FORUM REVIEW ARTICLE

Redox Signaling in Cardiac Renewal

Wataru Kimura,¹ Shalini Muralidhar,¹ Diana C. Canseco,¹ Bao Puente,¹ Cheng Cheng Zhang,²
Feng Xiao,¹ Yezan H. Abderrahman,¹ and Hesham A. Sadek¹

Abstract

Significance: Utilizing oxygen (O₂) through mitochondrial oxidative phosphorylation enables organisms to generate adenosine triphosphate (ATP) with a higher efficiency than glycolysis, but it results in increased reactive oxygen species production from mitochondria, which can result in stem cell dysfunction and senescence. **Recent Advances:** In the postnatal organism, the hematopoietic system represents a classic example of the role of stem cells in cellular turnover and regeneration. However, in other organs such as the heart, both the degree and source of cellular turnover have been heavily contested. **Critical Issues:** Although recent evidence suggests that the major source of the limited cardiomyocyte turnover in the adult heart is cardiomyocyte proliferation, the identity and potential role of undifferentiated cardiac progenitor cells remain controversial. Several types of cardiac progenitor cells have been identified, and several studies have identified an important role of redox and metabolic regulation in survival and differentiation of cardiac progenitor cells. Perhaps a simple way to approach these controversies is to focus on the multipotentiality characteristics of a certain progenitor population, and not necessarily its ability to give rise to all cell types within the heart. In addition, it is important to note that cycling cells in the heart may express markers of differentiation or may be truly undifferentiated, and for the purpose of this review, we will refer to these cycling cells as progenitors. **Future Directions:** We propose that hypoxia, redox signaling, and metabolic phenotypes are major regulators of cardiac renewal, and may prove to be important therapeutic targets for heart regeneration. *Antioxid. Redox Signal.* 21, 1660–1673.

Introduction

THE ACCUMULATION OF O₂ in the atmosphere, which began about 2.5 billion years ago, enabled organisms to utilize aerobic respiration, producing much more adenosine triphosphate (ATP). However, during aerobic respiration, through mitochondrial oxidative phosphorylation, reactive oxygen species (ROS) are produced (27). Mitochondrial ROS, which are generated as a consequence of electron leak by the electron transport chain (77, 121), can promote widespread damage of proteins, nucleic acids, lipids, and so on, in particular when ROS production overwhelms the cellular antioxidant defense mechanisms (93, 103). On the other hand, a proper amount of ROS is known to act as a mediator of the cellular signaling pathway, including the response to growth factors or to form protein disulfides (88, 97, 170, 174).

Therefore, an adaptive antioxidant system that balances between ROS generation and ROS scavenging by antioxidant enzymes such as superoxide dismutases (SODs), catalases (CATs), glutathione peroxidases (Gpxes), peroxiredoxins (Prxes), and thioredoxins (Trxes) is essential for maintaining the critical redox balance (49).

In adult stem cells (tissue-specific stem cells), reduction of oxidative stress, as well as other types of cellular stresses, is especially critical, as these cells support self-renewal and tissue regeneration throughout the lifespan (139). Moreover, accumulation of cellular stress in stem cells might be an important mechanism of malignant transformation (72). Cellular ROS level is also suggested to be a critical regulator of stem cell fate. For example, moderate ROS production is correlated with stem cell proliferation and differentiation, while a high ROS level results in stem cell senescence,

¹Division of Cardiology, Department of Internal Medicine, UT Southwestern Medical Center, Dallas, Texas.

²Departments of Physiology and Developmental Biology, UT Southwestern Medical Center, Dallas, Texas.

premature exhaustion, and apoptotic death (Fig. 1) (20, 139). Several stem cells are located in environments with low oxygen tension (hypoxic) in tissues or organs; for example, ependymal zone of the central nervous system for neural stem cells or endosteal region of the bone marrow (BM) for long-term hematopoietic stem cells (LT-HSCs), which help shield them from oxidative stresses (83). In addition, stem cells have often developed systems to reduce oxidative stress and ensure long-term maintenance (73, 105).

The relationship between the regulation of ROS level, metabolic adaptation in a hypoxic environment, and stem cell quiescence has been extensively studied in several different types of stem cells, especially in hematopoietic stem cells (HSCs). On the other hand, characterization of redox signaling, metabolism, maintenance of quiescence, and differentiation of the stem or progenitor cells in the mammalian heart have only just begun. In this review, we provide a brief overview of mechanisms of redox regulation and metabolism and their role in maintenance, proliferation, and differentiation of HSCs, one of the best-characterized tissue-specific stem cells, and discuss the emerging role of these pathways in

resident cardiac progenitor cells by comparing each of these aspects with those in HSCs.

