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Mitochondria in Pluripotent Stem Cells: Stemness Regulators and Disease Targets

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Abstract

Beyond the 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 mitochondria DNA-based disease.

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

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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 (Table 1).

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.

Mitochondria as stemness regulators

Fundamental to nuclear reprogramming is the reduction in mtDNA copy numbers and regression in mitochondrial density, distribution and ultrastructure [3–5], 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 mitochondria-associated plasticity 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]. ESCs do not appear to completely oxidize glycolysis-derived pyruvate to CO_2 and H_20 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 results 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 does significantly modify 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 [22–25]. 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.

Correcting mitochondrial DNA defects in pluripotent stem cells

Mutations in mtDNA contribute to a wide range of life-threatening conditions. Among well-described mtDNA diseases are maternally inherited germline mutations associated with defects in oxidative energy metabolism [27]. Pathogenic mtDNA mutations may reside in tRNA, rRNA or protein coding genes and often persist in each cell as a mixture of mutated and healthy wild type molecules, a state known as heteroplasmy. Often, these two mtDNA types are unevenly segregated during mitosis, resulting in daughter cells with significantly different levels of heteroplasmy, which offers an opportunity to select against cells with high mutations loads. Mitochondrial dynamics underlying pluripotent induction, including a significant mtDNA copy number reduction followed by clonal cell expansion, in essence recapitulates a genetic bottleneck during nuclear reprogramming. As a consequence of this bottleneck, mtDNA type can drift toward homoplasmic mutant or wild type in individual iPSC clones. This phenomenon provides the opportunity to recover iPSC clones with normal mtDNA from patients with heteroplasmic mtDNA mutations [28–31]. However, this option is not sufficient when mtDNA mutation is homoplasmic or high heteroplasmic, and requires complementary approaches.

One strategy would be to correct the mtDNA mutation, and although initial proof of concept studies has demonstrated that mitochondrial-targeted endonucleases or TALENs may be utilized to shift heteroplasmy of mtDNA haplotypes/mutations in cells [32,33] and mouse embryos [34], whether these techniques can be utilized to generate mutation-free pluripotent stem cells have not been examined. Alternatively, a complete mitochondrial replacement strategy through SCNT may be required. Transplantation of a somatic cell nucleus into enucleated cytoplasm from unfertilized metaphase 2 (MII) arrested oocyte induces rapid epigenetic and cell cycle changes resulting in the erasure of somatic cell identity and formation of oocyte-like meiotic chromosomes. Reconstructed SCNT oocytes are functional as they can be triggered to develop into a diploid zygote and ultimately have been demonstrated to produce viable offspring in numerous mammalian species [35–38]. In addition, human SCNT oocytes can also be used to derive patient matched embryonic stem cells (NT-ESCs) [39]. A hallmark of NT-ESCs is that they are cytoplasmic hybrids (cybrids), which contain a somatic cell nuclear genome and the oocyte's mtDNA. This fundamental feature of SCNT allows replacement of the entire mtDNA complement in patient somatic cells carrying mtDNA mutations while producing high quality pluripotent cells [39]. NT-ESCs carry almost exclusively oocyte mtDNA ensuring that stem cells regain normal OXPHOS function, irrespective of mtDNA mutation type or heteroplasmy level in starting somatic cells. Technically, a whole somatic cell is fused to a cytoplast resulting in a small somatic cell/oocyte mtDNA heteroplasmy. However, due to significant mtDNA copy number

