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CellPress REVIEWS

Review

Nrf2: Redox and Metabolic Regulator of Stem Cell State and Function

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Nuclear factor erythroid 2-related factor 2 (Nrf2) is ubiquitously expressed in most eukaryotic cells and functions to induce a broad range of cellular defenses against exogenous and endogenous stresses, including oxidants, xenobiotics, and excessive nutrient/metabolite supply. Because the production and fate of stem cells are often modulated by cellular redox and metabolic homeostasis, important roles of Nrf2 have emerged in the regulation of stem cell quiescence, survival, self-renewal, proliferation, senescence, and differentiation. In a rapidly advancing field, this review summarizes Nrf2 signaling in the context of stem cell state and function and provides a rationale for Nrf2 as a therapeutic target in stem cell-based regenerative medicine.

Highlights

As a cellular metabolic and stress sensor, the transcription factor Nrf2 is a pivotal regulator of stem cell self-renewal, proliferation, and differentiation.

Nrf2 displays cell type-specific and/or stage-dependent impact on stem cell biology in response to various environmental cues.

Nrf2 modulates PSCs through the regulation of pluripotency factors, metabolism, redox homeostasis, and cellular stress responses.

Nrf2 maintain ASCs self-renewal, quiescence, and regenerative capacity while protecting against ASC depletion in response to stress and aging.

Nrf2: Effector and Regulator of Redox and Metabolic Homeostasis in Stem Cells

Nrf2 is a stress-responsive transcription factor encoded by the *NFE2L2* gene in humans [1]. Although previously considered to function primarily as an antioxidative transcription factor, Nrf2 is now recognized to be involved in the cellular response to multiple stressors including xenobiotics, excessive nutrient/metabolite supply, inflammation, and the accumulation of misfolded proteins (Box 1) [2–4]. Although Nrf2 regulation in cancer, diabetes, and aging is well studied [2,4–9], the role of Nrf2 signaling in stem cells is not clear.

Stem cells, including pluripotent stem cells (PSCs) and adult tissue stem cells (ASCs) (Box 2), possess unique metabolic programs and reduction–oxidation (redox) states to sustain proliferation while maintaining **pluripotency** (see Glossary) or **multipotency** and/or specified **differentiation** [10–12]. In stem cells, ATP is mainly produced by **glycolysis** and **oxidative phosphorylation (OXPHOS)** during **self-renewal** and differentiation [13]. Quiescent PSCs rely primarily on glycolysis for energy with lower respiration rates, reduced **reactive oxygen species (ROS)** production, and elevated antioxidant enzyme expression [10]. PSC differentiation results in a metabolic shift from glycolysis to OXPHOS, which has been shown to revert back to glycolysis after reprogramming to pluripotency [10,14]. Most quiescent ASCs in their **niches** tend to prefer glycolysis and fatty acid oxidation with high levels of transcription factors, such as Nrf2, driven antioxidant enzyme expression to suppress ROS signaling [13]. Upon activation by stress or injury, proliferating ASCs increase their oxygen use via the influence of growth factor kinase signaling, alter cell metabolite levels and redox states, lower their expression of antioxidant enzymes, and activate ROS signaling [10,13]. Therefore, the stem cell metabolic state and redox profile can be used as an index of stem cell self-renewal, pluripotency or multipotency, and differentiation. Consistent with an essential role for energy regulation in stem cell survival and function, redox biology and metabolic programming-related genes are among the most enriched transcripts and proteins present in stem cells [13,15–17]. Nrf2 is a common upstream regulator of many of these genes suggesting that Nrf2 can serve as a master regulator of stem cell redox and metabolic homeostasis [13,18,19]. In this review, we summarize and discuss recent progress in understanding Nrf2 regulatory signaling in stem cells, and highlight the role of Nrf2 in controlling stem cell redox states, metabolic homeostasis, survival, self-renewal, pluripotency, proliferation, differentiation, and reprogramming (Table 1).

Nrf2 Regulates the Balance between Embryonic Stem Cell Stemness and Germ Layer Differentiation by Finely Tuning the Expression of Pluripotency Genes

Embryonic stem cells (ESCs) are derived from the inner cell mass of an early-stage preimplantation embryo blastocyst and have provided critical insights into disease modeling and treatment due to their pluripotency. Nrf2 plays critical roles in human ESC fate determination (Figure 1A). Nrf2

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Box 1. Nrf2 Structure and Function

Nrf2 belongs to the cap 'n' collar transcription factor family. Human Nrf2 possesses a conserved basic leucine zipper structure and seven functional Nrf2-ECH homology (Neh) domains, Neh1–7 [98]: Neh1 interacts with small musculoaponeurotic fibrosarcoma (Maf) and binds to antioxidant response element (ARE)-DNA; Neh2 binds to Keap1; Neh3–5 are required for transactivation of Nrf2; Neh6 regulates stability of Nrf2; and Neh7 is involved in activation of transcription, retinoid X receptor α binds to the Neh7 domain and downregulates Nrf2, suggesting a mechanism for Nrf2 repression independent of Neh2-Keap1.

Nrf2 activity can be regulated at multiple levels, including Nrf2 transcription, post-transcriptional regulation, and post-translational modification. At the transcription level, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [99], aryl hydrocarbon receptor [100], Kras, Braf, Myc, and Jun [101] can bind to the *Nrf2* promoter to enhance *Nrf2* transcription. The phosphoinositide-3-kinase–protein kinase B (PI3K–Akt) [102] and Notch signaling pathways [103] have also been reported to augment *Nrf2* transcription. Nrf2 also appears to autoregulate its own expression through an ARE-like element located in the proximal promoter region, leading to persistent nuclear accumulation of Nrf2 and protracted induction of phase 2 genes in response to chemopreventative agents [104]. Nrf2 can also be transcriptionally repressed. For example, *Nrf2* promoter modifications, including hypermethylation or single nucleotide polymorphisms result in decreased *Nrf2* expression [105]. *Nrf2* post-transcriptional regulation occurs mainly via miRNA. Several miRNAs, including miR-27a, miR-144, and miR-93 have been identified to reduce *Nrf2* mRNA expression [106–108]. In addition, Nrf2 can be regulated through post-transcriptional alternative splicing, and *Nrf2* mRNA splice variants lack the Keap1 interaction domain resulting in Nrf2 stabilization [109]. Nrf2 is also subject to post-translational modifications including phosphorylation, acetylation, ubiquitination, sumoylation, and distinct chaperon interactions that may finely tune degradation, nuclear translocation, nuclear residence, nuclear export, or transactivation capacity [4].

Nrf2 is ubiquitously expressed in most eukaryotic cells and serves as a primary regulator of numerous inducible cell defense systems through the regulated expression of more than 200 downstream cytoprotective genes, including antioxidant proteins, detoxification and metabolism enzymes, transport proteins, proteasome subunits, chaperones, growth factors and their receptors, and transcription factors [90,110]. Extensive research has deciphered the signaling pathways regulated by Nrf2 that are involved in regulating redox homeostasis, detoxification, autophagy, mitochondrial bioenergetics, lipid synthesis, transport and degradation, fatty acid oxidation, gluconeogenesis, and metabolic reprogramming.

expression is highly enriched in human ESCs and decreases dramatically upon differentiation [18]. Nrf2 inhibition by siRNA knockdown or kelch-like ECH-associated protein 1 (Keap1) overexpression impairs both ESC self-renewal and cellular reprogramming, while Nrf2 activation by *tert*-butylhydroquinone (t-BHQ) or sulforaphane (SFN) can suppress differentiation [18]. Nrf2 controls the proliferation of self-renewing ESCs, three germ layer differentiation, and cellular reprogramming by regulating proteasome activity partially through proteasome maturation protein (POMP) [18].

