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Mitochondrial remodeling in hepatic differentiation and dedifferentiation

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ABSTRACT

Mitochondrial biogenesis and metabolism have recently emerged as important actors of stemness and differentiation. On the one hand, the differentiation of stem cells is associated with an induction of mitochondrial biogenesis and a shift from glycolysis toward oxidative phosphorylations (OXPHOS). In addition, interfering with mitochondrial biogenesis or function impacts stem cell differentiation. On the other hand, some inverse changes in mitochondrial abundance and function are observed during the reprogramming of somatic cells into induced pluripotent stem cells (iPSCs). Yet although great promises in cell therapy might generate better knowledge of the mechanisms regulating the stemness and differentiation of somatic stem cells (SSCs)—which are preferred over embryonic stem cells (ESCs) and iPSCs because of ethical and safety considerations—little interest was given to the study of their mitochondria. This study provides a detailed characterization of the mitochondrial biogenesis occurring during the hepatogenic differentiation of bone marrow-mesenchymal stem cells (BM-MSCs). During the hepatogenic differentiation of BM-MSCs, an increased abundance of mitochondrial DNA (mtDNA) is observed, as well as an increased expression of several mitochondrial proteins and biogenesis regulators, concomitant with increased OXPHOS activity, capacity, and efficiency. In addition, opposite changes in mitochondrial morphology and in the abundance of several OXPHOS subunits were found during the spontaneous dedifferentiation of primary hepatocytes. These data support reverse mitochondrial changes in a different context from genetically-engineered reprogramming. They argue in favor of a mitochondrial involvement in hepatic differentiation and dedifferentiation.

Abbreviations: α1AT, α-1-antitrypsin; BM-MSCs, bone-marrow mesenchymal stem cells; COX1, subunit I of the cytochrome c oxidase; COX2, subunit II of the cytochrome c oxidase; COX4, subunit IV of the cytochrome c oxidase; DRP1, dynamin-related protein 1; ECAR, extracellular acidification rate; ERR, estrogen-related receptors; ESCs, embryonic stem cells; FCCP, carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone; G6PC, glucose-6-phosphatase catalytic subunit; iPSCs, induced pluripotent stem cells; mtDNA, mitochondrial DNA; ND2, NADH dehydrogenase subunit 2; NRF-1 and-2, nuclear respiratory factor 1 and 2; OCR, oxygen consumption rate; OTC, ornithine transcarbamylase; nDNA, nuclear DNA; OXPHOS, oxidative phosphorylation; PEPCK1, phosphoenolpyruvate carboxykinase 1; PGC-1, PPAR gamma coactivator 1; PPAR, peroxisome proliferator-activated receptor; PPIE, peptidyl-prolyl cis-trans isomerase E; PRC, PGC-1-related coactivator; SSCs, somatic stem cells; TAT, tyrosine aminotransferase; TDO2, tryptophan 2,3-dioxygenase; TFs, transcription factors; TFAM, mitochondrial transcription factor A.

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1. Introduction

Depending on physiologic and cellular cues, mitochondria can display differences in their abundance, morphology and functions. Mitochondrial biogenesis is a complex process involving lipid membrane formation, mitochondrial DNA (mtDNA) replication and transcription, and coordinated synthesis of mitochondrial proteins encoded by both nuclear and mitochondrial genomes. The coordination between the nuclear and mitochondrial genomes is achieved through the expression of nucleus-encoded mitochondrial proteins which control mtDNA replication and transcription, such as the mitochondrial transcription factor A (TFAM). In the nucleus, the expression of mitochondrial genes is controlled by a limited number of transcription factors (TFs), including NRF-1 and -2 (nuclear respiratory factors 1 and 2), PPARs (peroxisome proliferator-activated receptors α, β/δ, and γ) and ERRs (estrogen-related receptors α, β, and γ). (Interested readers can refer to (Hock and Kralli, 2009) for a detailed review of the involvement of these TFs in mitochondrial biogenesis). The activity of these TFs is itself controlled by the PGC-1 (PPAR gamma coactivator 1) family of coactivators: PGC-1α; PGC-1β; and PRC (PGC-1-related coactivator) (Hock and Kralli, 2009). Among these, the best described is PGC-1α, a protein considered to be the master regulator of mitochondrial biogenesis and function (Fernandez-Marcos and Auwerx, 2011).