differences between somatic cells and MII oocytes (ratio <1:100) and a bottleneck effect during embryo and ESC development, the mutated mtDNA is diluted and often becomes practically undetectable in NT-ESCs [31]. A potential limitation of this approach is that the donor mtDNA haplotype in NT-ESCs may be different than the original in parental somatic cells due to natural sequence variation between the oocyte donors and patients affected by mtDNA mutations. Hypothetically, this may result in metabolic insufficiency in NT-ESCs due to inadequate interactions between the donor mitochondrial and patient nuclear genomes [40]. However, there is no direct evidence of mtDNA incompatibility between human populations including in children born in families from distant haplogroups. This suggests that normal nuclear-to-mitochondrial interactions are highly conserved within species and any human haplotype may serve as a functional donor mtDNA for mitochondrial replacement therapy [40]. Indeed, NT-ESCs carrying “unmatched” donor mtDNA displayed differentiation potential and transcriptome profiles similar to control ESCs produced from in vitro fertilized embryos (IVF-ESCs)[31]. In addition their metabolic functions were also normal suggesting that donor mtDNA interacts normally with patient nuclear DNA [31]. Although functionally similar, recent evidence indicates that murine NT-ESCs induced an immune response that leads to rejection when administered to recipients that have matched nuclear genomes, but mismatched mtDNA, indicating that non-cell-autonomous effects must also be considered [41] (Table 2).

Both nuclear reprogramming and SCNT-based approaches to derive mutation free pluripotent stem cells from patients with mitochondrial disease offer potential benefits and limitations. While nuclear reprogramming is relatively simple to perform and does not require the same technical expertise as required for SCNT, NT-ESCs may more closely resemble IVF-ESCs, the ‘gold standard’ of pluripotency. Indeed, initial studies of transcription-factor-based nuclear reprogramming demonstrated that incomplete DNA methylation resulted in significant differences in the transcriptional signature of iPSCs versus ESCs [42–44]. In addition, iPSCs show significant differences in DNA methylation patterns when compared to genetically matched IVF-ESCs and NT-ESCs, including a three- and ten-fold exaggerated aberrancy in CG and non-CG methylation than NT-ESCs, respectively [45]. These studies suggest that oocyte-based reprogramming is more robust and capable of resetting the DNA methylation and corresponding gene expression program of somatic cells. While a separate study recapitulated the distinct clustering of these cell lines, they did not detect differences in coding mutations and loss of imprinting between iPSCs and NT-ESCs derived from other cells lines [46]. These different observations may be due to the iPSC derivation strategy and ages of the somatic cell source, but indicates that additional studies are required on a greater number of cell lines to examine the functional differences between iPSCs and NT-ESCs.

While the incidence of inherited mtDNA disease is relatively rare, acquired, somatic mtDNA mutations are more common and implicated in normal aging itself and in the etiology of diverse age-onset diseases including neurodegenerative conditions such as Parkinson’s and Alzheimer’s disease [47–51]. With age, mtDNA heteroplasmy can drift toward mutant types, particularly in post-mitotic neurons. When the proportion of mutant mtDNA reaches a critical threshold, the cell’s ability to supply minimum energy for vital functions is affected, leading to disease symptoms. Due to the random nature of somatic mtDNA mutations, the

mutation type may differ among individual cells within tissues, making difficult to detect mtDNA mutations in pooled cells or tissues and may pose a risk of uncovering rare mitochondrial mutations due to the clonal nature of iPSC generation. In contrast, female germ cells have relatively quiescent mitochondria that mainly rely on the glycolytic energy supply rather than on OXPHOS. Therefore, a female germline may evade accumulation of mutations and provide purifying selection to generate oocytes and subsequent SCNT-ESCs with less mtDNA mutation burden.

Summary

The present overview highlights advances in mitochondrial biology in the context of an emerging understanding of mitochondria-dependent control of stemness in health and disease. Decoding intimate events that repurpose mitochondria from traditional energy generators to enablers of bi-directional transitions between somatic versus pluripotent cell identities has now open new avenues in developmental biology with potential applications in regenerative medicine. Case in point, resetting or replacing disease-corrupted mitochondria, through nuclear reprogramming and somatic cell nuclear transfer respectively, to produce healthy patient derived stem cells underscore the most recent breakthrough in the field paving the way towards targeting the cause and potentially establishing future cures for patients with inherited or age-related mtDNA disorders.

