Cell Metabolism
Review

Mitochondrial Regulation in Pluripotent Stem Cells

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http://dx.doi.org/10.1016/j.cmet.2013.06.005

Due to their fundamental role in energy production, mitochondria have been traditionally known as the powerhouse of the cell. Recent discoveries have suggested crucial roles of mitochondria in the maintenance of pluripotency, differentiation, and reprogramming of induced pluripotent stem cells (iPSCs). While glycolytic energy production is observed at pluripotent states, an increase in mitochondrial oxidative phosphorylation is necessary for cell differentiation. Consequently, a transition from somatic mitochondrial oxidative metabolism to glycolysis seems to be required for successful reprogramming. Future research aiming to dissect the roles of mitochondria in the establishment and homeostasis of pluripotency, as well as combining cell reprogramming with gene editing technologies, may unearth novel insights into our understanding of mitochondrial diseases and aging.

Mitochondria are highly specialized and dynamic double-membrane organelles of bacterial origin that play fundamental roles in multiple processes in eukaryotic cells, including energy production, calcium homeostasis, cell signaling, and apoptosis (Dyall et al., 2004). In order to meet different demands of distinct cell types and tissues, cells modulate mitochondrial function through biogenesis and degradation as well as dynamic fusion and fission events. Mitochondria are semiautonomous organelles and contain their own genome of ~16.6 kb circular mitochondrial DNA (mtDNA), encoding for 13 essential protein subunits of complexes I, III, IV, and V of the respiratory chain as well as 22 tRNAs and 2 rRNAs necessary for the translation of mitochondrial subunits (Anderson et al., 1981). Mitochondrial biogenesis is accomplished by the coordinated expression of genes from the nuclear and mitochondrial genome. Transcriptional regulators of mitochondrial biogenesis include the nuclear transcription factors (NRF-1, NRF-2, and ERR1) and transcription cofactors (PGC1-α, PGC1-β, and PPRC) as well as nuclear-encoded assembly factors necessary for the correct assembly of the respiratory complexes. The nuclear-encoded DNA polymerase (POLG and POLG2) and mitochondrial transcription factor A (TFAM) are key regulators of mtDNA replication. In addition, mitochondria are highly dynamic organelles that undergo cycles of fusion and fission important for their function, maintenance, and quality control (Chen and Chan, 2009; Westermann, 2010). On the other hand, mitochondria are the major sources of endogenous reactive oxygen species (ROS), which are by-products of ATP production through oxidative phosphorylation (OXPHOS). Although high levels of ROS may cause protein carbonylation, lipid peroxidation, and DNA damage and have deleterious effects for the cells, recent studies have also suggested a physiological role for low levels of ROS (Sena and Chandel, 2012). Besides their key role in energy production through oxidative phosphorylation, mitochondria are also the sites of essential pathways of intermediate metabolism, amino acid biosynthesis, fatty acid oxidation, and steroid metabolism. In addition to these biochemical functions, mitochondria also possess important functions in many other essential processes including the maintenance of calcium homeostasis (Jacobson and Duchen, 2004), redox regulation (Dröge, 2002), and apoptosis (Danial and Korsmeyer, 2004). Recently, mitochondria have been closely linked to cell fate determination and development, and several reports have demonstrated important roles for mitochondria in stem cells (Folmes et al., 2012; Rafalski et al., 2012). In this review, we summarize recent discoveries from mitochondrial studies in stem cells, formulate open questions, and prospect future impacts of the novel findings in the clinic.

Mitochondria and Pluripotency

Pluripotent stem cells (PSCs) represent a remarkable cell type because of their ability to self-renew and differentiate into any tissue of the three germ layers, which confers on them great potential for disease modeling, drug screening, and cell-replacement therapies. Recent studies have demonstrated that there is a strong connection between mitochondrial function and pluripotency (Ahlqvist et al., 2012; Folmes et al., 2011; Lapasset et al., 2011; Prigione et al., 2010; Zhang et al., 2011; Zhou et al., 2012). Under self-renewing conditions characterized by high expression of pluripotent genes (*Nanog*, *Oct4*, and *Sox2*), cellular metabolism has been suggested to play an essential role in the regulation of pluripotency (Mandal et al., 2011). Compared to adult somatic cells that utilize an aerobic metabolism based on oxidative phosphorylation for energy production, PSCs rely heavily on anaerobic glycolysis (Folmes et al., 2011; Kondoh et al., 2007; Prigione et al., 2010). Glycolysis provides important cofactors and substrates to meet the biosynthetic demands and support cell proliferation of PSCs. More importantly, although glycolysis is less efficient in terms of energy production, it produces energy at a faster rate with lower ROS generation,

