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Mitochondria in pluripotent stem cells: stemness regulators and disease targets

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Beyond their canonical role in efficient ATP production through oxidative metabolism, mitochondria are increasingly recognized as critical in defining stem cell function and fate. Implicating a fundamental interplay within the epigenetics of eukaryotic cell systems, the integrity of mitochondria is found vital across the developmental/differentiation spectrum from securing pluripotency maintenance to informing organotypic decisions. This overview will discuss recent progress on examining the plasticity of mitochondria in enabling the execution of programming and reprogramming regimens, as well as the application of nuclear reprogramming and somatic cell nuclear transfer as rescue techniques to generate genetically and functionally corrected pluripotent stem cells from patients with mitochondrial DNA-based disease.

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1. Introduction

Pluripotent stem cells are defined by their capacity for indefinite self-renewal and the ability to give rise to all cell types within the three embryonic germ layers. This unique class of cells is epitomized by embryonic stem cells (ESCs), which are derived from the inner cell mass of the blastocyst stage of the embryo. Beyond the natural potential in deriving diverse lineages, the ability to revert the fate of a specialized differentiated cell back to a pluripotent ground state has the potential to revolutionize the fields of stem cell biology and regenerative medicine, and hence garnered Sir John Gurdon and Shinya Yamanaka the Nobel Prize in Physiology and Medicine. From Gurdon's initial work cloning frogs to optimization of the technique in sheep and other mammals has laid the groundwork for recent developments in utilizing somatic cell nuclear transfer (SCNT) into human oocytes for the derivation of stem cells [1]. This approach has been complemented by the discovery that nuclear reprogramming induced through expression of four embryonic transcription factors, Oct4, Sox2, Klf4 and cMyc, is sufficient to reset differentiated cells back into induced pluripotent stem cells (iPSCs), which mimic the features of their embryonic counterparts [2]. These discoveries have enabled the generation of patient-specific pluripotent stem cells, which offer unique platforms to examine mechanisms of disease pathogenesis in a dish, to screen small molecules to identify novel therapeutics for difficult to treat diseases, as well as for toxicity screening in lineages specified from pluripotent stem cells. Ultimately, these cells offer an unlimited and autologous cell source for regenerative applications across degenerative diseases for which curative therapies are currently lacking.

Over the past decade the field has made great strides in understanding the genetic and epigenetic mechanism by which nuclear reprogramming can reset the fate of a differentiated cell. Complementing these studies, is the emerging appreciation that mitochondrial function and energy metabolism are tightly linked to the fate and function of a stem cell. In this review we will discuss recent findings underscoring the enabling role of mitochondria and their dynamics in the acquisition of the pluripotent state and how nuclear reprogramming and SCNT can be leveraged to derive pluripotent stem cells from patients with mitochondrial DNA (mtDNA)-based disease.

2. Mitochondria as stemness regulators

Fundamental to nuclear reprogramming is the reduction in mtDNA copy numbers and regression in mitochondrial density, distribution and ultrastructure [3••,4,5], events that collectively recapitulate the mitochondrial features of ESCs [6]. Indeed recent evidence indicates that mitochondrial clearance through Atg5-independent autophagy

is essential for pluripotency induction and generation of iPSCs [7]. Moreover, proteomic profiling has identified a reduction in subunit expression of complex I and IV and an increase in II, III, V of the mitochondrial electron transport chain as an early reprogramming event preceding remodeling of other metabolic pathways and expression of pluripotency genes, indicating that mitochondrial remodeling is not simply a consequence of transition between cell identities, but may represent an initiating event [3••,8]. Functionally, this transition manifests as a suppression of cellular respiration in favor of glycolysis in iPSCs, with somatic sources having a greater glycolytic and lower oxidative capacity displaying a higher reprogramming efficiency [3••,5]. Although on the surface the presence of immature mitochondria may be interpreted to indicate that pluripotent stem cells may minimize their requirement for mitochondria, it has been demonstrated that mitochondrial homeostasis is necessary for maintenance of the pluripotent state as excessive mitochondrial fission or knockdown of the mtDNA specific polymerase gamma leads to loss of pluripotency [9,10]. Stem cells actually appear to actively maintain their mitochondria, potentially even hydrolyzing ATP through ATP synthase to support high mitochondrial membrane potential [3••,11–14]. Consistent with these observations, stem-like cells asymmetrically segregate their mitochondria during cell division, with a greater proportion of young mitochondria observed in daughter cells displaying stem cell traits, while impaired segregation leading to loss of stem cell properties in the cell progeny [15•]. Therefore stem cells may repurpose mitochondria from their canonical role of energy generators to alternative functions in support of stem cell function and maintenance of pluripotency.

