to oxidative phosphorylation, thereby reducing the production of ROS.[14,24]

Extrinsic Stress to Stem Cells

Extrinsic stress to the stem cells includes systemic stresses such as changes in circulating hormones as well as environmental stresses that cause tissue and cellular damage. For example, immune challenge and inflammation can stimulate mammalian HSC proliferation to support cellular immune response[25] and the release of stem cells into the circulation.[26] Inflammation in response to tissue damage signals stem cell activation and repair in muscle and other tissues.[27] Chronic inflammation, such as that associated with aging, is implicated in cancer progression and the maintenance of cancer stem cells.[28] Cancer stem cells are hypothesized to support the growth of specific tumors analogous to the way adult stem cells support normal tissues.[29] The cancer stem cells may derive from adult stem cell populations or their descendants through a process involving multiple mutations. Aging results in additional changes to systemic signals that can negatively impact stem cell function, for example, circulating factors in serum from old mice can inhibit muscle stem cell function.[30] Environmental stresses include radiation and xenobiotic toxins, often used as anticancer therapies.[31] Anticancer therapies typically function by hyper-stimulating otherwise repressed apoptosis or senescence pathways in dividing cells,[32,33] and because stem cells are dividing, they are particularly susceptible to damage from these interventions. Many extrinsic stresses have tissuespecific consequences for stem cells through increased demand for cell division and replacement. Notably, in both invertebrates and mammals, nutrition and consequent metabolic stress can regulate stem cell dynamics through insulin-like signaling and other mechanisms.[34,35]

Biomechanical stresses and physical interactions with the extracellular matrix are emerging as important factors in regulating stem cell biology. While severe biomechanical stress such as shear stress and hydrodynamic stress can disrupt or destroy the cell, within a homeostatic range, these same stresses can regulate proliferation and differentiation to meet the needs for normal cell turnover.[36-38] For example, mammalian stem cell differentiation is affected by the elasticity of the extracellular matrix: soft matrices that mimicked brain promoted neurogenesis, moderately stiff matrices that mimicked muscle were myogenic, whereas stiff matrices akin to bone were osteogenic.[39,40]

TISSUE-SPECIFIC STEM CELLS AND STRESSES

Representative tissues illustrate tissue-general and tissue-specific features of the response of stem cells to stress.

Hematopoetic Stem Cells

The mammalian HSC was the first stem cell identified and is among the best studied, in particular in humans and mouse models. Blood cells turn over rapidly and are replenished from a small reserve of HSCs located in the bone marrow (Figure 1).

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Hematopoietic stem Cells, Replicative Stress, and Senescence—Bone marrow transplants facilitate the assay of HSC self-renewal and pluripotency and the responses of these cells to stress. A single transplanted HSC is capable of replenishing a mouse host in which the endogenous HSCs have been ablated by sublethal irradiation.[41,42] Serial bone marrow transfer experiments in mice indicate that HSCs have a finite replication potential; however, this limit is not reached until several mouse lifetimes.[23,43-46] This tends to suggest that replicative senescence does not necessarily limit HSC function; however, during mouse aging, the HSCs show extensive evidence of age-related declines in function, including a decrease in the number of cells with long-term repopulating potential in serial transplant experiments.[43,47] These data indicate that the *in vivo* setting and age-related stresses are causing a specific cellular senescence phenotype and a progressive loss of functional HSCs.

Most HSCs are quiescent (in the G0 phase of the cell cycle), and this slow-cycling state appears to function to reduce metabolic and ROS stress and ensure long-term maintenance of the stem cells.[48,49] Several studies indicate that HSCs can be further subdivided into relatively more active and dormant populations (Figure 1), although this conclusion is controversial and the extent to which it is observed may vary by mouse strain. The active HSCs can enter the cell cycle and reconstitute mice in transplantation experiments but do not sustain serial transplantation. In contrast, the dormant population appears to cycle only about five times through the mouse life span, and this population may be responsible for injury repair, but not the daily regeneration of the blood.[50] Stem cell populations in the skin and gut tissues may also be subdivided into relatively dormant and active subsets (Figure 1). These dormant populations of stem cells can be interpreted as a further mechanism to reduce stress on the cells and ensure their long-term maintenance.