which is important for maintaining pluripotency under hypoxic conditions. Along this line, while stimulation of glycolysis by hypoxia or inhibition of mitochondrial respiration promotes pluripotency (Ezashi et al., 2005; Varum et al., 2009), inhibition of glycolysis or enhancement of mitochondrial function, by either chemical treatment or overexpression of transcriptional factors, impairs stemness and differentiation efficiency of PSCs (Kondoh et al., 2007; Prowse et al., 2012). While mitochondria in adult somatic cells possess a complex morphology with a well-developed cristae, denser matrix, and elongated or branched appearance, consistent with their metabolic state, PSCs contain functionally immature mitochondria with a globular shape, poorly developed cristae, and perinuclear localization, all indicative of a less active mitochondrial state (Chung et al., 2010; Facucho-Oliveira and St John, 2009; Prigione et al., 2010; St John et al., 2005; Suhr et al., 2010). It has been suggested that the morphology, localization, abundance, and function of mitochondria could be used as markers of pluripotency (Lonergan et al., 2007). Indeed, mitochondrial staining has been used as a stem cell indicator in the enrichment of somatic stem cells, such as hematopoietic stem cells (Romero-Moya et al., 2013). Interestingly, although the energy production of PSCs favors glycolysis over OXPHOS, mitochondria in PSCs still possess functional respiratory complexes. While regulation of the preference for energy production in metabolism is still unclear, several clues have been suggested, including the decoupling of glycolysis from OXPHOS by shunting pyruvate out of the mitochondria by mitochondrial uncoupling protein 2 (UCP2) (Zhang et al., 2011), along with higher levels of the glycolytic enzyme Hexokinase II and lower levels of Pyruvate Dehydrogenase (PDH) in PSCs (Varum et al., 2011).

Mitochondrial homeostasis in the pluripotent state relies on mitochondrial biogenesis and dynamics (fission and fusion), as well as degradation through mitochondria autophagy (mitophagy). Although some initial studies have shown that PSCs contain less mitochondrial content (Prigione et al., 2010; St John et al., 2006) and lower mtDNA copy number (Facucho-Oliveira et al., 2007) when compared with somatic cells, recent publications have clarified that when mitochondrial mass, as measured by mitochondrial proteins, mitochondrial labeling, and mtDNA copy number, is normalized to total cellular mass, the ratio between these cellular parameters is similar in PSCs and differentiated cells (Birket et al., 2011; Zhang et al., 2011). The regulation of mtDNA copy number seems to be linked with the epigenetic methylation of the nuclear-encoded mitochondria DNA polymerase gamma catalytic subunit (POLG) in a tissue-specific manner (Kelly et al., 2012a). Knockdown of mitochondria DNA polymerase POLG in mouse embryonic stem cells (ESCs) results in reduced OCT4 expression and slightly increased levels of the mesodermal marker Brachyury (Facucho-Oliveira et al., 2007). In addition to biogenesis, mitochondrial dynamics is also actively involved in stem cell biology. Gene knockdown of the mitochondrial protein Gfer (growth factor erv1-like) in mouse ESCs leads to decreased levels of pluripotent markers (NANOG, OCT4, SSEA) through the regulation of dynamin-related protein 1 (Drp1), an important mitochondrial fission GTPase. Inhibition of Drp1 activity rescues structural and functional mitochondrial dysfunctions and restores the pluripotent gene expression caused by Gfer deficiency. Unexpectedly, these data demonstrate, for the first time, that despite the glycolytic metabolic state of PSCs, mitochondrial dynamics and maintenance of proper mitochondrial network integrity are crucial for the maintenance of pluripotency (Todd et al., 2010).