Metabolism Regulation in Stem Cell Maintenance and Differentiation

Oxidative stress and metabolic regulation in HSCs

HSCs are some of the best-characterized tissue specific stem cells, and their functional properties, phenotype, and regulatory mechanisms have been extensively used as a model to study adult tissue renewal and regeneration. HSCs have capacities for both long-term self-renewal and lineage contribution to all types of blood cells (1, 63). It is becoming increasingly clear that intracellular ROS level is finely regulated in HSCs, and changes in redox regulation alter the HSC phenotype, including their quiescence, proliferation, differentiation, and senescence (Fig. 2). HSCs that have greater self-renewal and reconstitution capacity after serial BM transplantation exhibit lower ROS level (58), and, by contrast, during their differentiation, ROS level markedly increases (108). Mouse models defective in ROS response or DNA repair demonstrate that these pathways are required for long-term maintenance of the HSCs (42, 122). HSC senescence is promoted by ROS through activation of p38/MAPK pathway and upregulation of p16 cell cycle inhibitor (9, 113, 137, 166, 178), and abnormal accumulation of ROS in HSCs leads to defects in hematopoiesis *in vivo* (101, 158). Thus, tight regulation of oxidative stress in HSCs is essential for normal control of homeostasis in hematopoietic tissues.

Redox signaling and regulation of oxidative stress in cardiac progenitors

Over the past decade, several studies provided evidence that progenitor cells may be present in the adult heart (13, 26), including c-Kit positive cells (7, 10, 52), stem cell antigen-1 (Sca-1)-positive cells (94, 106, 152), side population (SP) cells (4, 109, 113, 114, 127), and cardiosphere-derived cells (23, 99), among others [reviewed by Smart *et al.* (142); Kimura and Sadek (71)]. All of these cardiac resident progenitor cells possess capabilities of both self-renewal and differentiation into multiple cardiovascular lineages, including endothelial, smooth muscle, and myocardial cells both *in vitro* and *in vivo* after cell delivery (177). However, there has been significant controversy in this field over whether these cells are true cardiac progenitors or not.

Although studies exploring the role of metabolic regulation or redox signaling in maintenance of cardiac progenitor cell quiescence, stemness, and differentiation are very few, several *in vitro* studies using embryonic stem (ES) cells have shown that redox balance is a critical regulator of cardiomyocyte differentiation (15, 129). ROS mediate mechanical strain and electrical stimulation-induced cardiomyocyte differentiation in ES cells (117, 130), and treatment with agents such as catalase or N-acetylcysteine (NAC), which reduce ROS levels, impairs cardiomyocyte formation in embryoid bodies (82, 129). Physiological levels of H₂O₂ are required to maintain genomic stability by activating the DNA repair pathway *via* ataxia telangiectasia mutated (ATM) (85), which also plays an important role in ROS regulation in HSCs, as will be discussed in the next section. In addition, switching from mitochondrial oxidative phosphorylation to glycolytic

FIG. 1. Redox regulation, cellular metabolism, and stem cell status. Quiescent stem cells possess a well-organized antioxidant defense system, including niches which protect stem cells from various extrinsic cellular stresses, signaling pathways that activate free-radical scavenging enzymes, and energy metabolism depending on glycolysis rather than oxidative phosphorylation which reduces oxidative stress caused by ROS generated from mitochondria. The redox state in stem cells modulates a balance between quiescence *versus* proliferation and differentiation, and excess amounts of ROS result in cellular senescence and apoptotic death. LT-HSCs, long-term hematopoietic stem cells; ROS, reactive oxygen species. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