Box 2. Stem Cells and Progenitor Cells

In mammals, there are two basic types of stem cells: PSCs and ASCs. PSCs can be subcategorized into germline or ESCs and iPSCs. ESCs are derived from the inner cell mass of preimplantation embryos. iPSCs are reprogrammed from adult somatic cells *in vitro* through simultaneous overexpression of four core pluripotent factors: *Oct4*, *Sox2*, *Klf4*, and *c-Myc*. Both ESCs and iPSCs can be indefinitely maintained and expanded in a pluripotent state *in vitro* and are capable of differentiating into the derivatives of all three germ layers.

ASCs are characterized by their ability to self-renew and differentiate to generate all the cell types in a tissue. Based on their locations and turnover rates in various tissues/organs, ASCs can be subcategorized into HSCs, MSCs, EPCs, and NSCs.

During embryonic development, stem cells differentiate into organ- and tissue-specialized cells but are also maintained in stem cell niches capable of regenerative repair for most organs, including the BM, most solid organs, and skin, but not the postnatal heart. Stem and progenitor cells support the repair and replenishment of most adult tissues, though this capacity declines with age.

Glossary

Differentiation: ability of stem cells form into more specialized cells present in a specific tissue or organ *in vivo* or under specific stimulating conditions *in vitro*. It occurs during development of an organism or in adult tissues to replenish damaged and/or lost cells.

Glycolysis: process in which generates ATP and converts one molecule of glucose into two molecules of pyruvate or lactate. This process takes place in the cytoplasm outside the mitochondria with (aerobic glycolysis) or without (anaerobic glycolysis) oxygen.

Long-term hematopoietic stem cells (LT-HSCs): population of multipotent HSCs that are characterized by their extensive self-renewal capacity and ability to differentiate into short-term HSCs and lineage-restricted progenitors and finally to give rise to all blood lineage.

Metabolic reprogramming: ability of stem cells, as they alter their state from quiescence, self-renewal, proliferation, differentiation, and senescence, to shift their dependency on specific metabolic pathways and substrates such as glucose and fatty acids in order to support metabolic demand.

Multipotency: capability of stem cells to develop into multiple specialized cell types present in a specific tissue or organ *in vivo* or under specific stimulating conditions *in vitro*. Most adult stem cells (ASCs) have multipotency.

Niche: *in vivo* specific microenvironment in which stem cells reside in an undifferentiated and self-renewable state. Somatic cells within the microenvironment interact with the stem cells to maintain their survival and self-renewal.

Oxidative phosphorylation (OXPHOS): process in which cells oxidize nutrient substrates such as glucose, lipids, and pyruvate to produce ATP form of energy. This process takes place inside of mitochondria in most eukaryotes.

Pluripotency: ability of stem cells to develop into all the three basic body layers (endoderm, mesoderm, and ectoderm) of the early embryo and therefore into all cells

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Even modest proteasome inhibition by lactacystin or siRNA skews the balance of early ESC differentiation toward a mesendoderm rather than ectodermal fate by decreasing the protein level of cyclin D1 and delaying the degradation of octamer-binding transcription factor 4 (OCT4) and NANOG proteins [18]. Nrf2 can regulate human ESC early lineage specification by directly binding to upstream regions of pluripotency genes *OCT4* and *NANOG* to promote their expression and repress neuroectoderm derivation [20]. For example, during induced neural lineage specification, increased ciliation in neuroectoderm precursors induces autophagy that results in the inactivation of Nrf2 and thereby relieves transcriptional activation of *OCT4* and *NANOG* and facilitates neural lineage differentiation, demonstrating a cilium–autophagy–Nrf2 control axis connects the ESC cell cycle to the neuroectoderm fate [20]. Moreover, Nrf2 influences ESC osteogenic and neuronal differentiation. Mild oxidative stress activates Nrf2/heme oxygenase-1 (HO-1) signaling, facilitating osteogenic differentiation and mineralization of human ESCs [21]; while knockdown of Nrf2 with siRNA enhances the role of ROS-mediated ESC neuronal differentiation [22].

These results provide evidence that Nrf2 regulates ESC stemness and differentiation by finely tuning the expression of pluripotency genes *OCT4* and *NANOG* via Nrf2 control of their expression and proteasome-mediated ubiquitination and degradation (Figure 1A). However, all these mechanistic insights were derived from *ex vivo* culture conditions. Whether and how these mechanisms are finely tuned to regulate *OCT4* and *NANOG* expression and determine ESC fate during development and disease requires *in vivo* investigation and validation.

Nrf2 Regulates Induced Pluripotent Stem Cell Redox Homeostasis, Metabolic Reprogramming, and Differentiation

Induced PSCs (iPSCs) are a class of PSCs generated directly from adult tissue cells. The potential of iPSCs in disease modeling and regenerative medicine is vast, but current methodologies for iPSC generation, lineage induction, and maturation remain inefficient [23,24]. A number of studies have revealed that Nrf2 signaling and ROS play critical roles in iPSC reprogramming [25,26] (Figure 1B). At the onset of reprogramming, ROS generation increases substantially, influencing nuclear reprogramming of somatic cells, and ROS depletion by antioxidants or NADPH oxidase (NOX) inhibitors decreases reprogramming efficiency [25]. Initial iPSCs reprogramming is associated with a metabolic shift from OXPHOS to glycolysis [26]. In the early stages of reprogramming, Yamanaka factors induce cell proliferation which increases mitochondrial respiration and shuttles glucose to the pentose phosphate pathway to meet increased nucleotide synthetic demand [26]. Peaks in cell proliferation and OXPHOS contribute to ROS accumulation and correlate with upregulated Nrf2 activity and with Nrf2 activated hypoxia-inducible factor 1α (HIF-1α), which drives the metabolic switch toward glycolytic energy production [26]. Nrf2 inhibition by Keap1 overexpression compromises **metabolic reprogramming** and reduces the efficiency of iPSC colony formation [26]. During late reprogramming, Nrf2 expression plateaus along with upregulation of antioxidant enzymes that orchestrate relatively low ROS levels [25]. These findings are consistent with an essential role for Nrf2 in regulating the metabolic switch during the initiation of iPSC reprogramming and as a key mediator to maintain the redox homeostasis during late-stage iPSC reprogramming (Figure 1B).