More recently, direct evidence for the effect of mtDNA haplotypes on ESCs was demonstrated in mouse ESCs. Kelly et al. established isogenic mouse ESC lines that were different in their mtDNA haplotypes and found that expression of some pluripotent genes in those lines was different, which may partially reflect epigenetic alternations of these genes. Moreover, they found that mtDNA haplotypes influence not only chromosomal gene expression but also cell fate determination upon differentiation. Kelly's study presents evidence that mtDNA may regulate nuclear gene expression at the pluripotent state and suggests a potential interesting crosstalk between mtDNA and nuclear DNA in PSCs (Kelly et al., 2012b). Since a similar communication between mitochondria and the nucleus known as mitochondria retrograde pathway has been already described in yeast and mammalian cells, it would be of great interest to investigate if this pathway could be involved in the regulation of pluripotency by mitochondria (Butow and Avadhani, 2004).

Mitochondria and Differentiation

During the establishment of pluripotency, a specific remodeling of mitochondria and metabolism occurs in order to meet the energetic and anabolic demands of the pluripotent state. Consequently, differentiation of pluripotent cells requires remodeling of mitochondrial dynamics and bioenergetic system. Upon cell differentiation, higher amounts of energy are necessary to sustain specialized functions in different tissues while the lower proliferation capacity and necessity of anabolic precursors allow for a more efficient conversion of metabolic substrates into ATP (Folmes et al., 2012). Therefore, mitochondria undergo significant changes during cellular differentiation, mtDNA copy number is elevated, and mitochondrial morphology displays a structurally mature state with dense matrix, complex cristae, and dispersed cytoplasmic localization (Facucho-Oliveira et al., 2007; Lonergan et al., 2007; Prigione et al., 2010; Suhr et al., 2010).

In addition, several studies have demonstrated that differentiation of PSCs induces a spectrum of mitochondrial functional changes, including increased mitochondrial mass and function, upregulation of enzymes of the tricarboxylic acid (TCA) cycle and subunits of the mitochondrial respiratory chain, increased oxygen consumption and ROS levels, and reduced reliance on anaerobic glycolysis-based energy generation with the consequent downregulation of glycolytic enzymes (Armstrong et al., 2010; Chung et al., 2007; Prigione et al., 2010; Tormos et al., 2011). Once again, metabolic transition from glycolysis to oxidative phosphorylation is necessary for cellular differentiation. While inhibition of key glycolytic enzymes promotes differentiation, impairment of mitochondrial function with respiratory inhibitors improves pluripotency gene expression and blocks PSC differentiation (Chung et al., 2007; Mandal et al., 2011). Nevertheless, the disturbance of differentiation caused by the respiratory inhibitors used in these studies, such as CCCP (carbonyl cyanide m-chlorophenylhydrazone), antimycin A, or rotenone, may have not only been caused by the inhibition of mitochondrial respiratory chain, but also by the toxic and pleiotropic effects of

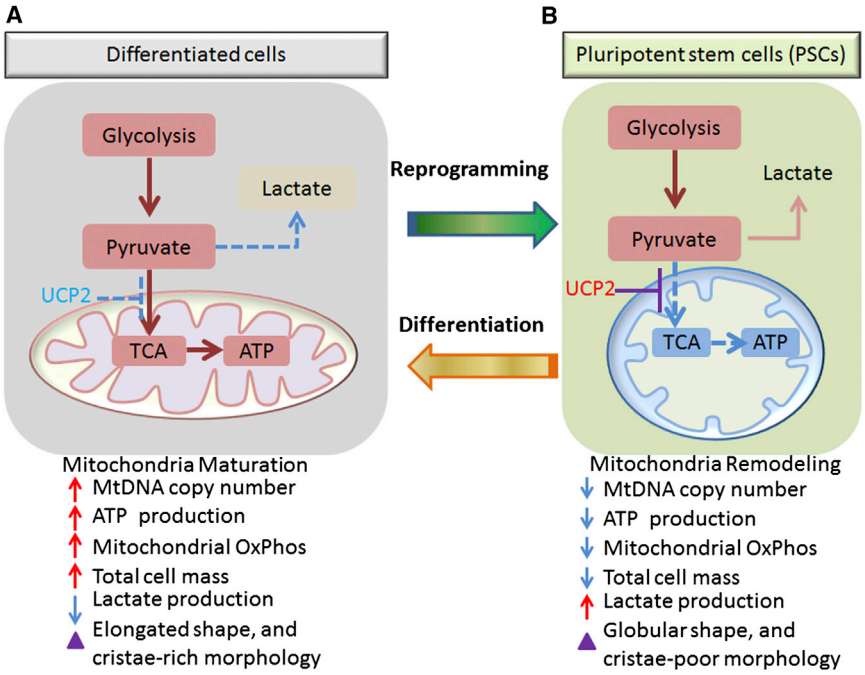

Figure 1. Simplified Scheme of Mitochondrial and Metabolic Differences between Differentiated Cells and Pluripotent Stem Cells