FIG. 2. Hematopoietic stem cells (HSCs) in hypoxic bone marrow niche. Both low oxygen tension and transcriptional regulation, which is mediated by homeodomain transcription factor Meis1 (and its unknown cofactor, indicated by “cofactor?”), are essential for the activation of Hif-1 α and Hif-2 α . Hif-1 α promotes an oxidative metabolism to glycolysis switch through transcriptional activation of genes that regulate glucose uptake (Glut1), glycolytic regulatory enzymes (PFKFBs, HKII), pyruvate disposal (Ldha and Pdk1), and repressor genes against mitochondrial oxidative metabolism (PDK1). Hif-2 α upregulates several antioxidant enzymes to reduce cellular ROS levels. Meis1-Hifs-mediated metabolism and redox regulation is crucial for the maintenance of LT-HSC quiescence and for avoidance of oxidative stress-induced senescence. Hif-1 α , hypoxia-inducible factor-1 α . To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

metabolism occurs during reprogramming of fibroblasts into induced pluripotent stem cells, and stimulation of glycolysis promotes *in vitro* reprogramming from somatic cells into induced pluripotent stem cells (37). Moreover, our group recently reported that postnatal terminal differentiation of cardiomyocyte and cell cycle arrest is triggered by mitochondrial ROS-mediated oxidative DNA damage (118). These studies strongly suggest that ROS may play diverse roles in cell cycle regulation and differentiation during different stages of cardiac development. It is, therefore, plausible that cells responsible for cellular turnover in the heart, whether they are immature myocytes or a true progenitor population, may reside in an environment with a lower oxygen concentration, similar to the hypoxic microenvironment where LT-HSCs are maintained, as discussed later in great detail.

Hypoxic Niche and Metabolism Regulation in Stem/Progenitor Cells

HSCs in hypoxic niche

Since the concept of the specific stem cell microenvironment, or niche, of HSCs was introduced in 1978 (131), mounting evidence indicates that the niche plays a crucial role in HSC self-renewal and differentiation (39, 40, 146). Since ROS is produced as a by-product of electron transport chain in the mitochondria, oxygen-rich condition produces more ROS in HSCs (156, 180). HSCs reside in a specific microenvironment that likely serves to reduce cellular stresses and damages in the HSCs, and to prevent premature differentiation (34). One of the hallmarks of the HSC niche is its low oxygen tension, hence the term “hypoxic niche” (34). In fact, hypoxic regions in the BM have been detected with gas analysis (50) and also by reduced accessibility to the circulating dye Hoechst and by marking with the hypoxia-sensitive reagent pimonidazole (110).

Although it has been widely accepted that HSCs reside within hypoxic niches in the BM, the metabolic adaptation of HSCs to the hypoxia has not been exploited until recently. During the last few years, it has become clear that HSCs harbor unique biological properties by which one can distinguish LT-HSCs from committed progenitors or mature blood cells (150, 156). A previous study has shown that LT-HSCs utilize anaerobic glycolysis, rather than mitochondrial oxidative phosphorylation, to support ATP production (140); anaerobic glycolysis is sufficient for LT-HSCs undergoing self-renewal; and mitochondrial oxidative phosphorylation is required for lineage commitment of HSCs (176).

Recently, our group and others showed that hypoxia-inducible factor-1 α (Hif-1 α), a basic helix-loop-helix (bHLH) transcription factor, plays a crucial role in maintaining metabolic program in LT-HSCs (140, 154, 155). Hif-1 α activates the transcription of several hundreds of target genes that enhance glycolysis, diminish mitochondrial activation, or regulate cellular proliferation, differentiation, and migration, including growth factor receptors (6, 57, 169). Hif-1 α activity is known to be regulated at multiple levels (91, 134). One of the best-known regulators of Hif-1 α is von Hippel Lindau (VHL) E3 ubiquitin ligase-dependent protein degradation pathway (66, 95, 124), which is known to be important for LT-HSC maintenance (154). In addition, Hif-1 α expression is regulated at the transcriptional level in LT-HSCs. Recently, we found that the three amino-acid loop extension (TALE) superclass homeodomain transcription factor Meis1 directly activates transcription of Hif-1 α in LT-HSCs and regulates HSCs metabolism (154). Meis1 deletion in HSCs results in the loss of both quiescence and reconstitution abilities of HSCs, and switches HSCs metabolism from glycolysis to mitochondrial oxidative phosphorylation (75). Therefore, an additional level of transcriptional regulation of metabolism, proximal to hypoxic response elements, exists in HSCs,

likely providing an additional level of redundancy to maintain glycolytic metabolism and quiescence. Taking together, hypoxic niche supports HSC quiescence by reducing mitochondrial respiration, a main source of ROS production, *via* activation of Hif-1-mediated metabolic switch into anaerobic glycolysis.