As within ESCs, Nrf2 also plays a critical role during iPSCs differentiation by repressing neuroectoderm differentiation and promoting mesendoderm differentiation [20]. Nrf2 activity levels are highly predictive of neuroectoderm differentiation potential in contrast to core pluripotency gene products, and Nrf2 inhibition is sufficient to rescue poorly neurogenic iPSC lines [20]. These findings support the key role of Nrf2 in early iPSC lineage determination. In addition, Nrf2 activation during later-stage human iPSC-derived neural stem cell (NSC) differentiation potentiates neuron and astrocyte lineages [27,28]. Thus, Nrf2 plays critical regulatory roles in iPSC generation, metabolic reprogramming, early lineage determination, and late lineage differentiation (Figure 1B). However, current knowledge regarding the regulatory roles of Nrf2 in iPSC generation and differentiation remains fragmented. Further studies are required to define how Nrf2 balances the redox status and metabolic reprogramming in iPSCs generation and regulates type- and stage-specific lineage differentiation.

and tissues of the adult body, excluding the placenta. ESCs and iPSCs have pluripotency.
Quiescence: reversible stem cell state in which it does not divide and proliferate, and reduces metabolic activity and vulnerability to stimuli. Some ASCs exit their quiescent state rapidly when stimulated, for example by injury to the tissues in which they reside.
Reactive oxygen species (ROS): reactive-oxygen-containing molecules, such as hydroxyl radical, superoxide, singlet oxygen, and hydrogen peroxide. ROS form as byproducts of cellular respiration and ionizing radiation and other stresses, which are essential for normal cell function within physiological extent; however, overproduction of ROS damages to other molecules and to cellular structures.
Self-renewal: stem cell property to divide and multiply without differentiation.

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Stem cells/ progenitor cells	Species origin	Nrf2 function	Manipulating approach	Mechanism	Refs
ESCs	Human embryo	Self-renewal ↑ Re-establishment of pluripotency ↑ Differentiation ↓	*Nrf2* siRNA or shRNA knockdown or *Keap1* overexpression or pharmacological activation	Nrf2 maintains and regulates proteasome activity through POMP	[18]
		Neuroectoderm differentiation ↓	*Nrf2* shRNA or siRNA knockdown	Nrf2 binds directly to upstream regions of pluripotency genes *OCT4* and *NANOG* to promote their expression and repress neuroectoderm derivation	[20]
		Osteogenic differentiation ↑	*Nrf2* siRNA knockdown	Nrf2 increases the expression of Runx2 to facilitates the mineralization of ESCs	[21]
		Neuronal differentiation ↓	*Nrf2* siRNA knockdown	Nrf2 decreases ROS level to inhibit neuronal differentiation	[22]
iPSCs	Human fibroblasts	Reprograming ↑	*Keap1* overexpression	Nrf2 promotes metabolic reprogramming of iPSCs via *HIF-1α*	[26]
	Human or chimpanzee skin fibroblast	Neuroectoderm differentiation ↓ Mesendoderm differentiation ↑	*Nrf2* shRNA knockdown	Nrf2 represses neuroectoderm differentiation and promotes mesendoderm differentiation by regulating pluripotency genes	[20]
HSCs	Mouse BM	Survival ↑	*Nrf2* knockout	Nrf2 promotes HSC survival via enhancing prosurvival cytokine (such as G-CSF) signaling levels	[34]
		Proliferation ↓ Expansion ↓ Differentiation ↓ Self-renewal ↑ Quiescence ↑ Migration ↑	*Nrf2* knockout	Nrf2 regulates cell quiescence, differentiation, and migration partially via regulating *CXCR4* transcription	[19]
		Quiescence and Maintenance ↓ Differentiation ↑	*Keap1* knockout or pharmacological activation of Nrf2	Nrf2 drives cell cycle entry and differentiation possibly via activation of JAK–STAT3 pathway	[36]

Table 1. Function of Nrf2 in Stem Cells and Progenitor Cells

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Stem cells/ progenitor cells	Species origin	Nrf2 function	Manipulating approach	Mechanism	Refs
		Function↑ Survival ↑ Expansion ↑	*Keap1* knockout or pharmacological activations of Nrf2 or *Nrf2* knockout	Nrf2-mediated Notch signaling improves HSC function following ionizing radiation exposure; Nrf2 increases the expression of antioxidative proteins under stress conditions	[41,43]
MSCs	Human BM	Apoptosis ↓ Oxidative stress ↓	*Nrf2* overexpression	Nrf2 upregulates *SOD* and *HO-1* expression	[52]
	Human umbilical cord	Proliferation ↑ Stemness ↑ Osteogenesis ↑ Apoptosis ↓	*Nrf2* overexpression or shRNA knockdown	Nrf2 might lead alterations in MSC genome sequences	[53]
	Human amnion	Homing ↑ Differentiation↑ Apoptosis ↓	*Nrf2* overexpression	Upregulation of Nrf2 DNA binding activity increases the cytoprotective genes expression	[54]
	Human BM	Self-renewal ↑ Osteogenic Differentiation ↑	*Nrf2* shRNA knockdown or pharmacological activation	Nrf2 maintains self-renewal and osteogenic differentiation of MSCs via p53-SIRT1 signaling	[61]
	Rat adipose	Osteoblastic Differentiation ↓	*Nrf2* siRNA knockdown	Nrf2 inhibits ROS-induced osteogenic differentiation of adipose derived MSCs by downregulating the expression of *BMP2* and *Runx2*	[62]
NSCs	Rat/mouse subventricular zone	Survival ↑ Proliferation ↑ Differentiation ↑ Regeneration ↑	*Nrf2* overexpression or *Nrf2* knockout or shRNA knockdown	The mechanism is not clear	[64]
	Rat/mouse dentate gyrus of the hippocampus	Proliferation ↑ Neuronal differentiation ↑ Regeneration ↑	*Nrf2* overexpression or *Nrf2* knockout or siRNA knockdown	The mechanism is not clear	[70]
	Mouse subgranular zone	Proliferation ↑ Differentiation ↑	*Nrf2* shRNA knockdown *Nrf2*-knockout or pharmacological activation	The mechanism is not clear	[65]
	Mouse hippocampi cerebellum	Survival ↑ Proliferation ↑ Differentiation ↑ Migration ↑	*Nrf2* overexpression or *Nrf2* knockout	The mechanisms is not clear	[69]

Table 1. *Continued*

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Stem cells/ progenitor cells	Species origin	Nrf2 function	Manipulating approach	Mechanism	Refs
EPCs	Human/mouse peripheral blood or BM	Survival ↑ Angiogenic function ↑	*Nrf2* overexpression or *Nrf2* shRNA knockdown or pharmacological activation	Nrf2 augments the angiogenic capacity of EPCs by inhibiting cell senescence and oxidative stress, improving cell survival, migration, proliferation and secretion of proangiogenic factors such as VEGF, SDF-1, and NO	[79–82]
ISCs	*Drosophila* or *Aedes aegypti* intestine	Proliferation ↓	*CncC* overexpression or *Keap1* overexpression or *CncC* dsRNA knockdown	CncC exerts function in ISCs by regulating the intracellular redox balance	[83,84]
	Drosophila Intestine	Cell cycle control ↑	*CncC* overexpression or *Keap1* overexpression or pharmacological activation	CncC limits age-related intestinal barrier dysfunction and extends lifespan via transcriptional activation of dacapo and proteolytic genes	[85]
	Mouse intestine	Proliferation ↓ Differentiation ↓	*Nrf2* knockout	The mechanisms is not clear	[86]

Table 1. Continued

Abbreviations: JAK, Janus kinase; Runx2, runt-related transcription factor 2; STAT3, signal transducer and activator of transcription 3.

Nrf2 Regulates ASC Quiescence, Survival, Self-Renewal, and Regenerative Capacity

ASCs include hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), NSCs, endothelial progenitor cells (EPCs), and intestinal stem cells (ISCs) that persist throughout life and provide critical replacement cells in response to homeostatic turnover, disease, and injury (Box 2). Reductions in ASC number and/or function have been linked to many human diseases [29,30]. While tissue-specific ASC populations have varying proliferative plasticity and distinct turnover patterns, their **quiescence**, proliferation, self-renewal, and differentiation are delicately balanced by common mechanisms [29,30]. While less advanced than other stem cell lineages, emerging data show that Nrf2 can protect against ASC depletion in response to stress and aging through maintenance of their quiescence, survival, self-renewal, and regenerative capacity.