(A) Upon differentiation, PSCs undergo mitochondrial maturation and bioenergetic transition from anaerobic to aerobic metabolism. Mitochondrial biogenesis increases mitochondrial number, respiratory chain complex density, and ATP production. Morphologically, mitochondria become elongated and cristae-rich. Additionally, the TCA cycle is activated as result of low levels of UCP2 (blue). (B) Upon reprogramming, differentiated cells exhibit mitochondrial resetting to a functionally immature state and a transition from aerobic to anaerobic metabolism. The mitochondrial morphology is altered, and mitochondrial content is reduced. As the consequence of an increase in glycolysis and decrease in oxidative phosphorylation, ATP level decreases, and lactate production increases. Meanwhile, TCA cycle is suppressed due to high levels of UCP2 (red). Abbreviations: PSCs, pluripotent stem cells; mtDNA, mitochondrial DNA; TCA, tricarboxylic acid cycle.

these inhibitors on cells. Therefore, the definitive mechanism of how respiratory inhibitors block PSC differentiation may need further investigation. The main differences of mitochondrial function and structure between PSCs and differentiated cells are summarized in Figure 1. Despite the changes observed in mitochondrial function and metabolism between PSCs and differentiated cells, mechanisms responsible for the switch of bioenergetic metabolism from glycolysis to the more efficient oxidative phosphorylation remain unclear. Nevertheless, a recent study by Zhang et al. (2011) showed that mitochondria of PSCs are capable of consuming oxygen at rates similar to differentiated cells. Moreover, UCP2, a member of the mitochondrial uncoupling protein (UCP) family, plays crucial roles in coupling glycolysis to OXPHOS during PSC differentiation (Figure 1). In differentiated cells, glycolysis is more coupled to OXPHOS, whereas the uptake of glucose is less coupled to OXPHOS in PSCs. Zhang et al. found that at early stage of differentiation, PSC proliferation slowed down, energy metabolism decreased, and UCP2 expression was reduced. These changes accompanied with in a switch from efficient glycolysis to increased mitochondrial glucose oxidation. Furthermore, UCP2 knockdown decreased the production of lactate, while ectopic expression of UCP2 suppressed OXPHOS and impeded PSC differentiation. On the other hand, UCP2 knockdown did not impair self-renewal of PSCs. Taken together, these findings demonstrate an important role of UCP2 in PSC differentiation and show that UCP2-mediated suppression of OXPHOS is required for the maintenance of pluripotency. In a similar manner, mitochondrial maturation plays a fundamental role during differentiation, and closing of the mitochondrial permeability transition pore (mPTP), as part of mitochondrial maturation, has been shown to be required during cardiomyocyte differentiation. Hom et al. found that functionally and structurally immature mitochondria, characteristic of early embryonic cardiomyocytes, displayed open mPTP and consequently uncoupled mitochondrial respiratory chain (Hom et al., 2011). While closing of the mPTP occurred during normal cardiomyocyte maturation, chemical and genetic closing of the mPTP led to mitochondrial maturation and increased cardiomyocyte differentiation (Hom et al., 2011). In conclusion, transition to an oxidative mitochondrial metabolism accompanied with the necessary functional and structural maturation of mitochondria is key for the differentiation of PSCs into specialized cell types.

The importance of mitochondria in cell differentiation has also been demonstrated in developmental studies. Gene knockout of essential factors (TFAM, POLG, POLG2, and NRF1) or cofactors (PPRC1) for mitochondria biogenesis did not affect embryo implantation but caused embryonic lethality at a later stage due to mtDNA depletion, as well as insufficient supply of energy and metabolites required for cell differentiation (Hance et al., 2005; Humble et al., 2013; Huo and Scarpulla, 2001; Larsson et al., 1998). Interestingly, a homozygous mutation (D257A) of POLG in mice resulted in decreased DNA proofreading capacity without apparently affecting polymerase activity, which subsequently caused an accumulation of mtDNA mutations (Trifunovic et al., 2004). It is also reported that mitochondrial POLG mutant mice did not exhibit severe phenotype at fetal stage, but showed premature aging syndrome at adult stage presumably caused by defects of adult stem cell populations (Ahlqvist et al., 2012). Therefore, these models may provide ideal opportunities to study the effects of mitochondrial dysfunction caused by the accumulation of mtDNA mutations during aging on stem cells.

