Review

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A ROS rheostat for cell fate regulation

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Cellular reactive oxygen species (ROS) are tightly regulated to prevent tissue damage. ROS also help to monitor different cell fates, suggesting that a 'ROS rheostat' exists in cells. It is well established that ROS are crucial for stem cell biology; in this review, we discuss how mitochondrial ROS influence hematopoietic cell fates. We also examine the importance in this process of BID and other BCL-2 family members, many of which have been implicated in regulating cell fates by modulating mitochondrial integrity/activity and cell cycle progression in the hematopoietic lineage. Based on this knowledge, we propose that selected BCL-2 proteins coordinate mitochondria and nuclear activities via ROS to enable 'synchronized' cell fate decisions.

Mitochondria and redox regulation

Mitochondria are multifunctional organelles that regulate pivotal processes controlling cellular homeostasis. Their complex and diverse functions comprise sustaining the balance between bioenergetic metabolism and cellular death programs. Under physiological conditions, the mitochondria are the dominant source of ROS generation. These reactive oxygen moieties act as second messengers and when overproduced provoke a state of oxidative stress, associated with many pathologies [1–3]. ROS are generated by the respiratory chain during oxidative phosphorylation in the form of the superoxide anion (O_2^-) and are immediately transformed by mitochondrial superoxide dismutase (MnSOD) to hydrogen peroxide (H_2O_2) [4]. Mitochondrial ROS (mitoROS) result in oxidative damage to mitochondrial membranes, proteins, and DNA, impairing the ability of the mitochondrion to synthesize ATP and carry out its metabolic functions. Mitochondrial oxidative damage can also increase mitochondrial outer membrane permeabilization (MOMP) and activate apoptosis [4]. The redox signal generated not only influences the mitochondria, but also a vast range of cytosolic and nuclear processes leading to changes in cellular behavior and fate, suggesting that a ROS rheostat exists in cells (Figure 1).

Oxidative stress not only alters signaling pathways in the cell, but can also influence genetic and epigenetic changes thereby modulating cellular proliferation and differentiation [3,5]. Of special interest are stem cells, in which it was shown that ROS regulate stem cell self-renewal, differentiation, and apoptosis [1]. ROS may influence changes in gene expression both directly, by altering the function of many transcription factors, or indirectly by altering epigenetic regulatory elements. The mammalian genome is a highly organized chromatin structure where epigenetic regulatory elements are important for orchestrating gene expression. Histone modifications, DNA methylations, and ATP-dependent nucleosome remodeling regulate chromatin structure and hence the accessibility of DNA. It is feasible that ROS may influence several, if not all, of these nuclear processes. Indeed, it has been shown that histone deacetylases (HDACs) can be modified by oxidation of cysteine residues, which can inhibit their enzymatic activity [6].

Among the most sensitive signaling components to the cellular redox state are Ataxia-Telangiectasia Mutated (ATM) kinase, p53, and the nuclear factor kappa B (NF-κB) signaling pathway. ATM kinase, a master regulator of the cellular response to double-strand break (DSB) DNA damage, was recently shown to be differentially activated in response to cellular oxidative stress [7]. Interestingly, ATM substrate specificity activated by oxidative stress was completely altered compared with its substrate specificity following DSBs [8]. In addition, ATM controls the cellular oxidation machinery via a transcriptional arm. Mutating ATM triggers an increase in ROS levels due to hampered expression of the FOXO family of transcription factors (FOXO1, 2, 3a), which directly regulate expression of catalase and MnSOD [9]. Another redox-sensitive transcription factor is p53. It was demonstrated that oxidation of p53 alters its conformation and disrupts its DNA binding activity, resulting in a pattern change of p53-dependent gene expression [10,11]. Moreover, p53 was shown to act as a homeostatic regulator by lowering ROS levels in stem cells and controlling hematopoietic stem cell self-renewal [12–14]. Finally, the NF-κB signaling pathway is also significantly altered by dysregulated ROS. ROS activates NF-κB signaling through elimination of the IκB inhibitor. An increase in ROS levels induces the activation of the IκB kinase (IKK), which in turn phosphorylates IκB, leading to its proteasome-dependent degradation [15].