Aging is a particularly important stress for the HSCs. In humans, an aging-related loss of normal HSC function is indicated by decreased immunity, expansion of the myeloid lineage, and mild to moderate normocytic anemia.[51] The age-associated lineage skewing, toward myeloid rather than lymphoid lineages, is associated with reduced lymphopoiesis and adaptive immunity and increased myeloproliferative disease, including leukemias. Notably, increased donor age in human bone marrow transplants is a predictor of transplant-related mortality, consistent with cell-autonomous HSC defects.[52] Similarly, aging mice exhibit a decreased number of functionally competent HSCs and a skew toward myeloid lineages over lymphoid lineages. As in humans, these defects appear to be cell autonomous, as transplantation of HSCs from old mice into young recipients does not reverse the age-related changes.[53,54] Studies in mice suggest that clonal selection processes may contribute to this skew, as myeloid-biased HSCs appear to have greater self-renewal potential.[55]

Mouse models defective in DNA repair, ROS response, and telomere maintenance demonstrate that these pathways are required for long-term maintenance and stress responses of the HSCs.[56,57] Telomeres in humans and mice shorten with age in stem cell compartments, and telomere-deficient mice show eventual defects in tissue maintenance. Telomere erosion may signal through activation of p53/p21 to block self-renewal and function of HSCs, as deletion of p21 can rescue stem cell function and tissue maintenance in telomere-deficient mice.[58] In contrast, p21 also appears to play a beneficial role in HSC stress resistance.[59] Experiments in which isolated HSCs were challenged with ionizing radiation indicated that HSCs have an upregulated DNA damage response involving p53- and p21-mediated growth arrest and survival, relative to their more differentiated progeny, which tended to enter apoptosis.[60] Cycling and quiescent HSCs also differed in their DNA repair responses: dividing HSCs were able to use the homologous recombination pathway, whereas quiescent HSCs were dependent upon non-homologous end-joining, thereby increasing the chances for mutations and translocations.[60,61]

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Changes in epigenetic state are emerging as an important mechanism for aging-related changes in HSC function.[22] For example, the protein BMI1 is a component of the polycomb repressive complex 1 that methylates lysine 27 of histone H3 to repress gene expression. Mouse HSCs with reduced BMI1 activity have reduced long-term repopulating activity in transplantation assays and reduced differentiation potential *in vitro*, indicating an important role for BMI1 in HSC maintenance.[62] Targets of repression by BMI1 include the *Ink4a/Arf* locus, which encodes the senescence-promoting factors p16 and p19[63] and the *Chk2* locus, which encodes a cell cycle checkpoint protein.[64] Epigenetic derepression of these loci is implicated in HSC senescence, as a deletion of the *Ink4a/Arf* locus yielded improved functional capacity of HSCs in serial transfer experiments,[65] and deletion of the *Chk2* locus rescued the premature aging phenotype of BMI1 null-mutant mice.[64]

Hematopoetic Stem Cells and Oxidative Stress—The HSC system highlights the importance of oxidative stress in regulating stem cell function. HSC senescence is promoted by ROS, through activation of the p38/MAPK pathway and upregulation of the p16 cell cycle inhibitor.[23,43,66-69] The ROS nitric oxide is produced by the enzyme nitric oxide synthase (NOS). Experimentally reducing NOS activity in mice caused increased numbers of HSCs, suggesting that nitric oxide may normally function to limit HSC division, possibly through its ability to stimulate expression of the cell cycle inhibitor p21.

Several mechanisms and stress responses appear to function to limit oxidative stress to the mammalian HSCs. For example, HSCs preferentially use glycolysis rather than oxidative phosphoryation to make ATP, which likely contributes to lower ROS levels.[14] Notably, HSCs with lower ROS levels are observed to have greater self-renewal potential.[70] The HSCs have been found to reside in a hypoxic niche, which likely serves to reduce ROS and damage in the HSCs, and to prevent premature differentiation.[13,71] Most of the slow-cycling HSCs are located in zones near the bone surface and distal from capillaries (Figure 1), consistent with an antiproliferative effect of hypoxia.[72] Hypoxia of the bone marrow has been detected using gas analysis of human tissue samples,[73] and hypoxia of HSCs is indicated by reduced accessibility to the circulating dye Hoeschst, and by marking with the hypoxia-sensitive reagent pimonidazole.[74] The hypoxic niche appears to favor hypoxia within the HSCs and the consequent stabilization of the HIF-1alpha transcription factor, which in turn drives expression of cell-cycle inhibitors such as p16(Ink4a)/p19(Arf) to promote long-term maintenance.[75] Consistent with this conclusion, when cultured *in vitro*, hypoxic conditions reduced HSC proliferation and favored long-term repopulation potential.[76] In *Drosophila*, ROS favor differentiation of hematopoietic progenitor cells, suggesting a conserved role for ROS in regulating proliferation and differentiation of the hematopoietic lineage across species.[77]