Mitochondria and Reprogramming

The reprogramming of somatic cells into a pluripotent state by the expression of four transcription factors was successfully established by Takahashi et al. in 2006 (Takahashi and Yamanaka, 2006; Yu et al., 2007). Thereafter, the role of mitochondria in the process of reprogramming has attracted tremendous interest in the stem cell field. Previous studies have

demonstrated that the process of nuclear reprogramming leads to structural and functional remodeling of parental mitochondria in both mouse and human somatic cells to a state resembling PSCs. These changes include the transition from somatic oxidative phosphorylation to glycolysis achieved through transcriptional and epigenetic regulation of gene expression, the consequent upregulation of glycolytic genes and downregulation of mitochondrial respiratory chain complexes, and reduction in mtDNA copy number and changes in the structure and morphology of mitochondria to a functionally immature state (Folmes et al., 2011; Prigione et al., 2010; Suhr et al., 2010; Varum et al., 2011). As expected, several studies have shown that a switch from OXPHOS to glycolysis is necessary to reprogram somatic cells to pluripotency (Folmes et al., 2011; Panopoulos et al., 2012; Prigione et al., 2010; Varum et al., 2011), and the key molecular pathways involved in this metabolic transition have been discussed (Zhang et al., 2012a). By comparing metabolic and protein profiles in mouse iPSCs with their somatic counterparts, Folmes et al. demonstrated increased expressions of glycolytic enzymes and decreased mitochondrial activities in iPSCs. Interestingly, when monitoring the time course of reprogramming, researchers found that metabolic transition from OXPHOS to glycolysis was prior to the induction of pluripotency. In cells with higher mitochondrial membrane potential, expressions of glycolytic genes (*Glut1*, *Hxk2*, *Pfkm*, and *Ldha*) were significantly increased within the first week of reprogramming, whereas expressions of pluripotent genes (*Fgf4*, *Nanog*, *Oct4*, and *Sox2*) remained at low levels (Folmes et al., 2011, 2012; Panopoulos and Izpisua Belmonte, 2011). Panopoulos et al. also reported changes in the epigenetic status of genes involved in glycolysis and mitochondrial OXPHOS pathway during reprogramming (Panopoulos et al., 2012). In addition, comparing global metabolite profiles of human iPSCs, ESCs, and fibroblasts, researchers found that although iPSCs and ESCs have similar glycolytic metabolism, differences at the levels of unsaturated fatty acids and S-adenosyl methionine may play important roles during reprogramming (Panopoulos et al., 2012). As expected, somatic cells with higher glycolytic metabolism displayed higher reprogramming efficiency than their highly oxidative counterparts (Panopoulos et al., 2012). In this line, Zhu et al. demonstrated that induced pluripotency could be achieved with a combination of only one transcription factor (OCT4) and a cocktail of small molecules including PS48, a potent activator of PDK1 (pyruvate dehydrogenase kinase 1) that facilitates the metabolic transition from mitochondrial oxidation to glycolysis. Consequently, reprogramming efficiency was stimulated when glycolysis was induced and inhibited when glycolysis was blocked by small compounds (Zhu et al., 2010). This study not only highlights the importance of mitochondrial function and metabolism during reprogramming but also demonstrates that the metabolic transition can induce cellular changes capable of driving cells to a pluripotent state. Varum et al. found that PSCs exhibited elevated levels of phosphorylated PDH that blocked PDH complex activity and resulted in fewer substrates entering the TCA cycle. Authors also demonstrated that the levels of Hexokinase II were higher in PSCs than in fibroblasts (Varum et al., 2011). More recently, Vazquez-Martin and colleagues revealed that the expression of ATPase inhibitor factor 1 (IF1) was significantly increased in iPSCs whereas the level of catalytic β-F1-ATPase subunit was drastically decreased. When activities of the acetyl-CoA carboxylase (ACACA) and fatty acid synthase (FASN) lipogenic enzymes are inhibited, reprogramming efficiency is significantly decreased. Coincidentally, ACACA and FASN are highly expressed in iPSCs (Vazquez-Martin et al., 2013). Interestingly, mitochondrial parameters observed in cancer stem cells (CSCs) including mitochondrial mass, mitochondrial morphology, mtDNA copy number, and oxygen consumption are similar to those observed in iPSCs, which may imply some common metabolic features between iPSCs and CSCs that may help in their further characterization and study (Varum et al., 2011; Ye et al., 2011).