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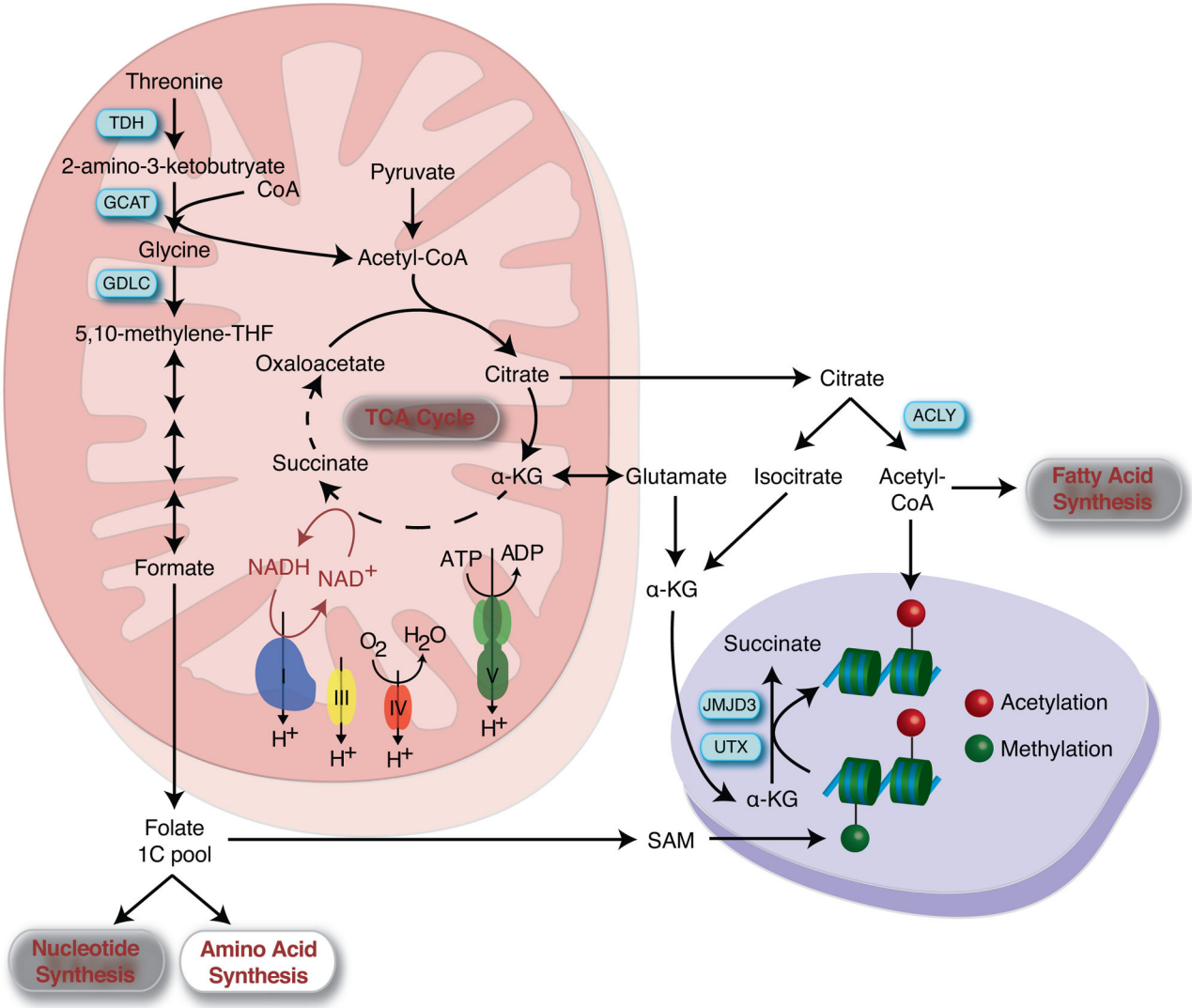

Figure 1.

Mitochondria contribute to stemness maintenance. By reducing their reliance on oxidative metabolism for ATP generation and potentially utilizing ATP to maintain mitochondria membrane potential, stem cells can repurpose their mitochondria to cataplerosis. These mitochondria-derived metabolites are utilized as precursors for the anabolic generation of cell building blocks and serve as substrates for reactions that add or remove epigenetic marks that are critical for directing cell fate.