FOXO family transcription factors regulate cell cycle arrest, DNA repair, differentiation, and apoptosis and also protect quiescent cells from oxidative stress.[13,78] *Foxo3a* deletion mice have impaired HSC function, increased ROS, and p38/MAPK pathway activation.[79] The mice are defective in maintaining HSC quiescence and are susceptible to myelotoxic injury. Similarly, conditional inactivation of Foxo1,3,4 in the adult mouse hematopoietic system caused defects in long-term repopulating activity and increased ROS and apoptosis that could be partially rescued by antioxidant treatment.[80]

In addition to FOXO, several other signaling and transcription factors function to reduce ROS levels in mammalian HSCs. The HIF-1alpha transcription factor regulates genes involved in energy metabolism, angiogenesis, and apoptosis. As discussed above, HSCs maintain lower ROS by stabilizing HIF under hypoxic conditions, and consequently deletion of HIF in mice leads to loss of HSC stem cell quiescence.[75] The ATM kinase regulates cell cycle checkpoints in response to DNA damage and disruptions in chromatin structure. ATM

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mutant mice have defects in HSC renewal, increased ROS levels, and activation of the p38/MAPK stress response pathway, and notably, these defects are partially rescued by antioxidant treatment.[81] Finally, the BMI1 transcriptional repressor discussed above is involved in self-renewal of HSCs, as well as neural stem cells (NSCs), intestinal stem cells (ISCs), and leukemic cells.[22] BMI1 functions as a p16 repressor, and BMI1 overexpression can extend the replicative life span of fibroblasts, consistent with a role in regulation of senescence.[63] BMI1 knockout mice exhibit increased ROS levels, DNA damage, and defects in HSC self-renewal, and these tissue homeostasis defects could be partially blocked by antioxidant treatment.[64] Therefore, HIF, ATM, BMI1, and FOXO factors modulate ROS at the stem cell level to promote maintenance, and an important question for the future is to identify the relevant targets of these factors that function to reduce ROS.

Muscle Stem Cells

Muscle stem cells (satellite cells), along with other myogenic progenitor cells, can regenerate new mammalian muscle fibers in response to stress caused by exercise, injury, or degenerative disease.[82] Several signals derived from damaged muscle fibers and infiltrating cells are involved in activating proliferation and differentiation of muscle stem cells, including the growth factors IGF and HGF, and the ROS nitric oxide. Within the muscle stem cells, TGFβ and Notch signaling pathways are involved in regulating proliferation, whereas WNT signaling is implicated in the transition to differentiation.[83]

Muscle stem cell function and signaling pathways are negatively affected by the stress of aging and aging-related systemic factors. Most studies indicate a decrease in muscle stem cell numbers in old mammals. During aging, damaged muscle fibers are less efficiently replaced, and the inflammatory response is prolonged.[84] Serum from old mice could convert myogenic precursors toward a fibrogenic phenotype, thereby impairing muscle regeneration.[85] WNT signaling is implicated in this mechanism, as the effect of old serum could be reproduced by WNT3a, and the effect of old serum could be reduced by a WNT inhibitor. In addition, old serum contained elevated levels of TGFβ, which can synergize with WNT signaling.[30]

The high metabolic activity of muscle tissue may subject muscle stem cells to oxidative stress, and in transplantation experiments, mouse muscle stem cells with relatively higher expression of the antioxidants superoxide dismutase and glutathione had a survival and differentiation advantage.[86]

Intestinal Stem Cells

The intestine is a site of rapid cellular turnover, and the ISCs exhibit regulation by several kinds of stress. *Drosophila* has emerged as a particularly tractable system in which to study ISCs and the effects of stress. In both *Drosophila* and humans, ISC proliferation and differentiation are controlled by the WNT, NOTCH, and JAK/STAT pathways.[87] The adult *Drosophila* midgut is maintained by continued division of ISCs.[88,89] ISCs divide to produce an ISC and an enter-oblast cell, and the enteroblast can then differentiate into either an enterocyte or an enteroendocrine cell (diagrammed in Figure 2). During normal aging and in response to the dietary oxidative stressor paraquat, the ISCs hyperproliferate to produce undifferentiated and misdifferentiated enteroblasts, coincident with disruption of normal gut structure (intestinal dysplasia).[90] The *escargot* gene is abundantly expressed in the ISCs and enteroblasts, and *escargot*-GFP transgenic reporter constructs allow the ISCs and intestinal dysplasia to be visualized using fluorescence microscopy (Figure 3). ISC proliferation and dysplasia of the gut in response to aging and environmental stresses is promoted by the JNK oxidative stress-response pathway. In contrast, the redox-regulatory transcription factor Nrf2 is constitutively active in the *Drosophila* ISCs, where it acts to reduce ROS levels and limit