Regarding mitochondrial dynamics, mitochondrial fission and fusion events that regulate mitochondrial distribution have been suggested to play important roles in the reprogramming process as well. When fission is blocked using a Drp-1 inhibitor, somatic cells fail to form net-like mitochondria, which consequently leads to a decrease in reprogramming efficiency of more than 95%. While the precise mechanism is still elusive, disturbance of the metabolic transition is suggested to be involved in this correlation (Vazquez-Martin et al., 2012). These data indicate that not only functional but also structural changes in mitochondria represent a requirement for successful nuclear reprogramming. Finally, Prigione et al. showed that induced pluripotency by nuclear reprogramming in iPSCs can introduce homoplasmic and heteroplasmic mtDNA mutations; however, probably due to the highly PSC-like glycolytic metabolism of iPSCs, these mutations do not affect metabolic reprogramming and induced pluripotency (Prigione et al., 2011).

Mitochondrial Disease Modeling and Gene Correction

The reprogramming of patient fibroblasts with mitochondrial defects provides a great model for pathological studies, drug screening, and cell-replacement therapy development. Since mitochondria are semiautonomous organelles and contain their own mitochondrial genetic material, mitochondrial disorders can be the results of mitochondrial dysfunction caused by mutations in the nuclear DNA (coding for multiple mitochondrial proteins) or mtDNA (coding for 13 proteins, 22 tRNAs, and 2 rRNAs). iPSCs from patients with mutations in the nuclear DNA have been widely established. For example, Cooper et al. (2012) generated iPSCs from Parkinson's disease (PD) patients carrying mutations in PINK1, a mitochondrial protein encoded by nuclear DNA, as well as LRRK2, a cytoplasmic protein that can interact with the mitochondrial outer membrane. Researchers found that neural cells differentiated from patient-specific iPSCs could be phenotypically rescued by certain chemicals. PD patient-specific iPSC-derived neural cells carrying PINK1 Q456X or LRRK2 mutations can be rescued by the antioxidant coenzyme Q10 or the LRRK2 inhibitor GW5074 in response to low concentrations of specific mitochondrial damage inducers such as valinomycin and concanamycin A. Additionally, rapamycin could reduce the vulnerability of neural cells derived from PD patient iPSCs with the LRRK2 G2019S or R1441C mutation to mitochondrial damage exposed to valinomycin. Another study by Jiang et al. demonstrated that in human midbrain dopaminergic (DA) neurons derived from iPSCs of patients with Parkin mutations (a mitochondria-related ubiquitin E3 ligase encoded by nuclear DNA), the levels of ROS,

