THE DIFFERENTIATION OF PLURIPOTENT STEM CELLS TO HEPATIC CELLS – PARALLELS BETWEEN MATURATION STATUS AND METABOLIC STATE

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Hepatocytes derived from human pluripotent stem cells (PSCs) hold great promise as an unlimited cell source for liver cell therapy and in vitro toxicity studies. Through the treatment of a series of cytokines and growth factors to mimic embryonic development, PSCs can be guided to differentiate through the endodermal and hepatic commitment stages to become hepatocytelike cells (HLCs). As PSCs differentiate toward endoderm, then to hepatic lineage, the glycolysis and amino acid metabolic rate decreased significantly. Flux analysis using a compartmentalized metabolic flux model that considers cytosolmitochondria interactions revealed that the progressive decline in glycolysis flux coincides with an increase in activities of oxidative phosphorylation (OxPhos) and TCA cycle. This increase in OxPhos activity was also accompanied by increased mitochondria activity. Transcriptome analysis showed that the expression of a number of enzymes and transporter in glucose metabolism decreased as PSCs differentiate toward HLCs. Using a kinetic model of energy metabolism, we showed that the decrease in the expression of those genes could account for the metabolic shift during the differentiation. Our results suggest that metabolic shift may play a role in in vitro PSC differentiation to HLC. Consistently, aborting the metabolic shift by culturing differentiating HLCs at a high glucose level showed a decreased degree of maturation. We then asked the question whether such metabolic shift occurred during embryonic liver development. Lacking fetal liver metabolism data, we examined the transcriptome data of developing liver in mouse embryo. We compiled the transcriptome data of human PSCs differentiation to HLCs and mouse embryonic liver development and performed cross-species in vivo vs. in vitro meta-analysis. After batch corrections on the combined data set cells at different stages of HLC differentiation and different embryonic days of mouse liver development aligned chronologically on a unified developmental “time” scale. The results show that in vitro HLC differentiation of human PSCs reached an equivalent period of E(Embryo day)13-E15 in mouse development, but lacked full maturity of hepatocytes. Furthermore, the enzymes of glucose metabolism behaved similarly in embryonic liver development and in HLC differentiation up to E15. In late stages of embryonic liver development, many of the metabolic enzymes subsequently switch their isoforms to those of the mature hepatocyte. The isoform switch of glycolytic enzymes may reflect the final switch to the mature metabolic nature of the liver. Although, we observe many similar trends in our differentiation, failure to switch isoforms in in vitro differentiation is a key contributor to the lack of maturity of HLCs. In conclusion, the energy metabolism undergoes significant changes over the course of in vitro differentiation from PSCs towards hepatocytes. The shift in energy metabolism is the result, but has also been proposed to be a possible driver, of the differentiation. To enhance the maturation of HLCs, correcting the expression of the genes that fail to progress concordantly as in mouse embryonic liver beyond E15 is a tempting proposition. However, this metabolic study also suggests that providing an appropriate environment to elicit a shift toward the metabolic state of mature hepatocytes may be equally important.