Nrf2 Regulates HSC Survival, Proliferation, and Differentiation through Redox-Dependent and -Independent Mechanisms

HSCs are characterized by their ability to self-renew and to reconstitute all the blood cell lineages, and the coordinated balance between HSC quiescence and self-renewal is crucial to maintain lifelong hematopoiesis. Intracellular ROS level has been shown to be a critical factor affecting HSC proliferation and differentiation [31,32]. Postnatally, quiescent undifferentiated HSCs reside in hypoxic bone marrow (BM) niches and have low ROS compared to their mature progeny [33].

Nrf2 is abundantly transcribed in HSCs and plays a regulatory role in all aspects of HSC homeostasis and function [19,34–36] (Figure 2). For example, Nrf2 deficiency does not increase mouse HSC basal ROS levels but increases spontaneous apoptosis and further decreases survival in response to oxidative stress [34]. Nrf2 deficiency also impairs HSC function, reduces chimerism after

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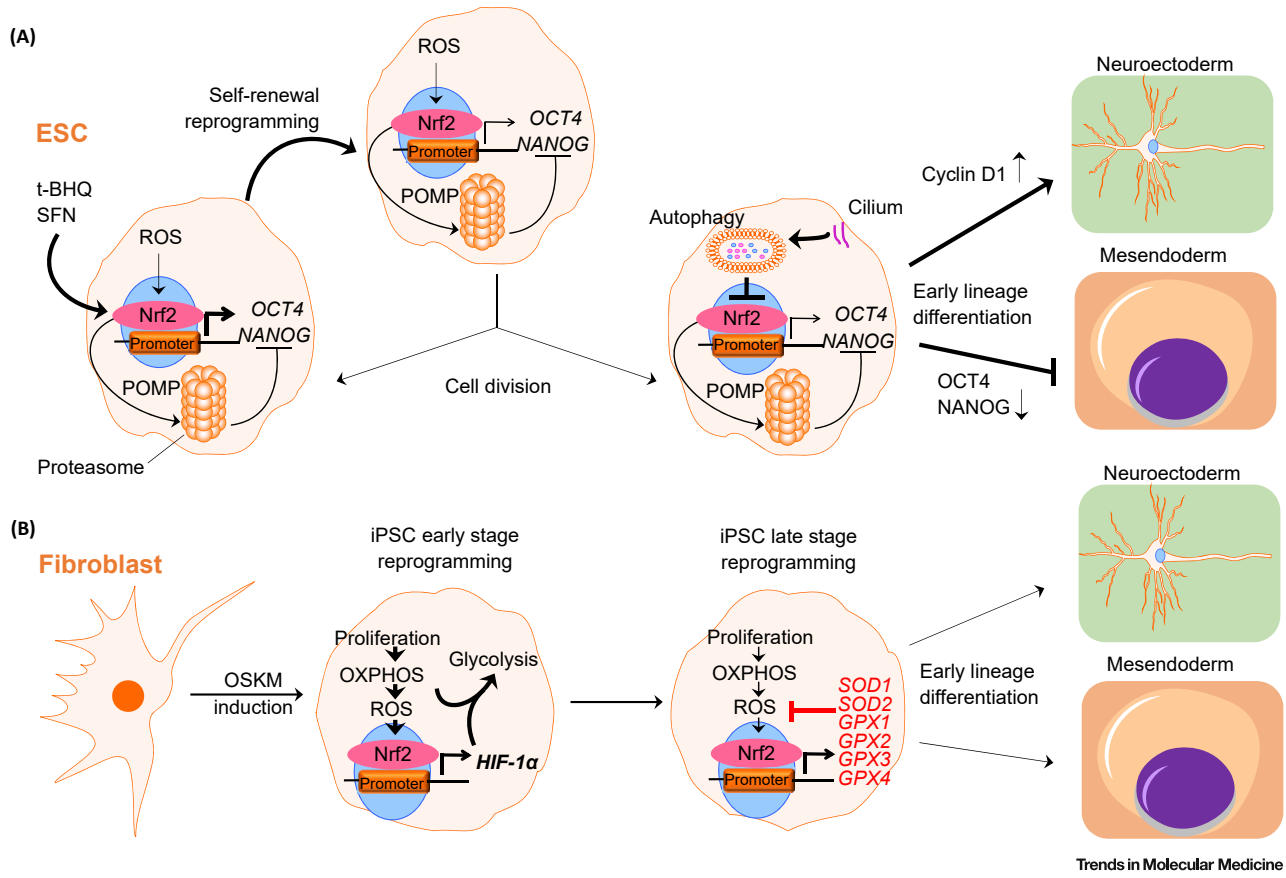

Figure 1. Role of Nrf2 in PSCs.

(A) In ESCs, Nrf2 senses the intracellular redox status and regulates ESC stemness and differentiation by tuning the expression of pluripotency genes *OCT4* and *NANOG* and by controlling their transcriptional expression and POMP-mediated proteasome ubiquitination and degradation. Nrf2 inducers (e.g., t-BHQ and SFN) upregulate Nrf2 activity and enhance ESC self-renewal and reprogramming, while cilium induction of autophagy downregulates Nrf2 levels (activity), disrupts the fine-tuned expression of pluripotency genes, and skews the balance of early differentiation toward mesendoderm at the expense of a neuroectoderm fate by decreasing cyclin D1 protein levels and delaying OCT4 and NANOG protein degradation. (B) At the onset stage of iPSC establishment, induction of Yamanaka factors [*OCT4*, *SOX2*, *KLF4*, and *c-MYC* (OSKM)] increases cell proliferation and OXPHOS, contributing to ROS accumulation and Nrf2 induction. Nrf2 upregulation activates *HIF-1α*, shifting OXPHOS toward glycolytic energy production and triggering iPSC metabolic and nuclear reprogramming. Increased Nrf2 expression (and activity) upregulates antioxidant enzyme genes (e.g., *SOD1*, *SOD2*, *GPX1*, *GPX2*, *GPX3*, and *GPX4*) to scavenge and modulate ROS levels during the later stage of iPSCs reprogramming. In addition, Nrf2 may regulate PSCs early lineage differentiation through similar mechanisms observed in ESCs. Arrow ↓ indicates decrease; ↑ indicates increase; thicker lines represent stronger signals. Abbreviations: ESC, embryonic stem cell; GPX, glutathione peroxidase; HIF-1α, hypoxia-inducible factor 1α; iPSC, induced PSC; KLF4, Kruppel-like factor 4; Nrf2, nuclear factor erythroid 2-related factor 2; OCT4, octamer-binding transcription factor 4; OXPHOS, oxidative phosphorylation; POMP, proteasome maturation protein; PSC, pluripotent stem cell; ROS, reactive oxygen species; SFN, sulforaphane; SOD, superoxide dismutase; SOX2, SRY (sex-determining region Y)-box 2; t-BHQ, *tert*-butylhydroquinone.

transplantation which can be rescued by prosurvival cytokine granulocyte-colony stimulating factor (G-CSF) but not antioxidant N-acetyl cysteine [34]. These findings suggest that Nrf2 may regulate HSC survival and function independent of ROS levels. Furthermore, Nrf2 deficiency increases mouse HSC hyperproliferation and differentiation at the expense of HSC quiescence and self-renewal but does not augment BM reconstitution [19]. Chromatin immunoprecipitation reveals that Nrf2 directly binds to the CXCR4 promoter in HSCs. Nrf2 deficiency significantly reduces CXCR4 expression, impacts HSC differentiation and homing, and can be rescued by lentiviral CXCR4 overexpression [19]. These findings suggest that Nrf2 regulates HSC homeostasis and function, in part, via the regulation of CXCR4 signaling.