Epicardial hypoxic microenvironment and cardiac progenitor cells

Since the heart is one of the organs with the highest O₂ consumption (25, 35, 128), shielding cells from oxidative stresses seems to be of particular importance, especially for cycling cells (126). Recently, it was reported that cardiac-derived cells cultured in cardiosphere three-dimensional cell aggregates are more resistant to oxidative stress and show enhanced functional benefit of stem cells than those cultured as a monolayer after transplantation into the injured heart (83). In addition, these cardiac progenitor cells expanded in hypoxic conditions (5% oxygen tension) show better functional recovery after engraftment than those expanded under 20% O₂ (84). In addition, similar hypoxic preconditioning of c-kit⁺ cardiac progenitor cells improves their survival and homing after the engraftment into an infarcted heart (173). Thus, at least in several cardiac resident progenitor cells, intracellular ROS level is highly likely to be kept at a low level to maintain their quiescence and/or multipotency.

During embryonic development, epicardium-derived cells contribute to cardiac fibroblasts, vascular smooth muscle, and endothelial cells, as well as to cardiomyocytes (16, 180). In the adult heart, the epicardium contains multipotent pro-

genitor cells (22) and on injury, epicardium-derived cells contribute to fibroblasts and perivascular smooth muscle cells without contribution to the cardiomyocyte pool (179), except after priming with thymosin β 4 (141). Therefore, there is evidence that the epicardium actually harbors multipotent cells in both the embryo and the adult. Moreover, Hif-1 α -mediated hypoxia response signal in the epicardium plays a crucial role in heart development during embryogenesis. Numerous epicardial genes, including Wilms tumor 1 (WT1) (164), vascular endothelial growth factors (VEGFs) (81), and Notch-1 (47, 123), control cardiomyogenesis, vasculogenesis, and epithelial-mesenchymal transition, and are direct target genes of Hif-1 α (Fig. 3). However, the role of epicardial Hif-1 α , or hypoxia-inducible signals in general, in cardiomyogenesis, especially in the adult heart, has not been explored.

Our group recently identified the epicardium and sub-epicardium regions as the possible hypoxic niche of the heart. We showed that Hif-1 α is localized to the epicardium and immediate subepicardial layers in the noninjured heart. In addition, epicardial (noncardiomyocyte) cells, with a low mitochondrial content, behave as cardiac progenitors *in vitro*, with the capacity of self-renewal and multipotency, including differentiation to endothelial, smooth muscle, and myocardial lineages (74) (Fig. 4). However, one caveat of these studies is the strict *in vitro* differentiation approach, which may not reflect the behavior or the differentiation potential of these cells *in vivo* in their native environment. These epicardial cells utilize glycolysis as a main source of energy instead of mitochondrial oxidation, hence the term glycolytic cardiac progenitor cells (GCPs), and Hif-1 α is required for maintaining their glycolytic metabolism as well

FIG. 3. Versatile functions of hypoxic epicardium in both the adult and embryonic heart. Hypoxia in the epicardium induces the expression of several different genes during embryogenesis. An Hif-1 α target gene WT1, bHLH transcription factor, regulates genes involved in proliferating cardiomyogenesis, vasculogenesis, and epithelial-mesenchymal transition (EMT), such as α 4integrin, RAR α 1, erythropoietin (Epo) receptor, and VEGFs. Thymosin β 4, which is also upregulated with hypoxia, from the myocardium induces epicardial EMT and coronary vessel formation. Hypoxia in the adult epicardium is highly likely to be essential for the maintenance of multi-potency of GCPs. Therefore, hypoxia in the epicardium/subepicardium actually supports quiescence of progenitor cells and their capacity of multi-lineage differentiation. bHLH, basic helix-loop-helix; GCPs, glycolytic cardiac progenitor cells; VEGFs, vascular endothelial growth factors; WT1, Wilms tumor 1. ? indicates unidentified hypoxia induced gene expression. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

FIG. 4. Epicardial and sub-epicardial hypoxic niche and glycolytic epicardial cardiac progenitor cells. The epicardium and subepicardium contain the lowest capillary density across the ventricles. More than 50% of epicardial cells express Hif-1 α proteins, and these cells isolated as low-mitochondrial contents are clonogenic, self-renewing and can differentiate into endothelial cells, smooth muscle cells, and cardiomyocytes *in vitro*. These cells utilize glycolysis rather than mitochondrial oxidative phosphorylation in an Hif-1 α -dependent manner (and are, therefore, named GCPs), which is required for the maintenance of quiescence. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

as proliferation and differentiation (74). It is important to note here that the rare Hif-1 α -expressing myocytes were also not examined in this study. These hypoxic cardiomyocytes may, in fact, represent a progenitor population and may contribute to cardiomyocyte turnover. These results suggest that similar to LT-HSCs, a hypoxic microenvironment and Hif-1 α -dependent glycolytic metabolism is required for quiescence of this metabolically sorted population of epicardial cells.

