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Empowering self-renewal and differentiation: the role of mitochondria in stem cells

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Abstract

Stem cells are characterized by their multi-lineage differentiation potential (pluripotency) and their ability for self-renewal, which permits them to proliferate while avoiding lineage commitment and senescence. Recent studies demonstrate that undifferentiated, pluripotent stem cells display lower levels of mitochondrial mass and oxidative phosphorylation, and instead preferentially use non-oxidative glycolysis as a major source of energy. Hypoxia is a potent suppressor of mitochondrial oxidation and appears to promote “stemness” in adult and embryonic stem cells. This has led to an emerging paradigm, that mitochondrial oxidative metabolism is not just an indicator of the undifferentiated state of stem cells, but may also regulate the pluripotency and self-renewal of stem cells. The identification of specific mitochondrial pathways that regulate stem cell fate may therefore enable metabolic programming and reprogramming of stem cells.

Keywords

Stem cells; Cardiovascular; Metabolism; Mitochondria; Differentiation; Self-renewal; Proliferation

Introduction

Stem cells constitute the corner-stone of regenerative medicine, because of the two key characteristics that define “stemness”: *pluripotency* and *self-renewal*. Pluripotency refers to the ability of undifferentiated stem cells to differentiate into a variety of cell lineages and tissues [1], while self-renewal describes the proliferation of undifferentiated stem cells without lineage commitment [2]. Self-renewal of stem frequently also entails a resistance to the cellular senescence seen in mature differentiated cells, which lose their ability to proliferate after a specific number of cell divisions [3]. Embryonic stem cells (ESCs) and adult stem cells are naturally occurring stem cells [1,2]. A third type of stem cells (induced pluripotent stem cells or iPSCs) can be generated in the laboratory by over-expressing ESC pluripotency regulators such as Oct-4 or Nanog in mature adult cells and thus reprogramming them to an ESC-like state [4,5]. Among human stem cells, human ESCs exhibit the potential for indefinite self-renewal as well as the highest degree of pluripotency, because tissues from all three germ layers (endoderm, mesoderm, and ectoderm) can be generated from them [1]. On the other hand, human adult stem cells, such as hematopoietic stem cells or mesenchymal stem cells (MSCs) are more limited in their self-renewal capacity and the number of target lineages they can differentiate into [1]. The pluripotency in ESCs appears to be based on the expression of a complex network of transcription factors, such as

Oct-4 and Nanog while the self-renewal capacity and resilience to senescence appear to be in part mediated by a suppression of senescence mediators such as p16 or p21 and an upregulation of telomerase [2,6,7].

For the purpose of regenerative cardiovascular therapies, human ESCs have been successfully used to generate functional cardiomyocytes and vascular cells [8–11]. Adult human stem cells, such as MSCs, are also emerging as therapeutic agents for patients with myocardial infarction or congestive heart failure [12]. Due to the limited differentiation capacity of adult stem cells, their therapeutic benefit may result from the secretion of important paracrine growth factors and does not necessarily depend on their differentiation into functional cardiovascular cells [13]. Multiple studies suggest that iPSCs display a high degree of pluripotency and self-renewal similar to what is seen in ESCs [4]. This is most likely due to the fact that, unlike adult stem cells, iPSCs express high levels of the pluripotency regulators Oct-4 and Nanog found in ESCs [4]. Therefore, iPSCs can also be successfully differentiated into functional cardiomyocytes and vascular cells [14–16] and are likely to play a key role in future cardiovascular regenerative therapies.

While the significant proliferative potential and pluripotency of stem cells (especially ESCs and iPSCs) form the basis of regenerative therapies, it is important to realize that these characteristics also represent obstacles for clinical applications. Broad pluripotency and self-renewal capacity permit ESCs and iPSCs to differentiate into unwanted cell types and form tumors such as teratomas in vivo [4,17]. One approach to reduce misdirected differentiation and teratoma formation is to pre-differentiate stem cells into a specific lineage prior to transplanting them into a recipient. Nevertheless, concerns still remain, since the degree of differentiation and maturation prior to transplantation can vary significantly and residual undifferentiated stem cells may still give rise to teratomas in vivo [7,18,19].