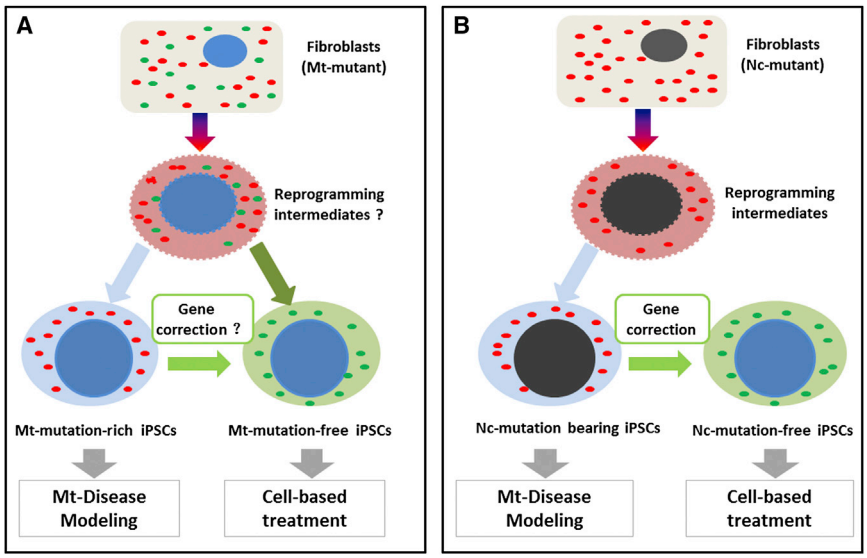

Figure 2. Mitochondrial Disease-Modeling and Gene Correction

(A) Reprogramming of fibroblasts from mitochondrial disease patients with mitochondrial dysfunction caused by mutations in mitochondrial DNA (Mt mutant). Mutant mtDNA may exist as a mixture of wild-type and mutant mtDNA (heteroplasmy). MtDNA mutant (Mt mutant) iPSCs derived from patients will give rise to two iPSC populations: Mt-mutation-rich iPSCs with high levels of mutant mtDNA and Mt-mutation-free-iPSCs with undetectable levels of mutant mtDNA. Mt-mutation-rich iPSCs represent valuable models for studying mitochondrial diseases, whereas the Mt-mutation-free iPSCs would be a promising resource for the potential autologous cell therapy. Alternatively, Mt mutations could be potentially corrected through gene targeting. Mt mutant mitochondria are colored in red; normal mitochondria are colored in green.

(B) Reprogramming of fibroblast from mitochondrial disease patients with mitochondrial dysfunction caused by mutations in the nuclear genome (Nc mutant). Nc-mutation-bearing iPSCs may be used as a model of mitochondrial diseases, whereas Nc-mutation-corrected iPSCs (Nc-mutation-free iPSCs) will be a promising resource for the autologous cell therapy. Defective mitochondria are colored in red; normal mitochondria are colored in green.

and monoamine oxidase transcripts were significantly elevated. Meanwhile, DA uptake was remarkably reduced with increased spontaneous DA release (Jiang et al., 2012). Finally, Hick et al. (2013) generated iPSCs from Friedreich's ataxia (FRDA) patients. Friedreich's ataxia is a neurodegenerative disease caused by expanded GAA codon repeats in the gene encoding for frataxin, a mitochondrial protein involved in the biosynthesis of iron-sulfur clusters. Neurons and cardiomyocytes derived from iPSCs from FRDA patients display mitochondrial dysfunction and recapitulate the mitochondrial degeneration characteristic of Friedreich's ataxia, indicating that iPSCs from these patients represent useful models for the study of this human disorder. These studies demonstrate that cells with mitochondrial deficits can be reprogrammed into a pluripotent state and that patient-specific iPSCs can be used as reliable models for disease study and drug discovery.

On the other hand, distinct from nuclear DNA mutations, mutations on mtDNA have also led to interesting observations during reprogramming to induced pluripotency. To date, only a few studies have been reported regarding iPSC reprogramming from patients with mtDNA mutation. Fujikura et al. (2012) reported the successful generation of mitochondrial disease-specific iPSCs (Mt-iPSC) from two diabetic patients carrying the mtDNA A3243G heteroplasmic mutation. Strikingly, they found that some of the Mt-iPSC clones became mutation-free during reprogramming while the others became mutation-rich. The authors also found that while the mtDNA copy number in Mt-iPSCs was similar to that of primitive fibroblasts at early stage, it became similar to that of human ESCs at later stage. Similar results were also found in another study where iPSCs were generated from fibroblasts of a Pearson marrow pancreas syndrome (PS) patient carrying heteroplasmic deletion in mtDNA. Compared to mtDNA deletion-free iPSCs, PS-iPSCs carrying deleted mtDNA displayed growth defects, mitochondrial dysfunction, and other disease-related phenotypes during in vitro hematopoietic differentiation (Cherry et al., 2013). More recently, Folmes et al. (2013) found that reprogramming fibroblasts derived from a MELAS patient (with a heteroplasmic mitochondrial mutation at position G13513A in the ND5 subunit of complex I) produced iPSCs with a spectrum of healthy and disease-causing mitochondrial heteroplasmy. Based on this observation, the authors proposed that disease-causing mitochondrial heteroplasmy could be segregated in patient-derived iPSCs. Therefore, iPSC-based mitochondrial disease models may provide fascinating tools to investigate molecular features of cellular dysfunction within the context of a native nuclear genome background where distinct levels of mitochondrial heteroplasmy could be segregated. Nevertheless, it is still unclear if heteroplasmy of mtDNA mutations in individual iPSC clones is inherited from the heteroplasmy of parental fibroblasts or generated de novo during the reprogramming process. Nevertheless, mutation-free iPSCs generated from patients may provide new promises for the autologous cell transplantation based therapy, as illustrated in Figure 2.