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proliferation.[91] In both *Drosophila* and humans, stressed and dying gut cells send signals to stimulate proliferation and differentiation of the ISCs to support tissue homeostasis (Figure 4), and chronic inflammation may cause abnormal activation of this pathway. The intestine houses and interacts with a complex microbial flora. Both normal and pathogenic bacteria as well as nutritional stress can stimulate division of the ISCs, through a mechanism that involves activation of the EGFR/RAS/MAPK stress-response pathway[92] (Figure 4).

In mammals, the intestinal epithelium exhibits the most rapid turnover among adult tissues, making ISCs particularly subject to replication stress and the accumulation of mutations,[93,94] including mitochondrial DNA mutations.[95] While replicative senescence of the ISCs has not been detected, functional deficits during aging are observed, including increased sensitivity to ionizing radiation, and a reduced rate of repopulation of damaged tissue.[96] Chronic inflammation, such as that associated with aging and inflammatory bowel disease, appears to favor the accumulation of mutations and the progression to colorectal cancer in the ISC lineage.[93]

Brain Stem Cells

In mammals, stem cell-based neurogenesis is limited to the subgranular zone of the dentate gyrus and the subependymal zone adjacent to the lateral ventrical.[97] Oxidative stress plays a key role in regulating NSCs. For example, mild hypoxia stimulates NSC self-renewal,[98] and consistent with this, the NSC niche has been found to be hypoxic and to favor HIF1-alpha stabilization.[99] FOXO family transcription factors have been found to be particularly important in maintaining NSC function, in part by reducing ROS.[100] FOXO1,3,4 knock-out mice have disrupted NSC function, characterized by enlarged brains and increased proliferation of NSCs in culture, but a decrease in NSC number and brain atrophy with age.[101] These results are consistent with a role for FOXO in maintaining NSCs by limiting ROS-induced differentiation and preventing premature exhaustion of functional NSC populations.

Bone and Endothelial Stem Cells

As the major structural support of the mammalian body, the bones are subject to significant mechanical forces and stress. Similarly, the blood vessels are subject to hydrodynamic stress. Correspondingly, division and differentiation of the bone stem cells and the vascular endothelial stem cells is found to be stimulated by mechanical stresses, including shear stress, and these effects are associated with increases in the ROS nitric oxide and activation of BMP and NFkappaB signaling.[36,102]

Hair Follicle Stem Cells and Melanocyte Stem Cells

The mammalian hair follicle is a tractable system for study of stem cells due to its accessibility and self-contained modular structure (Figure 1). Immune stress and altered systemic stress hormone signaling can inhibit hair follicle stem cell maintenance and lead to localized hair loss (alopecia areata).[103] Hair graying characterizes mammalian aging and is caused by loss of functional melanocyte stem cells. In mice, genotoxic stress caused by ionizing radiation or ATM gene mutation caused loss of functional melanocyte stem cells by inducing premature differentiation.[104]

STRESS RESPONSE PATHWAYS IN STEM CELLS

A number of stress-response pathways, including those described above, play well-characterized or emerging roles in regulating stem cell maintenance and differentiation. This includes a number of factors important in regulating stem cell maintenance and

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differentiation that have only recently been identified as components of stress-response pathways.

p53 and p21

The mammalian p53 transcription factor integrates multiple stress inputs and regulates survival and self-renewal in stem cells,[105,106] and negatively regulates reprogramming.[107] p53 regulates normal metabolism and cellular redox state, as well as apoptosis and cellular senescence in response to genotoxic and oxidative stress. p53 regulates cell senescence by activating expression of cell cycle inhibitors including p21. p21 plays a dual role in mammalian stem cells: it functions in maintaining acutely damaged stem cells in the quiescent state to avoid acute genotoxic damage, but also mediates cellular senescence during aging in response to chronic oxidative stress.[59]