For regenerative therapies, it is necessary to maximize the pluripotency and self-renewal capacity of stem cells when they are maintained in culture prior to their differentiation into a specific target lineage. However, once stem cells are chosen for a specific therapeutic purpose, it becomes important to maximize differentiation into the desired cell type and limit their pluripotency and proliferative potential. A significant amount of research has been conducted on identifying the “switches” that direct stem cell differentiation and regulate pluripotency or self-renewal. Most of the currently identified regulators of stem cell fate are transcription factors and cell cycle regulators such as Oct-4, Nanog, and c-Myc as well as the associated downstream signaling pathways [20]. Interestingly, recent studies now suggest that mitochondrial activity may also represent such an important regulatory mechanism that helps direct stem cell fate. The research on the role of mitochondria in stem cells has been fueled by a paradigm shift regarding the role of mitochondria. In addition to their traditionally ascribed anabolic/catabolic roles such as the production of ATP or synthesizing lipids, mitochondria also appear to regulate a variety of cellular processes such as cell proliferation and aging in many cell types [21]. This regulatory role of mitochondria is achieved through the controlled release of multiple signaling molecules, including reactive oxygen species (ROS) and calcium [21,22]. Novel mitochondria-targeted therapies for cancer utilize the regulatory role of mitochondria, such as therapies that enhance mitochondrial glucose oxidation and ROS production, which in turn suppresses cancer cell proliferation [23,24]. Whether mitochondrial modulation can similarly impact stem cell proliferation or differentiation has not yet been fully established. However, the recent studies reviewed below strongly suggest that mitochondria indeed contribute to the regulation of stem cell fate.

Mitochondrial metabolism in stem cells

Some recent studies have examined mitochondrial metabolism in stem cells and compared their metabolic activity to that of mature, differentiated cells. A study conducted in primate adult stromal cells (a form of MSCs derived from the adipose tissue) reported that low passage cell cultures containing a high proportion of undifferentiated stem cells have significant peri-nuclear clustering of mitochondria, when compared to late passage cells [25]. These late-passage cultures showed evidence of significant spontaneous differentiation into adipocytes and decreases in mitochondrial oxygen consumption [25]. A study examining the differentiation of human bone marrow derived adult MSCs showed that undifferentiated cells produce high levels of lactate, suggesting a reliance on non-aerobic glycolysis to cover the bioenergetic needs [26]. Upon osteogenic differentiation, however, there was a marked increase in the expression of the mitochondrial biogenesis regulator PGC-1-alpha and marked increases in mitochondrial mass and oxygen consumption [26]. Interestingly, the increase in mitochondrial mass was also accompanied by a marked increase in the expression of antioxidant enzymes such as catalase or superoxide dismutase (SOD) so that the levels of cellular ROS as measured by fluorescent ROS-sensitive dyes actually decreased in the initial days of differentiation in spite of the higher level of mitochondrial activity.

A study of human ESCs also showed an increase in mitochondrial mass and oxygen consumption with spontaneous ESC differentiation [27]. This study also demonstrated an increase in ROS production with differentiation and a concomitant increase in antioxidant defenses such as the expression of peroxiredoxin and SOD that are only expressed at low levels in undifferentiated ESCs [27]. Two recent papers published earlier this year strongly link the undifferentiated stem cell state with suppressed mitochondrial activity [28,29]. In one study, undifferentiated human ESCs and human iPSCs had a low mitochondrial mass, reduced mitochondrial ROS, and a substantial reduction in mitochondrial number when compared to mature fibroblasts or even ESCs and iPSCs undergoing differentiation [29]. The second study also demonstrated low mitochondrial mass in undifferentiated ESCs and iPSCs, but showed high levels of lactate production in these undifferentiated cells when compared to mature or differentiated cells, further underscoring the presence of a non-aerobic glycolytic metabolism in undifferentiated cells [28]. Furthermore, higher levels of oxidative damage were found in differentiating cells, possibly due to the higher mitochondrial activity. The increase in mitochondrial activity seen in ESCs undergoing differentiation is also associated with marked increases in mitochondrial DNA replication [30], which likely reflects the fact that key mitochondrial electron transport chain (ETC) components are encoded by mitochondrial DNA. The association between mitochondrial DNA and stem cell pluripotency has recently been reviewed elsewhere [31].