Although the findings of an epicardial hypoxic niche that harbors a form of progenitor cell or cycling cardiomyocyte are highly appealing, the biological characteristics of these cells, especially whether they are involved in maintenance of cardiac homeostasis and response of myocardial injury, are largely unknown. In addition, it is important to determine how these epicardial niche progenitor cells are regulated to differentiate and migrate toward damaged sites after myocardial injury. For example, redox changes at the injury site may regulate proliferation and differentiation of cycling cardiomyocytes or progenitor cells. Since ROS level is generally correlated with stem cell differentiation, as previously discussed, increased oxidative stress after cardiac injury (112, 153, 175) may well trigger GCPs proliferation and differentiation (71). Moreover, cardiac hypoxia, which is a typical form of cardiac injury (62, 64), may play a role in the recruitment of cycling cells or their progeny into injured sites (43, 71), akin to their hematopoietic counterparts (132, 138, 149). In that regard, Hif-1 α (148), stromal cell-derived factor 1 (SDF-1), and other chemokines are upregulated in the heart after myocardial injury, which have been shown to enhance cardiomyocyte migration into the injured region in zebrafish

(56). Therefore, hypoxia may be utilized by cycling cells as their niche in the normal heart and signaling center after the injury for their recruitment.

Antioxidant Response in Stem Cell Maintenance and Differentiation

Antioxidant defense system in HSCs

In addition to being in the optimal environment for their quiescence, hypoxic niche, stem cells have evolved antioxidant redox systems that protect against the oxidative stress to keep themselves from the accumulation of ROS which is detrimental to maintain their stemness. In LT-HSCs, several oxidative stress response pathways, such as forkhead box transcription factor FOXOs (125, 157) or ATM kinase (101, 172), play a crucial role in ROS regulation and are required for maintaining the self-renewal ability.

We have reported that Meis1 appears to play a critical role, not only in the metabolic phenotype but also in limiting ROS production in LT-HSCs (75, 140, 161). Meis1 is required for transcriptional activation of Hif-1 α and Hif-2 α , thereby repressing mitochondrial biogenesis and activating antioxidant defense mechanisms, respectively (96, 133, 150). HSC-specific Meis1 knockout results in downregulation of both Hif-1 α and Hif-2 α , increased mitochondrial respiration, oxygen consumption, and oxidative stress, as well as decreased glycolytic flux (75). Remarkably, ROS scavenger NAC administration rescues phenotype in HSCs with Meis1 deletion—restored quiescence, reconstitution activity after transplantation, and p16/Ink4a and p19/Arf expression. Altogether, Meis1 appears to be an important component of a

transcriptional network that regulates cellular metabolism and redox status, which are tightly coupled to cell cycle regulation.

Meis1 and cell cycle regulation in mammalian heart

As described thus far, our knowledge on tissue-specific stem cells, including their physiological localization, molecular basis of quiescence, differentiation, and metabolic properties, has significantly increased in the past two decades. However, little is known about metabolic and redox regulation of cardiac progenitor cells, perhaps owing to the inability of the adult heart to regenerate spontaneously in any meaningful way. The role of regulators of the oxidative stress response pathway, such as FOXOs, ATM, or Meis1 in quiescence, proliferation, and differentiation of cardiac progenitor cells, is yet to be determined. In contrast, an unexpected role of Meis1 has recently emerged in regulation of postnatal cell cycle arrest of cardiomyocytes in mammals (Fig. 5).