Redox regulation in mammalian hematopoietic stem cells (HSCs) has been a subject of intensive study in the past few years and has revealed that HSCs are extremely sensitive to ROS. To prevent excess ROS and maintain their stem cell characteristics (quiescence and self-renewal potential), HSCs have adapted to minimal mitochondrial activity and rely on glycolysis for energy production, along with maintaining an immense cellular antioxidant defense system [2]. It is still widely unknown how the mitochondria, by means of ROS, dictate changes in cellular fates. One possibility is that mitoROS reach the nucleus, where they induce DNA modifications that alter gene expression patterns. Supporting this notion are reports demonstrating that undifferentiated stem cells exhibit considerable

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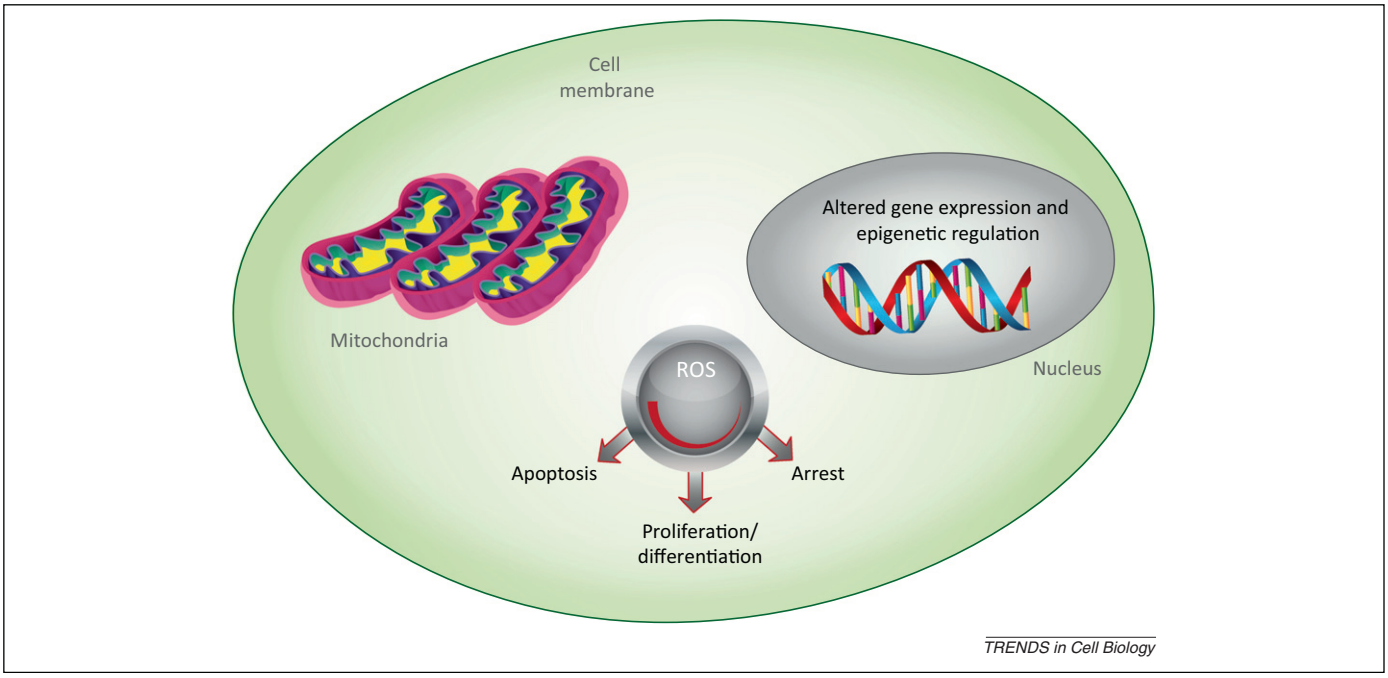