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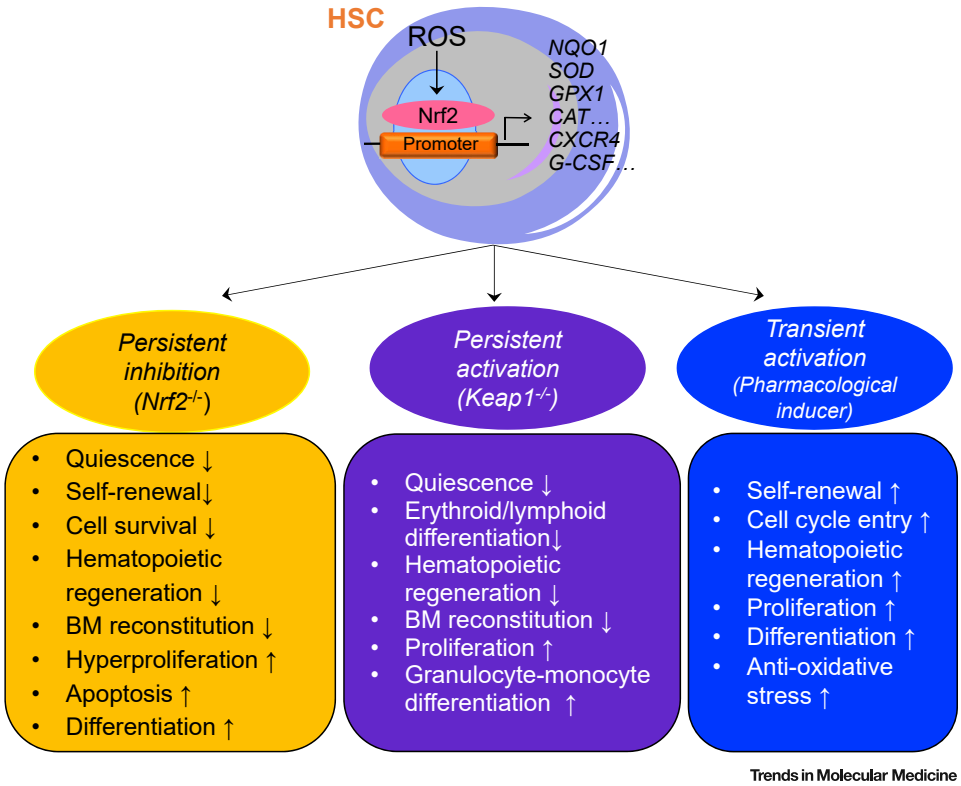

Figure 2. Role of Nrf2 in HSCs.
Under basal conditions, Nrf2 regulates the balance among HSC quiescence, self-renewal, and differentiation dependent on redox modulating expression and/or function of antioxidant genes (e.g., *NQO1*, *SOD*, *GPX1*, and *CAT*) and cytokines and their receptor genes (e.g., *G-CSF* and *CXCR4*). Nrf2 deficiency ($Nrf2^{-/-}$), persistent Nrf2 induction ($Keap1^{-/-}$), and pharmacological agents can disrupt the finely tuned status of HSCs. Arrow ↓ indicates decrease; ↑ indicates increase. Abbreviations: BM, bone marrow; CAT, catalase; CXCR4, C-X-C chemokine receptor 4; G-CSF, granulocyte-colony stimulating factor; GPX1, glutathione peroxidase 1; HSC, hematopoietic stem cell; Keap1, kelch-like ECH-associated protein 1; NQO1, NAD(P)H dehydrogenase (quinone 1); Nrf2, nuclear factor erythroid 2-related factor 2; SOD, superoxide dismutase.

Persistent activation of Nrf2 due to Keap1 deficiency enhances mouse HSC granulocyte–monocyte differentiation at the expense of erythroid and lymphoid differentiation [35] (Figure 2). Persistent activation of Nrf2 does not change the number of mouse **long-term (LT)-HSCs** under steady-state conditions, but enhances LT-HSC exit from quiescence into the cell cycle and upregulates cell proliferation pathways [36]. During hematopoietic regeneration after BM transplantation, Nrf2-depleted LT-HSCs are impaired in their ability to reconstitute BM. However, persistent activation of Nrf2 can be detrimental by inhibiting the quiescent survival of LT-HSCs, promoting differentiation, and leading to HSC exhaustion [36]. In agreement with these results, transient activation of Nrf2 by an electrophilic reagent can also promote mouse LT-HSC entry into the cell cycle [36], indicating that the physiological duration and amplitude of Nrf2 activation calibrate cell cycle entry of LT-HSCs and their quiescence and maintenance.

Taken together, these findings indicate that Nrf2 influences HSC homeostasis and function via redox-dependent or -independent pathways and the titration of Nrf2 activity by genetic or pharmacological strategies is important in the regulation of HSC stress-induced regenerative responses (Figure 2). However, most of these functional and mechanistic studies are performed in mouse HSCs, and some controversy remains regarding the role of Nrf2 in the regulation of HSC quiescence, activation, self-renewal, and differentiation, supporting the need for additional mechanistic studies in human HSCs.

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Nrf2 Impacts Hematopoietic Stem Cell Survival, Proliferation, and Differentiation Following Irradiation

The hematopoietic system is uniquely vulnerable to the damaging effects of accidental [37,38] or clinical [39] exposure to radiation that triggers ROS generation and the induction of HSC apoptosis and dysregulated differentiation [37,40], and Nrf2 plays a key role in the regulation of ROS levels and self-renewal and differentiation in response to irradiation (Figure 2). For example, augmenting Nrf2 signaling in mice, either by genetic deletion of *Keap1* or by pharmacological Nrf2 activation with 2-trifluoromethyl-2′-methoxychalone enhances HSC function and mitigates ionizing-radiation-induced myelosuppression and mortality [41]. Theaflavin treatment or hydrogen-rich water consumption reduces ROS levels and alleviates total-body-irradiation-induced HSCs oxidative stress and apoptosis by upregulating expression of Nrf2 and its targeted antioxidative proteins [40,42]. Similarly, astaxanthin increases activation of Nrf2 and its downstream antioxidative proteins to improve radiation-exposure-skewed differentiation of peripheral blood cells and ameliorate BM suppression by accelerating hematopoietic self-renewal and regeneration [43]. Furthermore, Vam3, a resveratrol dimer, activates Nrf2 and its target proteins and protects the proliferation of c-kit$^+$ HSCs receiving irradiation [44], confirming the direct protective effects of Nrf2 activation on HSCs.

These studies demonstrate that Nrf2 could be a therapeutic target for preventing radiation-induced HSC injury. However, most of the beneficial effects of pharmacological Nrf2 activation on total-body-irradiated animals or HSCs have been investigated in rodent models. Mechanistic and translational studies are required to determine if these functional and mechanistic features of pharmacological Nrf2 activation can be translated into the clinic.