Mitochondrial dysfunction is involved in many neurological diseases (Rugarli and Langer, 2012) and aging (George et al., 2011). In this regard, recent studies have thrown new light on the understanding of these diseases using iPSC disease modeling and gene targeting technology (Liu et al., 2011, 2012; Pan et al., 2011; Zhang et al., 2012b). The advancing technology of gene targeting in PSCs offers a novel approach to investigate mitochondrial function as well as mitochondria-related diseases. Despite the success achieved in nuclear gene editing, for many decades targeting mitochondrial genomes harboring pathologic mutations by gene editing tools has been a difficult task. Encouragingly, a recent report by Iyer et al. (2012) described a novel mitochondrial gene replacement technology to target mtDNA. In this study, authors introduced a Leber's hereditary optic neuropathy (LHON) pathogenic mtDNA containing a G11778A mutation. This pathogenic mtDNA was transfected

into dideoxycytidine-treated human PSC-derived neural progenitor cells with reduced endogenous mtDNA. This LHON-hNP line contained LHON mtDNA and had the capacity to differentiate into neurons. Interestingly, Wang et al. (2012) reported successful mitochondrial targeting of nuclear-encoded mRNAs and tRNAS when they were appended to a 20-ribonucleotide stem-loop sequence from the H1 RNA or a 3′ UTR localization sequence that confers localization to the mitochondrial outer membrane. Consequently, RNA fusion transcripts were directed into mitochondria. This study demonstrated that translocation of a wide range of RNAs with or without mitochondrial localization sequence can be achieved by appending a mitochondrial targeting sequence and potentially provides an exciting tool for correcting mitochondrial genetic disorders. Despite many years of unsuccessful attempts for the manipulation of mtDNA, these novel mtDNA editing-based approaches have opened the door for the creation of novel in vitro stem cell models and the discovery of therapies for mitochondrial disorders and neurodegenerative diseases. Nevertheless, validation of successful and stable mtDNA manipulation as well as application of these strategies to multiple mtDNA genes will need to be validated before the field can move forward.

Future Prospects

Mitochondria play vital roles in the induction and maintenance of pluripotency, as well as in the differentiation of PSCs. While recent advances have started to provide insights into these processes, we are far from a comprehensive understanding of mitochondria physiological functions in stem cells. Questions that need to be addressed include: (1) What is the role of mitochondria in regulating reprogramming and differentiation? (2) Are the functional and structural changes of mitochondria observed in the pluripotent state and during differentiation the cause or consequence of these processes? (3) What is the driving mechanism for the conversion of functionally mature mitochondria into an immature state and vice versa during reprogramming and differentiation, respectively? (4) What signaling pathways are determining these changes? (5) How is the mitochondrial network remodeled during reprogramming and differentiation? (6) What are the epigenetic modifications associated with changes in mitochondrial biogenesis, function, and structure? (7) Is mitophagy a crucial cellular process during nuclear reprogramming and induced pluripotency? Besides these open questions, due to the specific anabolic and catabolic requirements of PSCs when compared with their differentiated counterparts, it is evident that there is a metabolic transition from mitochondrial oxidative phosphorylation to glycolysis during the establishment of pluripotency. This metabolic switch is accompanied by significant changes in mitochondrial function, composition, structure, and maturation in order to meet the specific cellular demands of this cellular state. Understanding in more detail the role of mitochondria during these events and the mechanism behind these changes will not only provide important insights into the relationship between mitochondria and pluripotency, but may also contribute specifically to our understanding of induced pluripotency, embryonic development, and disease pathogenesis. Furthermore, the fascinating advances of gene targeting technology in the stem cell field will substantially accelerate mechanistic studies of aging and mitochondrial diseases, which in turn may facilitate the development of new treatments in the not too distant future.

ACKNOWLEDGMENTS

G.-H.L. is supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA01020312), NSFC (81271266, 31222039), the Thousand Young Talents program of China, National Laboratory of Biomacromolecules (2013kf05, 2013kf11), and State Key Laboratory of Drug Research (SIMM1302KF-17). X.X. was supported by NSFC (31201111). J.C.I.B. was supported by TERCEL-ISCIII-MINECO, Fundacion Cellex, G. Harold and Leila Y. Mathers Charitable Foundation, The Leona M. and Harry B. Helmsley Charitable Trust, The Glenn Center for Aging Research and The Ellison Medical Foundation.

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