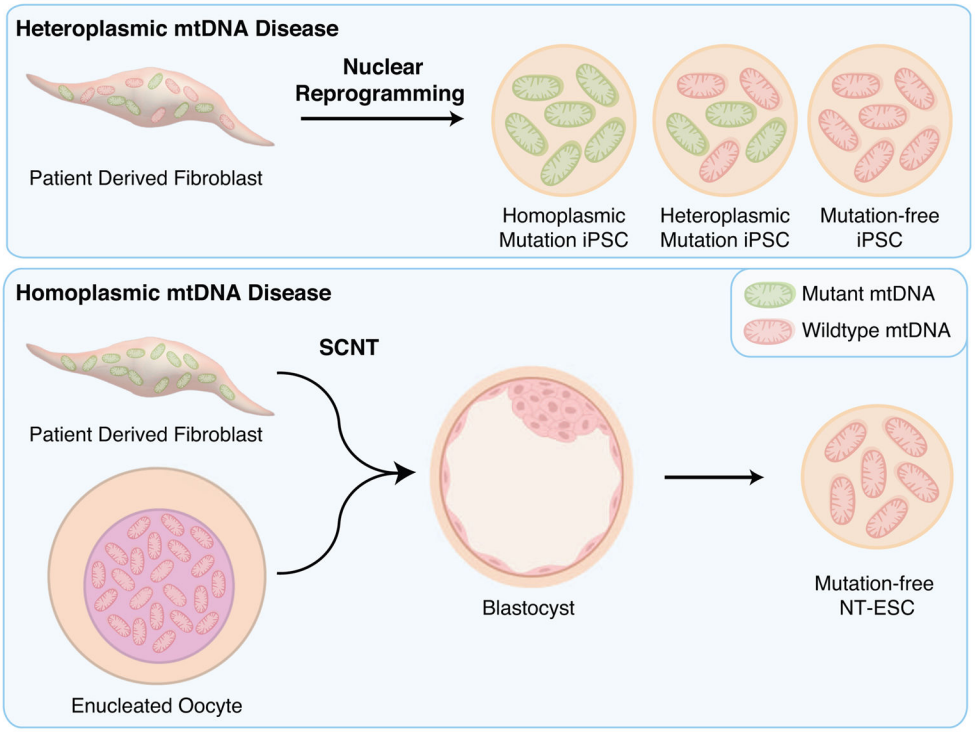

Figure 2.

Creating disease-free pluripotent stem cells from patients with mitochondrial DNAN (mtDNA)-based disease. Nuclear reprogramming segregates wildtype and mutant mt-DNA enabling the clonal expansion of mutation-free induced pluripotent stem cells (iPSC) from patients with heteroplasmic mt-DNA disease. Homoplasmic mtDNA disease requires a complete mitochondria replacement strategy using somatic cell nuclear transfer (SCNT) to generate mutation free nuclear transfer embryonic stem cells (NT-ESC).

Table 1

Comparison of different pluripotent stem cell types

	IVF-ESCs	NT-ESCs	iPSCs
Stem cell-based therapy	Heterologous	Autologous	Autologous
mtDNA origin	Oocyte	Oocyte	Somatic cells
mtDNA mutation frequency	Germline, Low	Germline Low	Somatic, accumulate with age
Reprogramming	Natural oocyte factors	Natural oocyte factors	Induced by transcription factors
de novo copy number variations (per cell line)	0.5	0.8	1.8
CG differentially methylated regions (DMRs)	Baseline control	212	619
Recurrent (hotspot) CG DMRs	Baseline control	50	110
Non-CG mega DMRs	Baseline control	7	70
Differentially expressed genes	Baseline control	48	629

Table 2

Methods for eliminating mtDNA mutations

	Nuclear transfer (MRT)	Mito TALEN
Efficiency of eliminating mutations	80–99%	65–85%
Applicable for germ line therapy	Yes	Yes
Applicable for heteroplasmic mutations	Yes	Yes
Applicable for homoplasmic mutations	Yes	No
Applicable for pluripotent stem cells	Yes	Not tested
Potential side effects	mtDNA haplotype mismatch	Off-target effects