FOXO and Insulin-Like Signaling

FOXO family transcription factors are activated by oxidative stress response pathways including JNK and repressed by growth-promoting pathways including insulin-like signaling.[13,108] They protect quiescent stem cells from oxidative stress and suppress ROS-induced differentiation, thereby facilitating long-term maintenance of stem cells. Insulin-like signaling is a key nutrient sensing pathway and can regulate stem cell dynamics in response to nutrient availability.[109]

WNT

The WNT pathway regulates both stem cell maintenance and differentiation and overactivation of WNT depletes stem cells in several compartments in mammals.[110,111] WNT signaling is activated by ROS through the thioredoxin-related protein nucleoredoxin.[112]

Nrf2/Keap1

Nrf2 is a key regulator of cellular redox state. In *Drosophila* ISCs, Nrf2 is constitutively active and reduces ROS levels, and repression of Nrf2 by Keap1 is required for ISC proliferation.[91]

Heat shock transcription factor

Heat shock transcription factor regulates expression of heat shock proteins and molecular chaperones and is a key component of the response to proteotoxic stress. Mammalian ESCs are characterized by high levels of chaperone expression and stress tolerance.[15]

NFkappaB

NFkappaB transcription factor is activated by stresses including UV radiation, oxidative stress, and viral infection, regulates inflammation, innate immunity, development, and apoptosis, and can promote differentiation in stem cells.[113]

OCT

OCT4 regulates pluripotency in mammalian ESCs and may be regulated by oxidative stress (hypoxia) through HIF2alpha.[114]

Bone morphogenetic protein

Bone morphogenetic proteins (BMPs) belong to the TGFβ superfamily and regulate mammalian stem cell maintenance and differentiation in several systems, including bone

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and vascular systems, where BMP signaling can be induced by mechanosensitive generation of ROS.[115]

Lamin A

LAMIN A is emerging as a transducer of stress-responsive signaling pathways involved in mammalian stem cell maintenance and differentiation, including pRb, MAPK, p53, WNT, and BMP.[116]

Hippo

The Hippo pathway regulates organ size in *Drosophila* and mammals by inhibiting cell proliferation and promoting apoptosis and also regulates stem and progenitor cell self-renewal and expansion. In response to tissue damage, Hippo functions to promote ISC proliferation.[117]

PLURIPOTENCY, REPROGRAMMING, AND STRESS

As discussed above, mammalian ESCs have the ability to differentiate into all of the adult cell lineages (pluripotency) and are characterized by essentially unlimited replication potential (immortal), as evidenced by long-term *in vitro* culture experiments. Stress pathway signaling, including p38/MAPK and JNK, have been shown to promote differentiation of ESCs toward specific lineages.[118]

Simultaneous forced expression of a small number of transcription factors, including OCT4 and SOX2, can reprogram differentiated mammalian cells back to a pluripotent state called induced pluripotent stem cells (iPS), and these cells have significant implications for clinical interventions. Recently, the oxidative stress-response regulator FOXO1 has been shown to be an essential positive regulator of pluripotency in human and mouse ESCs, where it acts in part by promoting expression of OCT4 and SOX2.[119,120] In contrast, the redox-regulatory factor p53 acts as a negative regulator of reprogramming, by responding to moderate DNA damage and telomere erosion in otherwise reprogrammable cells.[107] These results suggest the importance of stress-response pathways and (oxidative) stress reduction in maintaining the pluripotent state. Consistent with this, a shift from oxidative phosphorylation metabolism to glycolysis is associated with the acquisition of pluripotency upon reprogramming of mouse embryonic fibroblasts.[24]

Maintaining function in cultured and stored embryos, ESCs, and iPS cells is challenging because it requires stringent optimization of conditions to reduce the harmful effects of unwanted stress. When grown in culture, ESCs can accumulate chromosomal aberrations and become aneuploid, presumably due to the stress of *in vitro* conditions. For this reason, researchers typically limit ESC passage number and routinely check karyotypes. Conditioning hormesis is the ability of low doses of a stress to protect against subsequent higher doses of that stress or other stresses,[121] apparently by upregulating protective stress response pathways. Notably, carefully administered, sub-lethal doses of various stresses, including hydrostatic pressure, osmotic, heat, and oxidative stress, have been found to improve viability and differentiation potential of embryos and ESCs during various manipulations.[122]

STEM CELL SEX AND STRESS RESISTANCE

Systemic sex hormones and the sex of the stem cell are emerging as potentially important factors in stem cell biology and stress responses. Sex of the patient is a significant predictor of clinical outcome in cardiovascular disease, and sexual dimorphism is observed in the function of muscle stem cells and endothelial stem cells in the repair and regeneration of

