Review

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Mitochondrial pathology: stress signals from the energy factory

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Mitochondria are undergoing a renaissance. The cellular power plant is now recognized as a key cellular signaling platform. The signals released by mitochondria are currently an area of intense research. A complex network is emerging involving metabolic intermediates, the roles of the mitochondrial unfolded protein response, and the interaction of mitochondria with other organelles and with the cellular autophagic system. Despite the diversity of the perturbations leading to mitochondrial diseases, some emerging trends are apparent. The long-held notion that mitochondrial diseases result from decreased mitochondrial energy output has been challenged by new data showing that mitochondrial pathological signaling can cause disease irrespective of the energy output. This review proposes a novel integrative view of mitochondrial signaling in physiology and disease.

Mitochondria: energy supply and beyond

Mitochondria are long known as cellular 'power plants' because they harbor the processes of respiration and oxidative phosphorylation (OXPHOS), which harvest chemical energy from nutrients and use it to generate adenosine triphosphate (ATP), the cellular 'energy currency'. However, the roles of mitochondria go well beyond energy metabolism [1]. These organelles are responsible for an array of cellular functions, including sugar and fatty acid catabolism, amino acid metabolism, calcium homeostasis, synthesis of heme, iron–sulfur (Fe–S) clusters and steroids, as well as regulation of apoptosis [2]. Furthermore, mitochondria are now recognized as a fundamental platform in cellular signaling, with pivotal roles in processes such as cell proliferation, differentiation, autophagy, and cellular immunity [1–3].

Mitochondria contain their own genetic material, mitochondrial DNA (mtDNA), which encodes for 13 polypeptides of the respiratory chain/OXPHOS, as well as the two ribosomal RNAs (rRNAs) and the 22 transfer RNAs (tRNA) necessary for the translation of those polypeptides inside mitochondria [4]. All other proteins functioning in mitochondria (~1500) are encoded by the nuclear genome, translated in the cytoplasmic ribosomes, and imported to mitochondria through the translocator complexes of the outer and inner membranes (TOM and TIM, respectively) [5].

Mitochondrial diseases can be caused by mtDNA mutations in protein-coding genes, rRNAs and tRNAs, and by defects in nuclear-encoded genes affecting aerobic metabolism (e.g., pyruvate dehydrogenase, citrate cycle, respiratory chain, OXPHOS, and Fe–S maturation), mitochondrial lipid synthesis (e.g., cardiolipin and ubiquinone), mtDNA maintenance, deoxynucleotide metabolism, mtDNA translation, mitochondrial protein import, and mitochondrial dynamics [6,7]. The currently accepted unifying point of mitochondrial disease is a decreased performance of the respiratory chain/OXPHOS [8], and a typical outcome of mitochondrial disease involves pathology in the skeletal muscle, heart, or central and peripheral nervous system, although the clinical presentations are broad and other organs are also affected [6].

The energy factory takes a seat in the boardroom

The long-standing paradigm of mitochondrial diseases posits that pathology arises due to decreased capacity for ATP synthesis. It is typically assumed that the tissue or cells where the pathology arises have high energy needs, which are being unmet due to mitochondrial malfunction. Common examples include cardiomyocytes, myocytes, neurons, and cochlear hair cells, just to name a few. However, it is important to note that, although many of these cell types and tissues are indeed affected in many mitochondrial diseases, they are not affected in all mitochondrial diseases. For example, myopathies and cardiomyopathies are major clinical presentations of many mitochondrial diseases, but there are also numerous mitochondrial diseases that do not affect the heart, despite its high demands for energetic supply [7]. Numerous similar observations in other tissues underscore that the energy requirement of a given cell type or tissue is not sufficient to determine its susceptibility to pathology in response to mitochondrial malfunction. Instead, multiple cellular and mouse models of mitochondrial malfunction support the notion that the signals released by mitochondria are the essential triggers for the development of pathologies. This paradigm was first demonstrated in cancer cells under hypoxia, in the context

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of the activation of the hypoxia-inducible factor (HIF) pathway by mitochondria. Cells devoid of mtDNA are unable to stabilize HIF-1α [9], which is a key step in the HIF signaling pathway [10]. Naturally, these mtDNA-null cells also do not have a functional mitochondrial electron transport chain (mETC) or OXPHOS. An elegant subsequent study then took advantage of cells with the absence of a key subunit of the mETC, in which the ETC is still not functional from an energy generation perspective, but is able to produce superoxide [11]. These cells were able to stabilize HIF-1α under hypoxia, thus showing that the reactive oxygen species (ROS)-dependent signals, rather than the blockage in the mETC, were triggering the hypoxia response.