A number of recent studies evidenced an enhanced mitochondrial biogenesis in various stem cell differentiation models (Chen et al., 2012, Xu et al., 2013). In ESCs, the mitochondrial phenotype has been described as "immature," consisting of few mitochondria, containing poorly developed cristae, and displaying a perinuclear location (Cho et al., 2006, Lonergan et al., 2007, St John et al., 2005). While these cells mainly rely on glycolysis for their energy production, differentiating cells display an increased mitochondrial mass and mtDNA abundance, a more developed mitochondrial network, and a shift toward OXPHOS to meet their energy demands (Chung et al., 2010, Facucho-Oliveira et al., 2007, Lonergan, Bavister, 2007, Prigione et al., 2010, Suhr et al., 2010). In addition, the use of molecules promoting or inhibiting mitochondrial biogenesis or function, or interfering with the expression of mitochondrial biogenesis regulators or proteins involved in mitochondrial function, has been demonstrated to impact stemness and cell differentiation (Huang et al., 2011, Tormos et al., 2011, Xu et al., 2013). For instance, the attenuation of mitochondrial function using carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) in ESCs results in increased transcriptional levels of the pluripotency markers Sox2, Oct4, and Nanog, and in the repression of transcriptional programs associated with lineage differentiation (Mandal et al., 2011). Conversely, the reprogramming of somatic cells into iPSCs is accompanied by inverse modifications, in a process called "mitochondrial rejuvenation" (Folmes et al., 2011, Prigione, Fauler, 2010, Suhr, Chang, 2010, Varum et al., 2011). These data have led to the hypothesis that transitions in mitochondrial metabolism regulate, or are regulated by, differentiation and reprogramming events.

In order to better appreciate the involvement of mitochondria in differentiation processes, two main points remain to be addressed. On the one hand, due to the lack of an extensive characterization of the mitochondria in the different stem cell differentiation models previously studied, it is currently unclear if the mitochondrial biogenesis process always involves an increase in all or some of the constituents of mitochondria (membrane, protein and mtDNA) and if it systematically results in increased mitochondrial activity. In addition, the kinetics of the mitochondrial biogenesis has not been addressed in most studies, and it remains unclear when, and for how long, the mitochondrial biogenesis is induced. On the other hand, while most studies of mitochondrial biogenesis in stemness and differentiation have been performed on ESCs and iPSCs, few studies report an enhanced mitochondrial biogenesis during the differentiation of SSCs such as mesenchymal stem cells (MSCs) (Chen et al., 2008, Palomaki et al., 2013, Pietila et al., 2012, Tormos, Anso, 2011). If the mitochondrial biogenesis represents a hallmark of all types of stem cell differentiation processes therefore remains to be demonstrated. Although not as pluripotent as ESCs, SSCs offer several advantages over ESCs/iPSCs in cell therapy, such as better safety and fewer ethical concerns. Among SSCs, MSCs can differentiate into multiple cell types, including hepatocytes (Banas et al., 2007, Mosna et al., 2010). Interestingly, hepatocytes display a much higher expression of several mitochondrial OXPHOS subunits (Suppl. Fig. 1) than BM-MSCs, suggesting that a strong mitochondrial biogenesis might be associated with their differentiation.

Primary hepatocytes, however, cannot be maintained in culture in a fully differentiated and highly metabolic state. Hepatocytes are subject to a rapid dedifferentiation process during *in vitro* culture. This process is initiated during their isolation, and is characterized by: rapid loss of hepatic gene expression, polarity, and activity; re-entry into the cell cycle; and upregulation of cytoskeleton and mesenchymal proteins such as vimentin (Elaut et al., 2006, Meyer et al., 2013). Improving our understanding of the mechanisms involved in hepatocyte dedifferentiation, and eventually counteracting them, could thus lead to many important applications such as pharmacological testing. The demonstration of an involvement of mitochondria in hepatic differentiation and dedifferentiation processes might therefore be used as a starting point for studies aimed to improve/limit hepatocyte differentiation/dedifferentiation.

In this study, we provide a kinetic and detailed characterization of the mitochondrial biogenesis initiated during the hepatogenic differentiation of BM-MSCs. We provide evidence for inverse mitochondrial changes during the spontaneous dedifferentiation of hepatocytes. We initially found evidence for an induction of mitochondrial and mtDNA abundance and an increased expression of several mitochondrial proteins and mitochondrial biogenesis regulators, concomitant with an increased oxidative activity, during the early hepatogenic differentiation of BM-MSCs. In contrast, during primary hepatocyte dedifferentiation we found a reduction of several mitochondrial proteins involved in OXPHOS. Furthermore, while a fission of the mitochondrial network was observed in the differentiation model, a trend toward an increased fusion was observed during hepatocyte dedifferentiation. These data demonstrate the existence of opposite changes in mitochondrial morphology and function between the hepatic differentiation and hepatocyte dedifferentiation models. They further support the involvement of mitochondria in stemness and cell differentiation.

2. Material and methods

2.1. Ethical guidelines

This study was approved by the local ethics committee of the Jules Bordet Institute (Belgium) for obtaining human BM-MSCs after informed consent from donors. The use of human liver tissue for research was approved by the ethical committee of St-Luc Hospital and of the Université Catholique de Louvain, Belgium. Primary hepatocytes were obtained from the Hepatocyte and Liver Stem cell Tissue Bank of Cliniques St Luc, acting under the agreement of the Belgian Ministry of Health.

2.2. Cell culture and differentiation

Cultures of human BM-MSCs were established as previously described (Najar et al., 2013) from 4 different healthy donors (aged 2–22 years old). BM-MSCs were expanded in Dulbecco's Modified Eagle Medium low glucose (DMEM-LG) supplemented by 1%