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post-ischemic damage.[123] These differences are due in part to the different effects of male and female sex steroids on stem cells,[123,124] consistent with systemic effects of the organism's sex on stem cell function and stress responses. *In vitro* analyses with purified mammalian stem cell populations support a role for sex steroids in regulating proliferation and differentiation. For example, progesterone and estrogen favor proliferation and differentiation of mouse ESCs into neuronal lineages.[125] In addition to systemic effects, the sex of the stem cell itself may affect function. For example, the sex of donor cells affected outcomes in rat NSC transplantation experiments.[124] Muscle stem cells can be transplanted into dystrophic (*mdx*) mutant host mice where they will regenerate skeletal muscle. In this assay, female muscle stem cells were reported to have somewhat greater regeneration capacity compared to male cells.[126,127] Moreover, male muscle stem cells were found to more readily undergo differentiation *in vitro* in response to oxidative stress, suggesting a model in which male cells are less effective in transplantation experiments due to stress-induced premature exhaustion of the pool. Differences in stress resistance and drug responses between male and female animals are ubiquitous,[128-130] consistent with possible cell-autonomous differences in stress responses of male and female stem cells.

CONCLUSIONS

The analysis of stem cell model systems reveals the central importance of stress-response pathways in the maintenance and function of stem cells. Stress is a common mechanism involved in stimulating stem cell division and differentiation in response to tissue needs for normal homeostasis and injury repair. Controlling and responding to intrinsic and extrinsic stress is critical for the long-term maintenance of functional stem cell populations. Oxidative stress in particular has emerged as a common feature that limits stem cell maintenance and disrupts function. As stem cell biology moves into the clinic, controlling stress becomes increasingly important in producing and maintaining functional stem cell reagents and in controlling their differentiation to produce desired cell types for use as interventions in disease and aging.

Acknowledgments

I thank the reviewers for their detailed and insightful additions and corrections. The author is supported by a grant from the Department of Health and Human Services (AG011833).

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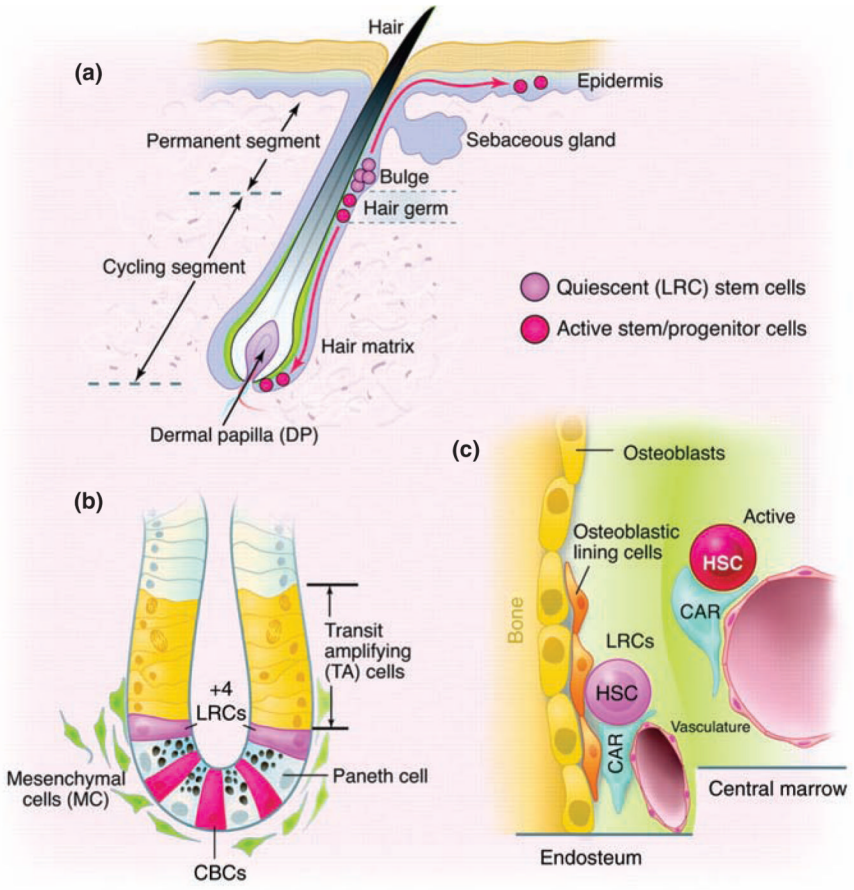

FIGURE 1.