Nrf2 Promotes MSC Stemness, Survival, Proliferation, Differentiation, Resistance to Oxidative Stress, and Senescence

MSCs are multipotent stromal cells that differentiate into various mesodermal cell types, including osteoblasts, chondrocytes, myocytes, and adipocytes [45]. MSCs are the most extensively investigated and clinically tested class of stem cells due to their high availability, beneficial immunomodulatory effects, and ability to support and replenish endogenous stem cell niches [46,47]. However, MSCs rapidly lose their original stemness characteristics when removed from their *in vivo* niche, which adversely impacts transplantation efficiency with respect to cell survival and differentiation [48,49]. A hypoxic microenvironment is essential to maintain MSC self-renewal and stemness [50], and exposure to exogenous oxidative stress or ROS during *in vitro* culture adversely affect MSC self-renewal and differentiation potential [51].

Several studies have confirmed that Nrf2 overexpression improves MSC survival and resistance to oxidative stress [52,53] (Figure 3A). Nrf2 overexpression upregulates superoxide dismutase (SOD) and HO-1 and protects human BM MSCs from cell death triggered by hypoxia and oxidative stress [52]. In contrast, knocking down Nrf2 expression in human umbilical cord MSCs impairs the expression of stem cell markers and the osteogenesis process [53]. In a mouse model of lipopolysaccharide-induced lung injury, treatment with Nrf2-overexpressing human amniotic MSCs reduced lung injury, fibrosis, and inflammation, and increased pulmonary MSC retention [54]. These results support a role for Nrf2 in modulating the efficiency of MSC-based cell therapies in adverse *in vivo* microenvironments.

Furthermore, pharmacological activation or inhibition of Nrf2 directly influences MSC survival and function in response to different stresses via various critical intracellular mechanisms (Figure 3A). For example, ginger oleoresin mitigates ionizing-radiation-induced oxidative stress and DNA breakage in human MSCs by inducing nuclear Nrf2 translocation and upregulation of cytoprotective gene including *HO-1* and *NQO-1* expression [55]. Melatonin prevents multiple-passage-induced senescence in canine adipose MSCs through Nrf2 activation and inhibition of endoplasmic reticulum stress [56]. Cyclic helix B peptide improves MSC viability and suppresses starvation-induced apoptosis by activating a Nrf2/sirtuin (SIRT)3/FoxO3a pathway [57]. The protective effect of 17β-estradiol (E2) on human umbilical cord blood MSCs against high glucose-induced mitochondrial ROS

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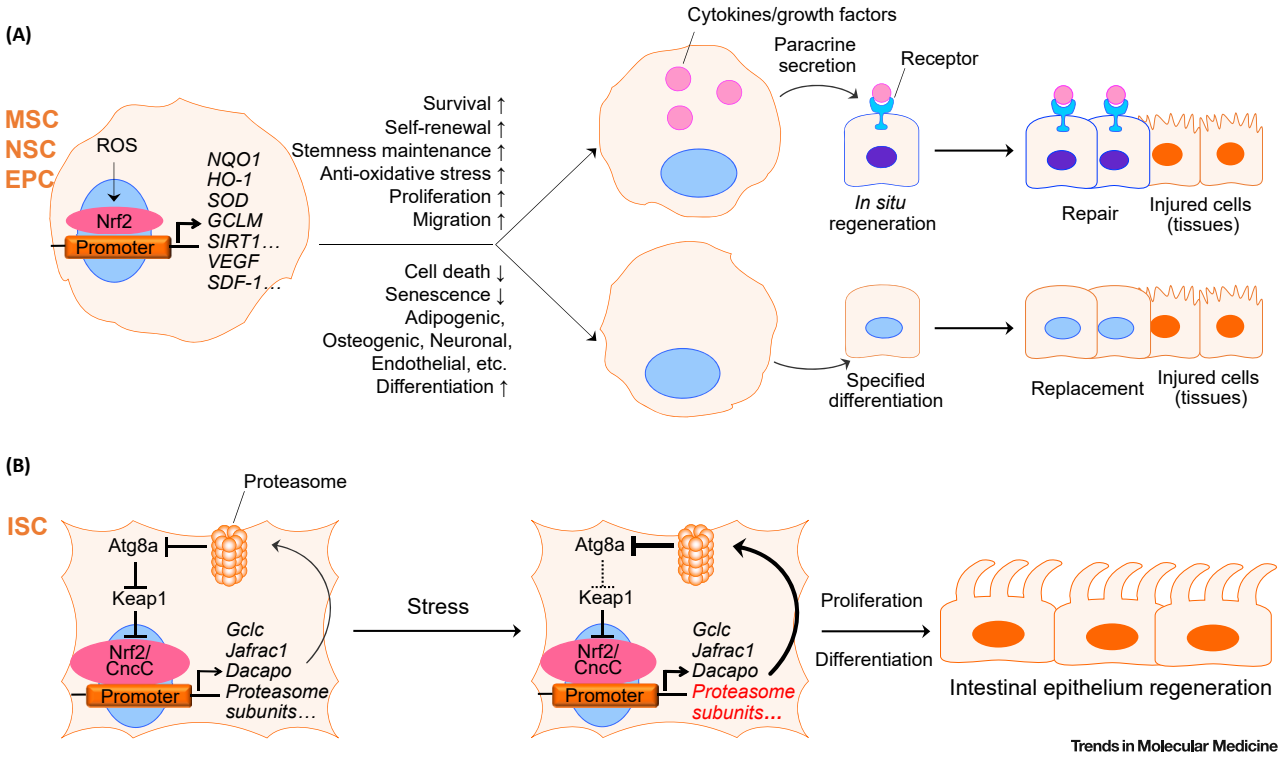

Figure 3. Role of Nrf2 in ASCs.

(A) Nrf2 senses the intracellular ROS and maintains intracellular redox homeostasis under basal conditions in MSCs, NSCs, and EPCs. Nrf2 is activated in response to tissue damage, extracellular hypoxia, oxidative stress, and pharmacological reagents and upregulates downstream cytoprotective genes (e.g., *NQO1*, *HO-1*, *SOD*, *GCLM* and *SIRT1*) and cytokines and growth factors (e.g., *VEGF* and *SDF-1*), resulting in inhibition of cell death and senescence, and the upregulation of antioxidative capacity, cell survival, self-renewal, stemness maintenance, proliferation, migration, and distinct lineage differentiation. These cellular responses repair injured cells (tissues) through paracrine secretion of cytokines and growth factors to improve *in situ* cell (tissue) regeneration, and direct differentiation into target cells to replace injured cells (tissues). (B) In ISCs, the autophagy protein Atg8a is required for Nrf2/CncC activation and is proposed to act by sequestering Keap1. Under the basal condition, Nrf2/CncC senses the intracellular redox status and induces expression of antioxidant genes (e.g., *Gclc* and *Jafrac 1*), cell cycle control gene (e.g., *Dacapo*), and proteasome subunits which subsequently regulates Keap1 abundance by proteasome-mediated degradation of autophagy protein Atg8a and produces a relative cytoprotective ISC state. In response to stress stimuli such as mitogenic signaling and/or proteostatic stress (i.e., presence of protein aggregates), Nrf2 differentially upregulates selective proteasome subunits that facilitate Atg8a degradation and Keap1 release. Keap1 represses Nrf2 transcription activity and elevates ROS levels, eventual resulting in ISCs proliferation, differentiation, and maintenance of ISC-mediated tissue regeneration and homeostasis. Arrow ↑ indicates increase; ↓ indicates decrease; thicker lines represent stronger signals. Abbreviations: ASC, adult tissue stem cell; Atg8a, autophagy-related 8a; CncC, cap 'n' collar isoform C; EPC, endothelial progenitor cell; Gclc, glutamate-cysteine ligase catalytic subunit; GCLM, glutamate-cysteine ligase modifier subunit; HO-1, Heme oxygenase-1; ISC, intestinal stem cell; Jafrac l, thioredoxin peroxidase 1; Keap1, kelch-like ECH-associated protein 1; MSC, mesenchymal stem cell; NQO1, NAD(P)H dehydrogenase (quinone 1); Nrf2, nuclear factor erythroid 2-related factor 2; NSC, neural stem cell; ROS, reactive oxygen species; SDF-1, stromal cell-derived factor 1; SIRT1, sirtuin 1; SOD, superoxide dismutase; VEGF, vascular endothelial growth factor.