The mammalian heart is one of the least regenerative organs, and, as a consequence, heart failure is a leading cause of morbidity and mortality worldwide. In stark contrast, urodele amphibians and teleost fish can undergo complete regeneration of their heart after multiple forms of injury (21, 90). Recent studies have revealed that the mammalian heart also has significant regenerative capacity in embryos (29) and neonates (115, 116). These findings may open new possibilities in regenerative therapy for heart failure. However, there are still significant challenges to be overcome before achieving heart regeneration in adult mammals. One of the biggest challenges is to understand why this remarkable regenerative capacity of the neonatal heart is lost by 1 week of age (115, 116), and ideally, how to re-activate the regenerative program in the neonatal heart again in the adult heart. The primary mechanism driving the regeneration of the neonatal mouse heart, similar to that of fish heart, is proliferation of pre-existing cardiomyocytes (61, 70, 115, 116) rather than stem or progenitor cells. In contrast, 1-week-old

mice heart fails to induce cardiomyocyte proliferation (115, 116). It is noteworthy that this timing of the loss in cardiac regenerative capacity in neonatal mice closely coincides with the time when rodent cardiomyocytes become binucleated and exit the cell cycle (82, 145). In larger mammals, including humans, many cardiomyocytes undergo a final round of DNA replication without nuclear division after birth, resulting in multiploid mononuclear cells (3, 102, 107), which are cell cycle arrested. After this postnatal cell cycle arrest, cardiomyocyte growth occurs through a hypertrophic increase in cardiomyocyte size with heightened organization of the sarcomere (104, 121, 171) and a metabolic switch from glucose to more energy efficient fatty acid oxidization (54, 89).

Recently, our group identified the transcription factor Meis1 as an important regulator that mediates the switch from embryonic/neonatal hyperplastic proliferation to postnatal hypertrophic growth (90). Meis1 expression is upregulated postnatally in cardiomyocytes, which is correlated with postnatal cell cycle arrest, and cardiomyocyte specific knockout of Meis1 induces cardiomyocyte proliferation. Remarkably, induced conditional knockout of Meis1 in adult mice activates cardiomyocyte cell cycle without a negative effect on cardiac function (90). In contrast, forced expression of Meis1 induces premature hypertrophic growth of cardiomyocytes and inhibits neonatal heart regeneration. Moreover, we found that Meis1 regulates cardiomyocyte cell cycle arrest through transcriptional activation of Ink4a-ARF-Ink4b and p21 cell cycle inhibitors.

Although Meis1 deletion in cardiomyocytes did not result in increased cell death, it would be important to examine how Meis1 deletion may affect the metabolic phenotype and redox status of cardiomyocytes, especially after cardiac injury. One critical distinction between the stem cell model and the cardiomyocyte model is the profound difference in the metabolic phenotype and oxygen tension of the environment. While HSCs are glycolytic with very low oxygen consumption rates, reflective of their hypoxic microenvironment, cardiomyocytes have the highest mitochondrial content in

FIG. 5. Meis1 and postnatal cardiomyocyte cell cycle arrest. Meis1 expression in cardiomyocytes is at a low level and Meis1 protein shows perinuclear localization just after birth, whereas it is localized in the nucleus at around P7 and expression levels increase significantly thereafter. This corresponds to the switch of cardiac growth from hyperplastic to hypertrophic, cardiomyocyte binucleation and also the loss of regenerative capacity after cardiac injury. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

any cell, and the tissue oxygen tension (pO_2) of the heart is the highest in the body. Therefore, any effect of Meis1 on Hif genes is likely to be negligible in uninjured cardiomyocytes, at least under normal conditions. However, it is still plausible that Meis1 deletion in cardiomyocytes may induce a modest increase in ROS production, which may have a proliferative effect on cardiomyocytes, especially in their early postnatal stages before cell cycle arrest. It is intriguing that Meis1 seems to be a critical mediator of HSCs quiescence and of cardiomyocyte cell cycle arrest. While HSCs can exit their state of quiescence on demand, cardiomyocytes are unable to do so. The discrepancy between Meis1 functions in these two systems may be related to the differential pattern of expression of Hox genes, which are known Meis1 cofactors that mediate its DNA binding and regulate its transcriptional activity (18, 19, 33). It remains to be seen whether Meis1 is important for transcriptional activation of Hif genes in cardiomyocytes, which may not have a significant effect on myocyte function under normal conditions, but could certainly impact myocytes profoundly in an injury setting where hypoxia signaling is required for myocyte survival.