Stem cells reside in protected locations and many rarely divide. (a) Hair follicle stem cells. Hair follicle structure with quiescent (bulge)and active (hair germ) stem/progenitor cells. Bulge area typically maintains quiescent stem cells, whereas DP provides stimulatory signals. Only during development and under injury condition, bulge stem cells give rise to stem cells in epidermis label-retaining cells (LRC). (b) Intestinal crypt and intestinal stem cells. Intestinal crypt structure with quiescent (+4) and active crypt-based columnar (CBC) (Lgr5+) stem cells, as well as TA and mesenchymal cells. (c) Bone marrow and hematopoietic stem cells (HSCs). Quiescent HSCs located in the endosteal region where osteoblastic lining, endothelial, CXCL12-abundant reticular (CAR), and other cells form the endosteal region and active HSCs located in the central marrow region, which lacks osteoblastic cells. (Reprinted with permission from Ref 11. Copyright 2010 The American Association for the Advancement of Science)

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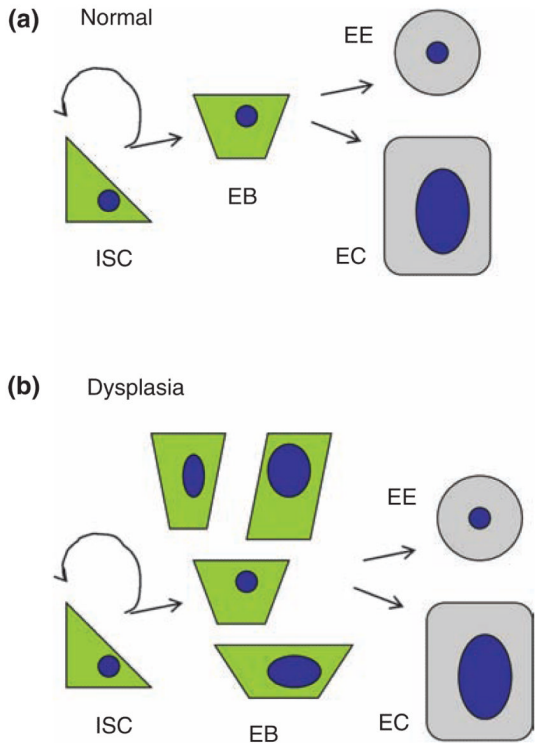

FIGURE 2.

Diagram of *Drosophila* intestinal stem cell (ISC) lineage and intestinal dysplasia. (a) Diagram of ISC lineage. (a) Normal conditions. The ISCs divide to produce an ISC and an enteroblast (EB) cell; the EB then differentiates into either an enterocyte (EC) or an enteroendocrine (EE) cell. (b) Dysplasia. During normal aging and in response to the dietary oxidative stressor paraquat, the ISCs hyperproliferate to produce undifferentiated and misdifferentiated EBs, coincident with disruption of normal gut structure (intestinal dysplasia). The *escargot* gene is abundantly expressed in the ISCs and EBs, and *escargot*-GFP transgenic reporter constructs allow the ISCs and intestinal dysplasia to be visualized using fluorescence microscopy (indicated in green).

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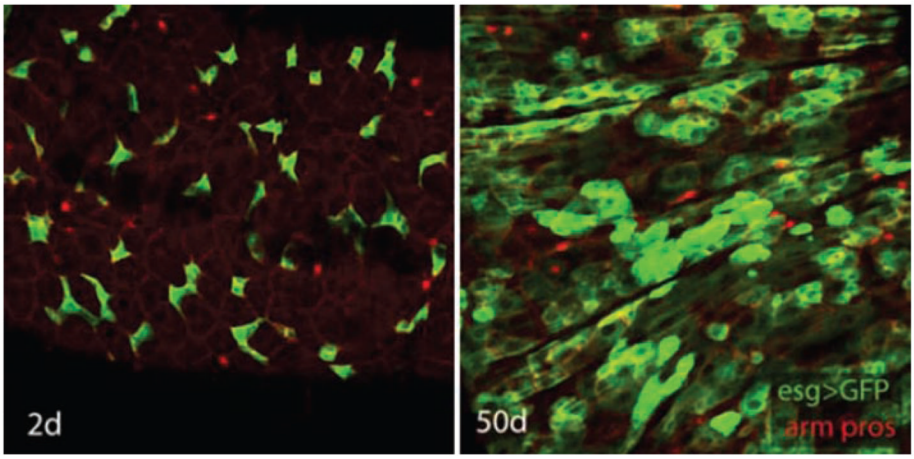

FIGURE 3.