Figure 1. A reactive oxygen species (ROS) rheostat for cell fate regulation. ROS, mainly generated in the mitochondria, are tightly regulated within the cell to prevent tissue damage. Many studies now indicate that ROS also help to monitor cell fates, and we propose that a 'ROS rheostat' exists in cells. Thus, a relatively small increase in cellular ROS, sufficient to alter gene expression and signaling cascades, may help to trigger proliferation/differentiation, whereas higher levels of cellular ROS may lead to outcomes such as apoptosis or necrosis.

perinuclear clustering of mitochondria [16,17]. Thus, it is feasible that mitoROS simply diffuse into the nuclear compartment. mitoROS may also activate redox-sensitive proteins that travel/signal to the nucleus and to other parts of the cell. Alternatively, nuclear factors may reach the mitochondria and become activated, as was recently reported for the ATM kinase [18]. In this respect, proteins from the BCL-2 family are strong candidates for such tasks, traveling/signaling between the mitochondria and the different cellular compartments (see below).

The BCL-2 family, mitochondria, and hematopoiesis

The BCL-2 family is divided into two major groups: the anti-apoptotic proteins (BCL-2, BCL-X_L, and MCL-1) and the pro-apoptotic proteins (BAX and BAK), which work together to control cell life and death decisions. The BH3-only proteins, a subset of the pro-apoptotic proteins (e.g., BID, BIM, BAD) act as sensors of intracellular damage. These proteins are located at distinct cellular locations at which they can sense specific damage and, once activated, translocate to the mitochondria to communicate the damage signal and execute apoptosis [19–21]. Recent studies demonstrate that selected BCL-2 family members are potent regulators of hematopoietic differentiation and are responsible for maintaining homeostasis at specific developmental stages. Moreover, many of these recently discovered activities of the BCL-2 family members were linked to their direct regulation of mitochondrial functions.

MCL-1, for example, was shown to be a crucial pro-survival molecule during early hematopoiesis. Inducible deletion of MCL-1 in MxCre mice resulted in a dramatic multilineage hematopoietic failure, causing loss of HSCs, common myeloid progenitors (CMPs), and common lymphoid progenitors (CLPs) [22]. In addition, ablation of MCL-1 was found to be critical for terminal granulocyte differentiation but dispensable for monocyte differentiation, indicating a selective role during myeloid development. MCL-1 was also recently demonstrated to localize to the mitochondrial matrix and to be necessary for basic mitochondrial functions such as respiration, ATP production, and mitochondrial fusion [23]. Another anti-apoptotic member of the family, BCL-X_L, localized at both the inner and the outer mitochondrial membrane, was recently shown to regulate mitochondrial energetics and metabolic efficiency [24,25]. Moreover, it was implicated in both cell cycle regulation and hematopoietic development, playing a role in G_2 arrest and DNA mismatch repair [26–28]. Thus, BCL-X_L is another family member that plays an important role in enabling mitochondrial and nuclear functions critical for proper hematopoiesis.

The BH3-only BCL-2 family members BIM, PUMA, and BID were also shown to play important roles in maintaining homeostasis of early bone marrow progenitors, HSCs, myeloid progenitors, and terminally differentiated myeloid and lymphoid cells [29–33]. PUMA, for example, was shown to play a crucial function during stress conditions in the hematopoietic system by being one of the most potent p53 target genes to induce apoptosis in HSCs following ionizing radiation [34,35]. In addition, $Puma^{-/-}$ HSCs show enhanced quiescence and more efficient DNA repair, indicating its critical role in maintaining hematopoiesis [36].

What are the molecular mechanisms by which BCL-2 proteins control cell fate decisions in the hematopoietic system? It is tempting to speculate that selected BCL-2 family members act as coordinators between the mitochondria and nucleus (mi-to-nuc coordinators) to 'synchronize' metabolism and transcription (Figure 2). Clearly ROS,