Antioxidant response in cardiac turnover

It is well accepted that the heart is a highly oxidative organ with high oxygen consumption. More importantly, myocardial injury such as acute myocardial infarction, ischemia/reperfusion, and chronic heart failure cause profound changes in oxidative stress and energy metabolism (8, 32, 38, 59, 147). Although low levels of ROS activate cell growth, intermediate or high levels of ROS trigger apoptotic or necrotic cell death in the c-kit⁺ cells (120). Accumulation of superoxide also has a negative impact on the survival of Sca-1⁺ cells (67). There is some evidence that cardiac progenitor cells, not unlike HSCs, are in tune to changes in redox status of their environment. Gurusamy *et al.* showed that redox effector protein-1 (Ref-1) plays an important role in maintaining the redox status of human c-kit⁺ cardiac progenitor cells, and protects them from oxidative injury-mediated cell death and differentiation (45, 46). c-kit⁺ cardiac progenitor cells are also known to express higher levels of SOD, and, therefore, coupled with their capacity to increase the expression of SOD in response to oxidative stress, are resistant to ROS-induced cell death (136). Khan *et al.* reported that c-kit⁺ cardiac progenitor cells switch the expression of the subtype of adrenergic receptor (AR) from β 2-AR to β 1-AR once they are committed to myocyte differentiation, and treatment of β -blockers, which are known to have antioxidant properties (76), can enhance their survival and proliferation (69). This might suggest that c-kit⁺ cells are especially sensitive to oxidative stress when they start differentiation.

Another resident cardiac progenitor population known as cardiac SP progenitor cells possesses mechanisms that support their survival against oxidative stress. SP cells were first identified in mouse BM by their ability to pump out fluorescent dye Hoechst 33342, which binds to DNA (44), and it turned out that the SP population is highly enriched for HSCs (17, 28, 68, 151, 168). The ability to actively efflux the dye is mainly dependent on ATP-binding cassette (ABC) transporters, which is essential for the maintenance of SP cells (14, 41, 44, 48, 51). One of the crucial role of ABC transporters in SP cells not only in BM, but also in multiple tissues, is

protecting cells from apoptotic or necrotic cell death under severe oxidative stress (36, 78). In line with these data, cardiac SP progenitor cells express a high level of *Abcg2*, which encodes an ABC transporter, and it plays an essential role in their proliferation, multipotency, and survival against oxidative stress (114). It is interesting that the transcription of *Abcg2* is activated by HIF-1-mediated hypoxia responsive signaling pathway (78). These findings suggest that the correlation between the hypoxic microenvironment and redox characteristics of stem/progenitor cells is not limited to HSCs, but may also exist in populations of cardiac progenitor cells.

An important factor that bridges redox signaling and cardiac regeneration is thymosin β 4, which is an actin monomer binding protein. Thymosin β 4 activates cell migration, angiogenesis, and epithelial-mesenchymal transition (12, 92, 143), and, interestingly, is activated by Hif-1 α (60). Thymosin β 4 is particularly attracting attention because of its potential role in cardiac regeneration. It was recently reported that thymosin β 4 can improve cardiac function by activating epicardial cell migration, vasculogenesis (119), and, in addition, inducing epicardium-derived progenitor cells to differentiate into cardiomyocytes (141). Moreover, several studies indicate that thymosin β 4 treatment decreases oxidative stress by activating antioxidant enzymes, such as SOD and catalase (167). Therefore, there appears to be an emergence of evidence that supports the role of redox regulation in cardiac progenitor cells' maintenance and function.

Concluding Remarks

A deeper understanding of the mechanisms of maintenance and differentiation of HSCs, including the role of redox signaling, has paved the way for successful stem cell therapy for hematopoietic disorders. So far, a similar effective therapeutic approach to heart disease has remained elusive.

It is important to note that the developmental origins of differentiated cardiac cells might provide clues to the question of the existence of a true cardiac stem cell. During embryonic development of hematopoietic system, all cellular blood components are derived from common progenitor cells, hemangioblast, similar to the adult organism (31, 53, 98, 160). In contrast, there are two distinct developmental origins that give rise to heart cells. The major source of epicardium, endothelial cells of coronary veins, smooth muscle cells, and fibroblasts, is the proepicardium that is a derivative of the splanchnic mesoderm; whereas cardiomyocytes, endocardial and coronary arterial and capillary cells are derived from the heart field mesoderm which is located in the anterior part of the lateral plate mesoderm (2, 24, 30, 55, 86) (Fig. 6). Therefore, one cannot simply apply the classic model of stem cells (where a single stem cell exists that gives rise to all cell lineages in an organ), as in hematopoiesis, to the cardiovascular system, at least if developmental origins provide any clues to cellular turnover in the adult organism.