An *escargot*-GFP transgenic reporter marks the *Drosophila* ISC lineage and stress dysplasia. Confocal images of guts dissected from 2-day old (2d) and 50-day old (50d) flies expressing GFP in the ISCs and enteroblasts (EBs) (genotype: w^{1118}; *esg-Gal4*, *UAS-GFP*). ISCs and EBs are labeled by GFP expression (green). Cell boundaries are labeled by immunostaining against Armadillo (membrane marker, red). Enteroendocrine cells are labeled by nuclear *pros* staining (nuclear marker, red). (Reprinted with permission from Ref 90. Copyright 2008 Elsevier)

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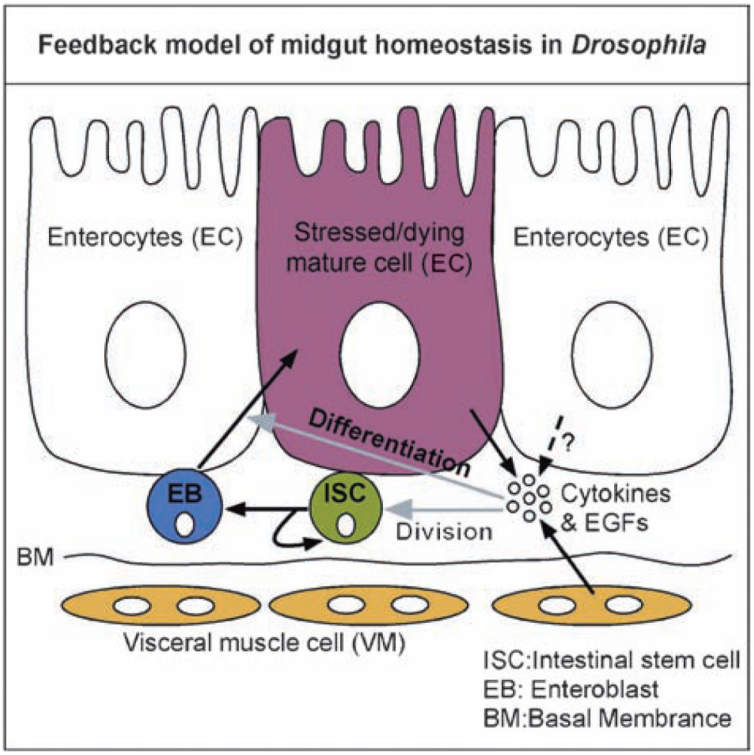

FIGURE 4.

Feedback regulation of intestinal stem cell (ISC) proliferation in response to stress. Stressed or dying enterocytes induce the expression of fly cytokines (such as Upd3 and Upd2) and EGFs (such as Krn and Vn) in the midgut, which activate the JAK/STAT and EGFR pathways in the midgut progenitor cells. Whereas EGFR signaling functions mainly to promote ISC proliferation, Jak/Stat signaling functions to promote both ISC proliferation and enteroblast differentiation. (Reprinted with permission from Ref 92. Copyright 2011 Elsevier)

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TABLE 1

Examples of stem cell stress

Intrinsic
Non-optimal genetic configuration (mutations, inbreeding depression, deleterious alleles).
Non-optimal epigenetic configuration (chromatin modifications, DNA methylation).
Reactive oxygen species (ROS; can damage all macromolecules, cause mutations, promote telomere erosion, promote apoptosis, promote premature/abnormal differentiation; normal levels required for signaling, proliferation, differentiation).
Other toxic metabolites (produced as byproducts of normal metabolism).
Cell division (favors mutations, epigenetic disruptions, telomere erosion).
Telomere erosion (ultimately signals DNA damage response and cellular senescence).
Extrinsic
Non-optimal physical contacts (cell—cell and cell—extracellular matrix interaction are required for normal signaling, adhesion, cellular orientation).
Non-optimal signals (includes hormones, intercellular signals, cytokines).
Inflammation (a non-specific immune response including specific cytokines).
Non-optimal temperature.
Non-optimal nutrients.
Mechanical forces (shear stress, hydrodynamic stress; can disrupt physical contacts).
Drugs and chemotherapeutics.
Radiation.
Non-optimal oxygen levels (increased ambient oxygen favors cellular ROS).