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The Missing Genome: Mitochondrial DNA Deletions in Stem Cells

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Induced pluripotent stem cells (iPSCs) have great potential for regenerative medicine, including the ability to avoid transplantation rejection, as well as relative ease of procurement compared to embryonic stem cells (ESCs). However, in comparison to ESCs, they have a higher rate of apoptosis, a decreased rate of proliferation, and premature aging behavior [1]. In his Free Radical Theory of Aging, Denham Harman proposed that organisms age due to mitochondrial damage that occurs over time. Harman stated that free radicals leak from the electron transport chain and react with mitochondria causing damage. Over time, this damage accumulates, causing mitochondria to cease producing ample energy for the cell, ultimately resulting in cell apoptosis and gradual somatic degradation; the process of aging [2].

Mitochondria operate semi-autonomously from the rest of the cell and have their own 16.5 kb genomic DNA, mitochondrial DNA (mtDNA) [3]. Dysfunction of either biogenesis, fission, or fusion of mitochondria is known to be associated with aging, diseases of the neuromuscular system, and genetic disorders such as cyclic vomiting syndrome, Parkinson's disease, non-syndromic deafness, and myoclonic epilepsy [4, 5]. MtDNA is especially prone to damage by reactive oxygen species (ROS) due to its localization near the inner mitochondrial membrane, the site of the electron transport chain and the origin of ROS production [6].

In this study, it was hypothesized that the detrimental properties of iPSCs are caused by mtDNA deletions, specifically a 4977 bp deletion known as the "common deletion", which is associated with aging and prevalent among patients with mitochondrial myopathies [7]. MtDNA was amplified by polymerase chain reaction (PCR), which showed that the common deletion was present in iPSCs generated with retroviral vectors, plasmids, and micro-RNA, but lacking in human foreskin fibroblasts (HFFs; source cells), embryonic stem cells (ESCs), the breast adenocarcinomas MCF-7 and MDA-MB-231, the lung adenocarcinoma A549, and the prostate adenocarcinoma PC-3. A real time (RT-) PCR-based assay was used to quantify mtDNA deletions within each cell type, revealing that while heteroplasmic, up to 60% of mtDNA molecules in iPSCs may contain the "common deletion" as compared to their source cells. Transmission electron microscopy was used to determine morphologic differences between mitochondria of iPSCs and their source cells, revealing disparities in terms of size and structural properties (Figures 1, 2). Results from the ESCs and cancer cells support the hypothesis that the deletions are a result of iPSC generation methodology and not attributed to the self-renewal and differentiation properties found in immortal cell lines.

These data show that iPSCs (compared to ESCs) have mtDNA deletions and morphologic changes that may be the basis for premature senescence, and that the mtDNA deletions are a function of iPSC generation methodology. New methods of generating iPSCs should be developed that retain mitochondrial genes. By comparing mtDNA deletion levels and morphologic differences, novel methods of generating iPSCs can be evaluated for their potential to maintain their clinical potential without producing rapid senescence.

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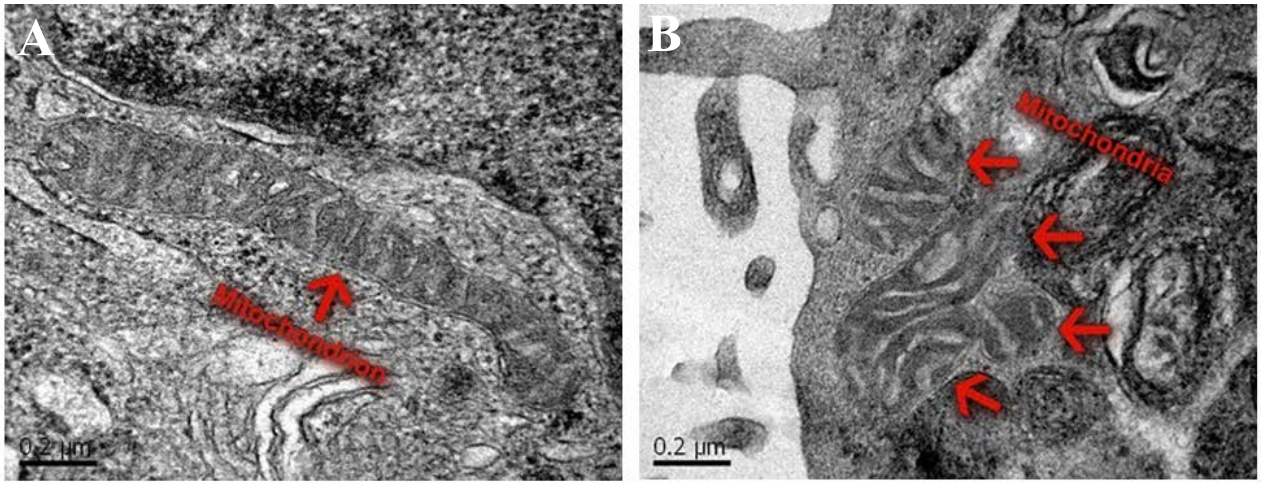

Figure 1: Mitochondrial morphology of (A) iPSCs and their source cells (B) HFF. The iPSC mitochondrion has poorly developed cristae, irregular shape, and is larger than most mitochondria. The mitochondria in HFFs are more typical in morphology. Scale = 0.2 µm.

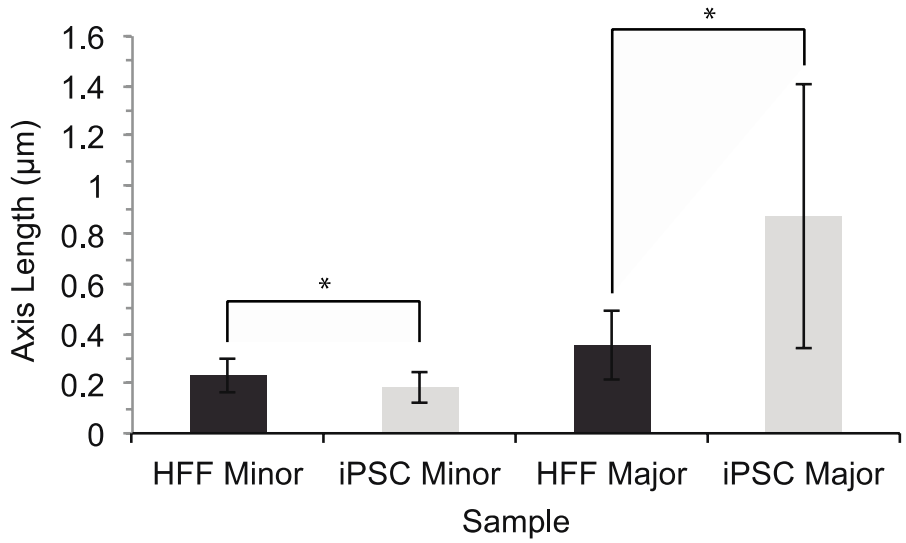

Figure 2. Major and Minor Diameters of Mitochondria in HFFs and iPSCs. The mean minor diameters are similar, while the major diameter of the iPSC sample appeared significantly larger. Bars represent standard deviation; n=25, *p < 0.01, two-tailed unpaired Student's *t*-test.