Another piece of evidence for the role of mitochondrial signals, independent of energy production efficiency, was recently provided by a model of mitochondrial ribosomal stress. Cells with the A1555G mtDNA mutation, in the 12S rRNA gene, present hypermethylation of 12S rRNA in a conserved stem loop [12]. This methylation is carried out by the mitochondrial rRNA methyltransferase TFB1M (transcription factor B1, also referred to as mitochondrial dimethyladenosine transferase 1) [13], which is essential for the maturation of the mitochondrial ribosome, and consequently for the expression of mtDNA-encoded mETC subunits [13]. 12S rRNA hypermethylation can be recapitulated in the absence of the mutation by overexpression of TFB1M [12], and increases in 12S rRNA methylation levels are associated with increases in apoptosis susceptibility. Recent data show that in response to ribosomal stress, mitochondrial superoxide production is increased, and a superoxide-dependent signal leads to the activation of AMP-dependent protein kinase (AMPK), which in turn induces a proapoptotic gene expression program dependent on the transcription factor E2F1 [14]. This pathway is functional both in cultured cells as well as *in vivo*, and increased apoptosis susceptibility causes pathology in the inner ear and consequent hearing loss in a mouse model of mitochondrial ribosomal stress (Tg-TFB1M) [14]. Importantly, blocking the pathway at any level in cultured cells (removal of superoxide, inhibition of AMPK, or knock-down of E2F1) ablates apoptosis susceptibility [14]. This is equally observed *in vivo*, because the knock-out of E2F1 in Tg-TFB1M mice rescues apoptosis in the inner ear as well as hearing loss [14]. Therefore, blocking the signaling pathway, even in the presence of a putative lower energy output, rescues cellular and animal phenotypes (Figure 1). These results underscore that it is the signaling arising from malfunctioning mitochondria, rather than lower energy production, which triggers pathology development. This is an important paradigm shift, because it changes the focus from decreased mitochondrial performance to pathogenic signaling elicited by malfunctioning mitochondria.

Furthermore, mutations in mitochondrial genes would typically have similar consequences in terms of decreased ATP output in different tissues, even in tissues that are not developing pathology. By contrast, the consequences of mitochondrial malfunction for cellular signaling pathways would have marked tissue specificity. Even if the same signaling pathways are activated by mitochondrial malfunction in different tissues, the final outcome in terms of pathology depends on the signaling context of each tissue. For example, pathological hyperactivation of one given signaling pathway may be of little significance in a tissue where that pathway is buffered by other signaling mediators. Therefore, signaling pathways triggered by mitochondrial malfunction will be interpreted differently in different tissues, with any resulting pathology dependent on the tissue-specific signaling context.

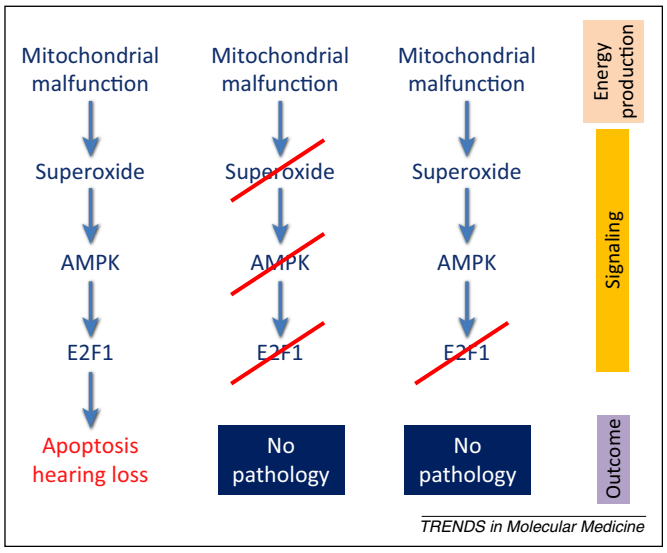

Figure 1. An example of pathology triggered by mitochondrial signaling independent of energy production deficits. A mouse model of mitochondrial malfunction causes deafness through the pathway outlined on the left [14]. The genetic removal of elements of this signaling pathway (superoxide, AMP-dependent protein kinase, transcription factor E2F1; indicated by red lines) blocks pathology development, but not mitochondrial malfunction.

Mitochondrial signaling

It is well established that the cytoplasm and the nucleus are constantly being informed on the state of mitochondrial performance, of which the programs of compensatory biogenesis in response to mitochondrial malfunction are a pivotal example [2]. A number of pathways have been implicated in the communication between mitochondria and the nucleus, such as AMPK signaling [15] or the mitochondrial unfolded protein response (mito^{UPR}) [16]. The nature of the mitochondrial signals that result in the modulation of signaling pathways in the cytoplasm is, however, much less defined. The highly restricted transport of molecules across the mitochondrial inner membrane further complicates this question, because not many signals can be physically released from the mitochondrial matrix to the cytoplasm.

A mitochondrial signal has to be intrinsically ephemeral, with quick production and removal, and to be able to transmit information across the inner mitochondrial membrane to the cytoplasm. Several molecules have properties of mitochondrial signals, including Ca^{2+} and the ratio of ATP/ADP, which have widespread roles in the regulation of signaling pathways involving calcineurin [17,18] and AMPK [15]. However, many other mitochondrial signals are less well characterized. For example, the ratio of citrate export from mitochondria will determine the levels of cytoplasmic acetyl-coenzyme A (acetyl-CoA), which determines the acetylation of diverse proteins, affecting the