While the studies with adult stem cells discussed above give conflicting results as to the effect of differentiation on mitochondrial oxidative metabolism, the studies with iPSCs and ESCs are quite consistent. They point to reduced mitochondrial activity, low mitochondrial ROS production, and a reliance on anaerobic glycolysis in undifferentiated stem cells, thus suggesting similarities to the metabolic profile of cancer cells [32,33]. The differences among the studies of adult stem cells may reflect properties of the tissues from which these adult stem cells are derived and the degree of mitochondrial activity in adult stem cells may also strongly depend on the target lineages into which these cells differentiate. Unlike adult stem cells, iPSCs and ESCs exhibit similarly high levels of expression of pluripotency regulators such as Oct-4 or Nanog and this may explain the consistent mitochondrial-metabolic signature in these two cell types.

Multiple studies have noted that the mitochondria in undifferentiated stem cells appear to exhibit peri-nuclear clustering with limited mitochondrial network formation [25,26,31]. Mitochondrial morphology and mitochondrial network dynamics are now being recognized as regulators of cellular processes such as proliferation and apoptosis in various cell types [34]. This suggests that the peri-nuclear clustering and limited mitochondrial networking may also have a functional significance in stem cells that still needs to be determined.

Hypoxia as a regulator of “stemness”

While the afore-mentioned studies link reduced mitochondrial oxidation and increased anaerobic glycolysis with the undifferentiated pluripotent state, they do not necessarily prove that the mitochondrial activity can regulate “stemness”. However, the use of hypoxia as a mitochondrial modulator highly suggests that the metabolic state can indeed affect stem cell fate. During mitochondrial oxidative phosphorylation, electrons derived from NADH travel down an electrochemical gradient in a step-wise manner from Complex I through IV, and the energy released during this process is by a proton pump to create a mitochondrial membrane potential ($\Delta\psi_m$) by pumping protons out of the mitochondrial matrix into the intermembrane space. Molecular oxygen is used during the final step of oxidative phosphorylation (Complex IV) as the terminal electron acceptor and thus presence of oxygen is necessary for a completion of the process (see [35] for detailed overview of oxidative phosphorylation). Mitochondrial ROS are generated as a by-product of oxidative phosphorylation [22]. Reactive oxygen species generated by mitochondria can leave the mitochondria and act as signaling molecules regulating a variety of cellular functions, including cell survival [22] or cell senescence [36]. Under hypoxic conditions, there is limited availability of molecular oxygen and thus the activity of the mitochondrial ETC is suppressed and cells switch to glycolysis to meet their energetic needs, since it does not require oxygen. Hypoxia not only directly suppresses ETC activity by reducing available oxygen, but also acts via hypoxia inducible factors (HIFs) [37–39]. These transcription factors reduce the expression of mitochondrial enzymes and further enhance the shift to glycolysis by upregulating glucose transporters and glycolytic enzymes [38,39].

When stem cells are exposed to hypoxia the self-renewal capacity and maintenance of pluripotency in culture is enhanced. In adult stem cells such as hematopoietic stem cells or mesenchymal stem cells, hypoxia prolongs the lifespan of the stem cells, increases their proliferative capacity, and reduces differentiation in culture [40,41]. ESCs are able to retain their pluripotency for a longer period of time when cultured in hypoxic conditions [42,43]. The oxygen sensitivity of stem cells has significant relevance for stem cells in vivo since embryonic stem cells and adult stem cells are exposed to varying oxygen concentrations and the respective oxygen “niches” may control stem cell fate [39]. The importance of hypoxia as an enhancer of “stemness” has been further strengthened with a recent publication showing that hypoxia substantially enhances the reprogramming of mouse embryonic fibroblasts to iPSCs [44].

Mitochondria in stem cell fate decisions: output or mediator

The role of hypoxia as a promoter of “stemness” strongly suggests that modulation of metabolic state can affect stem cell self-renewal and differentiation. However, it is important to realize that in addition to inducing a glycolytic shift, hypoxia may also directly act on stem cell fate decisions via activation of HIF-dependent pathways without necessarily acting through shifts in mitochondrial or metabolic activity. Very few studies have directly modulated mitochondrial function in stem cells or selected stem cells based on their mitochondrial function without the involvement of hypoxia. One study demonstrated that murine ESCs with low resting $\Delta\psi_m$ were more likely to differentiate into mesodermal

lineages in vitro and less likely to form teratomas in vivo than cells with high $\Delta\psi_m$. Another recent study suggested that inhibition of the mitochondrial electron transport chain promotes human ESC self-renewal [45]. Together with the “stemness”-promoting effects of hypoxia and the observed increase in mitochondrial oxidative phosphorylation with stem cell differentiation, these findings point to an important role of mitochondrial metabolism in regulating stem cell proliferation or differentiation.

