Methodology for visualization of mitochondria in induced pluripotent stem cells and induced trophoblast stem cells

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ABSTRACT

The cause of preeclampsia (PE), a common and potentially fatal pregnancy syndrome, is unknown. However, early-onset preeclampsia (EOPE) has been associated with mitochondrial dysfunction and oxidative stress. To study the etiology of PE, our lab has generated induced Pluripotent Stem Cells (iPSCs) by reprogramming umbilical cord (uc) fibroblasts from both preeclamptic and healthy pregnancies. Additionally, the uc fibroblasts were converted to induced trophoblast stem cells (iTSCs), which represent the placental cells of early pregnancy. While iTSCs are already trophoblasts (TBs), iPSCs must be differentiated to TB using the BAP treatment method established by our lab. The BAP treatment consists of bone morphogenetic protein 4 (BMP4) and signaling inhibitors A83-01 and PD173074, which inhibit ACTIVIN A and FGF2, respectively. This treatment differentiates the cells into trophoblast populations found in early pregnancy. The iPSCs can be maintained in the undifferentiated state using medium conditioned by irradiated mouse embryonic fibroblasts (iMEF) and supplemented with FGF2. These cells can be grown in 5% O_2 (physiological) or 20% O_2 (oxidative stress). We hypothesize that if EOPE affects the mitochondrial quality but not quantity, there will not be a difference in signal when we quantify the amount of MitoTrackerRed. To test that hypothesis, here, we have established methodology for identifying mitochondria in iPSCs differentiated to TB and iTSCs using MitoTrackerRed, DAPI, and Phalloidin. MitoTrackerRed reveals mitochondria by diffusing through the mitochondrial membrane, and then fluorescing red. DAPI stains nuclei, and Phalloidin stains filamentous actin, so individual and syncytialized trophoblasts can be distinguished. Confocal microscopy was used to create three-dimensional images that can be analyzed to compare the volume of mitochondria in control and EOPE TBs or iTSCs and support other mitochondrial analyses in our unique early pregnancy stem cell models. Understanding the cause of preeclampsia may lead to development of treatments to combat this fatal syndrome.