production and autophagic cell death occurs via Nrf2 nuclear translocation followed by *SIRT3* upregulation and manganese SOD activation [58]. In contrast, Nrf2 inhibition by triclosan impairs human BM MSC proliferation in a dose-dependent manner associated with increased oxidative stress [59]. It remains intriguing that Nrf2 activation has unique protective effects depending on the mechanism of injury with both species and cell lineage specificity. These studies indicate that pharmacological MSC preconditioning targeting Nrf2 is a feasible strategy to improve MSC survival and function.

In addition, Nrf2 plays an important role in MSC differentiation (Figure 3A). Nuclear Nrf2 protein levels continuously decrease during adipogenic differentiation in mouse BM MSCs [60], and reduced Nrf2 nuclear localization (activation) during prolonged cell passage influences human BM MSC osteogenic differentiation by regulating the p53–SIRT1 axis [61]. Blocking Nrf2 nuclear translocation using

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ochratoxin A activates p53, suppresses *SIRT1* promoter activity, and reduces MSC self-renewal and osteogenic potential. Conversely, increasing Nrf2 nuclear translocation via t-BHQ stimulation suppresses *p53* expression, activates *SIRT1* transcription, and enhances MSCs self-renewal and differentiation potential [61]. In agreement with these findings, Nrf2 deficiency impairs the beneficial effect of hypoxia on MSC stemness and osteogenic capacity, while Nrf2 overexpression promotes the maintenance of human umbilical cord MSC stemness and prevents apoptosis [53]. The role of Nrf2 in osteoblastic differentiation remains controversial, and previous research has demonstrated that Nrf2 negatively regulates rat adipose MSC osteoblast differentiation [62].

These findings confirm that Nrf2 plays a critical role in promoting MSC survival, proliferation, stemness, and differentiation, resistance to oxidative stress and senescence, and therapeutic capacity in response to tissue injury (Figure 3A) and deserves further translational investigation. The mechanistic functions of Nrf2 in MSCs appear discrepant due to experimental heterogeneity (different species, tissues, and/or protocols) and need to be investigated and validated using human MSCs.

Nrf2 Enhances NSC Survival, Proliferation, and Regenerative Capacity

NSCs play vital roles in the development and maintenance of central and peripheral neuronal tissues, in response to injury, and the maintenance and differentiation of NSCs is Nrf2 dependent [63–65] (Figure 3A). Mitochondrial dynamics regulate the fate and metabolic identity of NSCs by modifying physiological ROS signaling and activating developmental gene expression via a Nrf2-dependent retrograde pathway [63]. The decline in rat NSCs with age correlates with reduced Nrf2 expression and the Nrf2 target gene *GCLM* [64]. Suppressing or increasing Nrf2 expression results in a corresponding decrease or increase in newborn and middle-aged rat NSC survival and regeneration, respectively [64]. Nrf2 deficiency also decreases mouse NSC clonogenic, proliferative, and differentiating capacity, which could be rescued by ectopic expression of Nrf2 [65].

Successful NSC transplantation therapy for both structural and functional neurological recovery in neurodegenerative diseases and neural injury requires a delicate balance of NSC survival, expansion, and differentiation in disease states [66]. Manipulating NSC state and function is a key facilitating NSC transplantation [67,68]. Nrf2 overexpression mitigates amyloid β toxicity to mouse NSCs by preventing amyloid-β-induced reductions in NSC proliferation, survival, and neuronal differentiation [69]. Similarly, activating the Nrf2 signal pathway with pharmacological agent pyrrolidine dithiocarbamate also improves survival, proliferation, and differentiation of NSCs [69]. Studies using rats from multiple aging stages ranging from newborn to old age and aging Nrf2 knockout mice confirm that Nrf2 expression controls the proliferation and the balance of neuronal versus glial differentiation in rat dentate gyrus (DG) NSCs and impacts functional neurogenesis-related hippocampal behaviors [70]. More importantly, Nrf2-overexpressing NSC transplantation mitigates age-related declines in DG stem cell regeneration during the critical middle-age period and improved pattern separation abilities [70].

Taken together, these findings emphasize the importance of Nrf2 in regulating NSC survival, regeneration, proliferation, and differentiation, and support Nrf2 upregulation as a potential approach to modulate NSC activity and function to treat neural damage and diseases (Figure 3A). Additional disease-specific mechanistic studies are required to determine the translational potential of Nrf2 regulation on NSC function and repair.

Nrf2 Enhances Endothelial Progenitor Cell Survival, Proliferation, and Angiogenic Function

EPCs include several distinct populations of progenitor cells that differentiate into functional endothelium and sustain vasculogenesis [71,72]. These cell populations improve vascular repair through two principal mechanisms (Figure 3A): (1) formation of a functional blood vessel by direct incorporation into injured endothelium [73]; and (2) secretion of proangiogenic factors to facilitate local endothelial cell regeneration and repair [74]. EPCs have been proposed as potent cell-based therapies due to their capacity to stimulate vascular repair [75,76]. Because clinical conditions such as diabetes are

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associated with increased inflammation and oxidative stress and reduced EPC number and impaired functionality [77], enhanced EPC survival and function are essential for EPC-based cellular therapies.

Clinical studies demonstrate that impaired EPC function may occur, in part, via the suppression of the Nrf2 downstream gene *HO-1* and the adiponectin axis [78] with a critical role for Nrf2 in EPC function [79–81]. Preclinical studies confirm that Nrf2 knockout attenuates survival, proliferation, migration, and angiogenic potential of mouse proangiogenic cells and affected angiogenic transcriptome [79]. Nrf2 silencing by siRNA impairs mouse EPC or human cord blood endothelial colony-forming cell (ECFC) biological functions, accelerates cell senescence, increases oxidative stress, and inhibits migration, proliferation, and secretion of proangiogenic factors, including vascular endothelial growth factor (VEGF), stromal cell-derived factor 1 (SDF-1) and NO [80,81]. Conversely, Nrf2 activation via increased *CXCR7*-expression-mediated Nrf2 nuclear translocation and its downstream antioxidant gene upregulation improves mouse EPC survival and angiogenic function in diabetic conditions [82], supporting a critical role for Nrf2 in preserving EPC angiogenic functionality under conditions of increased stresses.