In pluripotent stem cells, like other populations of rapidly proliferating cells, the demand for anabolic precursors for biosynthesis of cellular component may outpace their requirement for energy in the form of ATP [16]. Therefore reducing their dependence on mitochondria-dependent ATP generation may enable stem cells to commission alternative mitochondrial pathways to support stemness, such as elevated cataplerosis, the extraction of incompletely oxidized substrates from the mitochondria, to serve as substrates for anabolic pathways and post-translational protein modifications [17] (Figure 1). ESCs do not appear to completely oxidize glycolysis-derived pyruvate to CO_2 and H_2O in the tricarboxylic acid cycle (TCA), but utilize pyruvate to generate TCA intermediates that can be exported out of the mitochondria and utilized for alternative purposes [18•]. For example, mitochondrial-derived citrate can be exported from the mitochondria and subsequently metabolized by ATP-citrate lyase to generate cytosolic acetyl-CoA, which serves as a substrate for protein and histone acetylation, as well as for fatty acid and cholesterol biosynthesis [19]. Early differentiation of ESCs is associated with a reduction in acetyl-CoA production and loss of histone H3 lysine 9 and lysine 27 acetylation, indicating that TCA-derived cytosolic acetyl-CoA may be critical for maintaining histone acetylation and an open chromatin state during pluripotency [18•]. Functionally, inhibition of ATP citrate lyase reduces acetyl-CoA content and histone acetylation resulting in induction of myogenic differentiation [20], while acetate supplementation impairs both early differentiation capacity and histone deacetylation [18•]. However this axis may not be exclusive to the pluripotent state as ATP-citrate lyase derived acetyl-CoA and increased histone acetylation is also associated with adipogenesis [19]. ESCs also metabolize both glucose and glutamine to generate alpha-ketoglutarate, another key metabolite that connects metabolism with the epigenetic regulation of the pluripotent state [21••]. A high alpha-ketoglutarate/succinate ratio is critical in regulating a number of enzymes that couple the conversion of alpha-ketoglutarate to succinate with their specific functions, such as HIFα prolyl hydroxylase and a class of alpha-ketoglutarate dependent dioxygenases, which include Jumonji C-domain-containing histones demethylases and the ten-eleven translocation (TET) family of DNA demethylases. Therefore high levels of alpha-ketoglutarate in ESCs favors demethylation of repressive chromatin marks including histone 3 lysine 9 and lysine 27 timethylation and histone 4 lysine 20 trimethylation and maintenance of the pluripotent state [21••]. Although these alternate roles of TCA cycle intermediates are established in maintaining pluripotency, it remains unexamined how they contribute to the acquisition of the pluripotent state during nuclear reprogramming, where global changes in DNA methylation and histone marks are occurring.

Nuclear reprogramming significantly modifies other metabolic pathways that regulate the epigenetic state, including increases in the expression of mitochondrial enzymes required for threonine catabolism, such as threonine dehydrogenase (TDH), glycine C-acetyltransferase (GCAT) and glycine decarboxylase (GLDC) [22••]. Activation of this pathway results in elevated conversion of threonine into acetyl-CoA and glycine, the latter of which is metabolized to 5,10-methylenetetrahydrofolate to supply single carbon equivalents to the folate pool. This pool then provides these one-carbon units to support the biosynthesis of nucleotides and amino acids, as well as generation of S-adenosylmethionine (SAM), which serves as the substrate for histone H3 lysine 4 trimethylation, a critical component for self-renewal of pluripotent stem cells [22••]. Compromising this pathway either through threonine restriction or modulation of TDH activity leads to loss of ESC colony growth and impairs nuclear reprogramming [22••,23–25]. Although humans do not express functional TDH, a parallel pathway relying on methionine appears to fuel the SAM pathway [26]. Taken together, by minimizing their reliance on mitochondrial oxidative ATP generation, pluripotent stem cells can exploit alternative mitochondrial functions to maintain stemness.