Historically, there has been a fierce debate over cardiomyocyte turnover in the adult heart (52, 79). The rate of cardiomyocyte turnover has been reported to range from less than 1% per year (11, 144, 165) to more than 40% per year (65). In addition, the source of newly formed cardiomyocytes has been attributed both to the division of existing myocytes (65) and to progenitors residing within the heart (8, 162). However, recent data indicate that the main source of

FIG. 6. Lineage correspondence of adult cardiac turnover with developmental origin. During cardiogenesis in the embryos, two major mesodermal sources provide cardiac progenitor cells: heart field mesoderm, which is derived from anterior lateral plate mesoderm, and proepicardium, which is derived from splanchnic mesoderm. Cells from heart field mesoderm give rise to cardiomyocytes, endocardial cells, and vascular endothelial cells. Proepicardium mainly contributes to epicardium, fibroblasts, and vascular smooth muscle cells (and myocardium, although there is controversy regarding epicardium-derived cardiomyocytes). These lineage origins seem to correspond roughly with adult cardiac cell turnover. For example, epicardial cells can contribute to cardiac fibroblasts and smooth muscle cells (and cardiomyocytes only after thymosin β_4 priming), and newly formed endothelial cells are derived from pre-existing endothelial cells, similar to embryonic development. Cardiomyogenesis in the adult can occur at a very low rate using pre-existing cardiomyocytes as a source of new cardiomyocytes. Limited contribution of epicardium to myocardium is indicated by broken lines. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

cardiomyocyte turnover in lower vertebrates (61, 70), neonatal (115, 116) as well as adult hearts is pre-existing cardiomyocytes, rather than an undifferentiated progenitor population (5, 135). In that sense, perhaps one should consider the population of cycling cardiomyocytes a true “cardiac progenitor population,” although no specific defining characteristics of these myocytes have been described thus far, other than perhaps their persistent mononucleation. Although these results indicate that cardiomyocytes are the major source of new cardiomyocyte formation, it is possible that there is a minor contribution of resident stem/progenitor cells to cardiomyogenesis. Recently, it was shown that c-kit⁺ cardiac progenitor cells widely contribute to nonmyocyte lineages, especially vascular endothelial cells, but rarely contribute to cardiomyocyte turnover (0.008%) even after injury (163). In contrast, fate mapping of Sca-1⁺ cardiac progenitor cells revealed contribution of these cells to cardiomyocyte at a rate of 4.6% per year (159). Whether cardiac stem or progenitor cells truly contribute meaningfully to cardiomyocyte turnover in the adult heart may not be the only deciding factor in their therapeutic potential. The beneficial effects of cell transplantation in the injured heart are due to not only the direct contribution of myocardial or vascular lineages, but also the secretion of

paracrine factors that can lead to cytoprotection, vascularization, and/or activation of endogenous repair mechanisms (87, 100, 111) and show measurable therapeutic benefits after direct intracardiac delivery (80, 100, 111).

Finally, the cardiac regeneration field has taken great strides in the past few years and there is real hope that regeneration of the adult heart may be within reach. Whether cardiac progenitor cells will emerge as a viable therapeutic option is still unclear; however, a greater understanding of the role of environmental cues and mechanisms of quiescence and differentiation of cycling cardiac cells undoubtedly remain a significant knowledge gap.

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Address correspondence to:

Dr. Hesham A. Sadek

Division of Cardiology

Department of Internal Medicine

UT Southwestern Medical Center

Dallas, TX 75390

E-mail: hesham.sadek@utsouthwestern.edu

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Abbreviations Used

ABC = ATP-binding cassette
AR = adrenergic receptor
ATM = ataxia telangiectasia mutated
ATP = adenosine triphosphate
bHLH = basic helix-loop-helix
BM = bone marrow
CAT = catalase
ES = embryonic stem
FOXO = forkhead box transcription factor o
GCP = glycolytic cardiac progenitor cell
Gpx = glutathione peroxidase
Hif-1 α = hypoxia-inducible factor-1 α
HSC = hematopoietic stem cell
LT-HSC = long-term hematopoietic stem cell
MAPK = mitogen-activated protein kinase
NAC = N-acetylcysteine
Prx = peroxiredoxin
ROS = reactive oxygen species
Sca-1 = stem cell antigen-1
SDF-1 = stromal cell-derived factor 1
SOD = superoxide dismutase
SP = side population
TALE = three amino-acid loop extension
Trx = thioredoxin
VEGF = vascular endothelial growth factor
VHL = von Hippel Lindau
WT1 = Wilms tumor 1