Emerging evidence also demonstrates that pharmacological Nrf2 activation protects EPC function. For example, preincubation of human ECFCs with the Nrf2 inducer SFN reduces intracellular ROS in the presence of H_2O_2 and preserves ECFC mediated scratch-wound closure and tubule formation [81]. Nrf2 activation with t-BHQ preconditioning improves mouse EPC function by protecting diabetic EPC from the effects of oxidative stress and cell senescence via increasing antioxidant gene expression and reducing ROS [80]. These studies indicate that preconditioning of EPCs with Nrf2 inducers is an efficient strategy to protect EPCs against stress conditions and support Nrf2 as a promising target to enhance the efficacy of EPC-based cell therapies. Whether *ex vivo* preconditioning of EPCs with Nrf2 inducers enhances the therapeutic efficacy of EPC transplantation needs systemic preclinical and/or clinical validation.

Nrf2 Suppresses ISC Proliferation, Differentiation, and Regenerative Function

ISCs have essential roles in the maintenance of intestinal homeostasis and intracellular redox balance in both insects and mammals. Cap 'n' collar isoform C (CncC), the *Drosophila* homolog to Nrf2, is constitutively active in *Drosophila* ISCs, and CncC repression by Keap1 triggers ISC proliferation by regulating intracellular redox balance, and *Gclc* or *Jafrac1* overexpression reduces oxidative stress and decreases Keap1-mediated ISC proliferation [83] (Figure 3B). In supporting this notion, Nrf2 silencing increases ROS levels and stimulates ISC proliferation in the *Aedes aegypti* midgut [84]. CncC also couples cell cycle control with proteostatic responses in *Drosophila* ISCs and contributes to age-related epithelial dysfunction [85]. Finally, CncC induces the accumulation of the p21 cell cycle inhibitor homolog, *Dacapo*, and the transcriptional activation of proteolytic genes, which is reduced in aging fly ISCs, can be restored by a CncC activator or by the overexpression of CncC or autophagy-related protein 8a (Atg8a), resulting in *Drosophila* lifespan extension [85]. Consistent with these findings, Nrf2 knockout also protects small intestine damage from abdominal irradiation in mice by promoting the proliferation and differentiation of ISCs and by NF-κB activation [86]. However, the critical role and the underlying mechanism of Nrf2 in regulating the state and function of ISCs in higher organisms is yet to be defined.

Taken together, these findings establish Nrf2 as a critical redox transcription factor that negatively regulates ISC proliferation, cell cycle progression, differentiation, and regenerative function in intestinal tissues (Figure 3B), although further studies are required to determine the translational relevance of Nrf2 in intestinal homeostasis and the response to injury.

Concluding Remarks

The critical roles for stem and progenitor cells during morphogenesis and growth and during repair of organs and tissues in response to inflammatory diseases are well established [87], and ROS signaling adversely affects stem cell proliferation, differentiation, senescence, longevity, and reprogramming, along with the interactions between stem cells and their niches [88,89]. Nrf2 transcriptional activity is

Clinician's Corner

As an effector and regulator of redox and metabolic homeostasis in stem cells, the transcription factor Nrf2 can regulate stem cell biology and function *in vitro* and *in vivo* in preclinical models.

The regulatory roles of Nrf2 in stem cell biology and function are context dependent and are influenced by stem cell type, development stage, and intracellular and extracellular microenvironment.

The beneficial effects of Nrf2 regulation on stem cell survival and function may be balanced by potential adverse effects on cancer cells and cancer stem cells.

Current research focuses on preconditioning of stem cells to upregulate Nrf2 signaling prior to stem cell transplantation to augment cell survival in tissue-engineered constructs or cell therapies.

Stem-cell-specific Nrf2 inducers and/or inhibitors are not yet available for therapeutic purposes.

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precisely regulated within stem cells, regulates multiple downstream antioxidant and metabolic pathways, and influences stem cell integrity, homeostasis [88], and metabolic programming [13,90]. This review focuses on the roles for Nrf2 redox and metabolic regulation of the state and function of ESCs, iPSCs, HSCs, MSCs, NSCs, EPCs, and ISCs (Table 1). Nrf2 also plays critical roles in the physiological and pathological function of other stem cells [91–95], beyond the scope of this review. These findings support a potential therapeutic role for Nrf2 in stem cell-based regenerative medicine (see Clinician's Corner).

Because Nrf2 plays unique type- and lineage-specific roles in regulating stem cell proliferation, differentiation, and reprogramming, refinement in standardized *in vitro* culture protocols is required [88]. Stem cell-related studies may be significantly refined by designing tunable Nrf2-mediated redox and metabolic balance-based culture conditions to support stem cell proliferation without impacting pluripotency or differentiation towards desired cell lineages relevant to regenerative medicine.

In vivo preclinical studies utilizing stem-cell-targeted therapies optimized via Nrf2 pathway regulation of downstream targets are likely to expand the opportunities for clinical translation. Notably, current strategies focus on transplantation of Nrf2-overexpressed MSCs [54] or pretreatment of EPCs to upregulate stem cell Nrf2 signaling [18,81,96] prior to implantation to augment cell survival in tissue-engineered constructs or cell therapies [81].

Additional preclinical studies should address the safety and risks of therapeutic strategies targeting stem cell Nrf2 regulation as Nrf2 can function both as a stem cell fate regulator and as an oncogene, and components of the Nrf2 signaling pathway may be dysregulated in various cancer cell lines [5,97]. Nrf2 pathway hyperactivation can help transformed/malignant cells escape oxidative stress through the expression of antioxidant target genes or by promoting cell survival and proliferation [5]. Moreover, Nrf2 plays an important role in chemoresistance and reducing cancer cell apoptosis [5]. Achievement of an ideal balance between the manipulations of stem cell fate while suppressing tumorigenesis will be required for Nrf2 related stem-cell-based therapies.

In summary, current knowledge about Nrf2 regulation suggests major opportunities in controlling stem cell redox states, metabolic homeostasis, survival, self-renewal, pluripotency, proliferation, differentiation, and reprogramming. Novel insights from ongoing *in vitro* and *in vivo* preclinical studies are likely to fill the missing gaps and lead to clinical translation towards improved stem cell efficacy (see Outstanding Questions).

Outstanding Questions

The role of Nrf2 in regulating stem cell proliferation, differentiation, and programing stages depends upon the stem cell class (ESC, iPSC, HSC, MSC, ESC, NSC, EPC, ISC, etc.). What are the lineage-specific pathways and mechanisms by which Nrf2 regulates stem cell redox and metabolic homeostasis and stem cell integrity and fate determination? Which Nrf2-specific mechanisms are shared between stem cell lineages and which are lineage specific? How are these pathways modified during stem cell lineage differentiation?

How do changes in stem cell microenvironments *in vivo* and *in vitro* impact Nrf2 regulation of stem cell fates, and what are the best methods to validate *in vitro* observations in preclinical, *in vivo* models?

At present, genetic modulation of Nrf2 expression/activity is feasible in preclinical models; however, there are no specific and effective small molecule Nrf2 inducers or inhibitors. Which pathways upstream or downstream of Nrf2 would be feasible for small molecule targeted strategies that could mimic Nrf2 regulation?

Nrf2 has also been proven to be an oncogene that regulates cancer stem cell transformation and/or chemoresistance. What treatment strategies could maintain the safety and effectiveness of beneficial Nrf2 effects on stem cells while avoiding Nrf2 adverse effects on cancer cells and cancer stem cells?

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