Title:

Label-free optical imaging probing of metabolic states of definitive endoderm differentiating human pluripotent stem cells

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250 Word Technical Review: (250)

The clinical potential and commercialization of human pluripotent stem cell (hPSC)-derived therapeutics hinges on the development of cost-effective, reproducible and scalable bioprocesses. Definitive endoderm (DE) cells are progenitors of a wide gamut of clinically targeted cells residing in organs such as the pancreas, liver and lungs. Capturing alterations in morphology, metabolic activity, and oxidative stress that accompany the transitions of cellular identity through imaging, can be a powerful non-invasive tool to improve the real-time monitoring of hPSCs' differentiation to DE. DE differentiation of hPSCs has been traditionally assessed via signal transduction changes and induction of specific endodermal related genes. Here, we elucidate the metabolic changes that hPSCs undergo during DE differentiation, based on label-free, intensity and lifetime two-photon excited fluorescence (TPEF) images. Specifically, label-free, TPEF images highlight morphological changes between differentiated and un-differentiated hPSCs, with the latter yielding bigger spheroids with limited size variations. Cell metabolic activity during DE differentiation is assessed by the fluorescence of NAD(P)H (755 nm excitation, 435-485 nm emission) and flavoproteins (860 nm excitation, 500-550 nm emission). Mitochondrial networking levels are quantified via analysis of NAD(P)H TPEF images and are enhanced in DE differentiated cells. NAD(P)H lifetime images indicate a shift towards longer lifetimes as well as a higher NAD(P)H bound fraction indicating that DE differentiated cells rely more on oxidative phosphorylation and possibly glutaminolysis for energy production; the non-differentiated cells exhibit higher levels of glycolysis. Thus, TPEF imaging has the potential to significantly advance our understanding of metabolic changes during differentiation hPSCs into DE lineages.

100 Word Summary:

The transition of hPSCs to definitive endoderm (DE) is a crucial step in a wide range of hPSC-derived therapeutics and disease modeling. Two-photon excited fluorescence (TPEF) imaging, as a non-invasive non-destructive method for metabolic studies, reveals the distinct metabolic switches during DE differentiation in a real-time monitoring mode. Since metabolic pathways are instigators of important regulatory mechanisms that can influence and determine stem cell fate, TPEF imaging serves as an important enabling technology in tissue engineering applications through non-invasive reporting of quantitative metabolic biomarkers associated with stem cell differentiation.

Keywords:

Human pluripotent stem cells, definitive endoderm stem cells, two-photon fluorescence microscopy, FLIM, metabolic imaging, redox ratio, oxido-reductive state, mitochondrial clustering.

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