The ultimate goal is to identify specific mitochondrial pathways that can act as metabolic switches and permit metabolic programming or reprogramming of stem cell fate. Future research on the role of mitochondria in stem cells will likely be guided by the success of recognizing the importance of mitochondria in cancer cells. The identification of specific mitochondrial pathways that control cell proliferation and apoptosis has resulted in a much better understanding of the pathophysiology of cancer cell growth and apoptosis resistance. This in turn has led to the development of novel therapeutic agents that supplement conventional chemotherapy or radiotherapy regimens. Such mitochondria-targeted therapies can act via multiple mechanisms, including the shifting of mitochondrial redox states to suppress cancer cell proliferation or by modulating the mitochondrial permeability transition (MPT) to enhance cancer cell apoptosis [46].

Undifferentiated ESCs and iPSCs share the potential for indefinite self-renewal and the predominance of glycolytic metabolism with cancer cells, therefore mitochondrial pathways involved in the regulation of cancer cell proliferation may also play an important role in the self-renewal of ESCs and iPSCs. The recognition that hydrogen peroxide and superoxide can differentially affect cell proliferation in mouse embryonic fibroblasts [47] suggests that redox sensitive pathways may also regulate the differentiation of ESCs. From a therapeutic perspective, identifying mitochondrial pathways could help facilitate stable differentiation of stem cells. It has been recently shown that oxidative mitochondrial metabolism is a prerequisite for embryonic stem cells to form sarcomeres and differentiate into cardiomyocytes [48]. This highlights the importance of ensuring adequate mitochondrial oxidation in stem cells that are used for cardiovascular regeneration and transplanted into the hypoxic environment of ischemic or infarcted myocardium. It is likely that stem cell fate decisions such as differentiation or proliferation are regulated by a complex set of mitochondrial pathways that may depend on the environment and age of the cells. For example, mitochondrial superoxide dismutase 2 (SOD2) improves the differentiation of myoblasts derived from young mice but this effect of mitochondrial SOD2 is blunted in aged mice [49]. It still needs to be determined whether SOD2 or other mitochondrial signaling pathways can similarly regulate differentiation in adult and embryonic stem cells.

In addition to enhancing stem cell differentiation, reducing stem cell apoptosis in the therapeutic setting may also be important to enhance the efficacy of regenerative therapies. Mitochondria-targeted cancer therapies attempt to increase apoptosis by modulating the MPT in apoptosis-resistant cancer cells [46]. Conversely, the MPT may be a potential target to reduce apoptosis in regenerative stem cells, although little is known about the MPT in stem cells.

Looking forward: some key open questions

This concise overview has highlighted some of the recent developments that have opened up mitochondrial metabolism of stem cells as a new field of inquiry (Fig. 1). However, numerous important questions remain unanswered and will form the basis of future research on the role of mitochondria in stem cells. Some of these key questions include (1) Are the specific mitochondrial pathways that regulate stem cell self-renewal distinct from those that regulate stem cell pluripotency? (2) Do hypoxia and mitochondrial metabolism regulate

stem cell fate primarily by modulating redox states and ATP levels, or are their effects also mediated by other signaling pathways such as mitochondrial calcium? (3) Are the shifts in mitochondrial redox states seen during stem cell differentiation mainly a result of changes in ROS production or of antioxidant defenses? (4) Do the roles of mitochondria as determinants of cell fate differ among distinct types of stem cells and the respective lineages they are differentiating into? (5) What role does the mitochondrial morphology and network structure play in regulating stem cell fate?

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Fig. 1.

Stem cells increase mitochondrial oxidation upon differentiation. This schematic figure depicts an overview of the mitochondrial activity shift that occurs with differentiation of an undifferentiated pluripotent stem cells. The immature stem cell (*top*) is characterized by high nuclear levels of pluripotency regulators such as Oct-4 and Nanog, lower mitochondrial mass and peri-nuclear mitochondria. In this undifferentiated state, cells rely on glycolysis for their energy needs and mitochondrial oxidation is suppressed, thus resulting in lower mitochondrial ATP production and ROS release. Upon differentiation (*bottom*), the pluripotency regulators decrease, while the mitochondrial mass, mitochondrial oxidation, and ROS production increase. Some studies suggest that mitochondrial ROS may act as signaling mediators that direct cell differentiation or self-renewal by shifting redox states. Additional mitochondrial signaling mediators and products such as calcium or ATP may also be important and the definitive roles of such mediators have not yet been established.