

Loss of FOXA2 induces ER stress and hepatic steatosis and alters developmental gene expression in human iPSC-derived hepatocytes

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Abstract:

Background and Aim: While FOXA2's significant role in rodent liver functions is well-documented, its functions in human hepatocytes remain incompletely understood. Our recent work involved generating induced pluripotent stem cell (iPSC) lines with FOXA2 mutations (*FOXA2*^{-/-} iPSCs) and revealing that FOXA2 deficiency leads to developmental defects in iPSC-derived pancreatic islet cells, potentially contributing to monogenic diabetes. In this study, our aim was to investigate FOXA2's impact on the development and functionality of human hepatocytes using *FOXA2*^{-/-} iPSC lines.

Methods: We differentiated two *FOXA2*^{-/-} iPSC lines along with their wild-type (WT) counterparts into hepatic progenitors (HP) and mature hepatocytes (MH). At various developmental stages, we assessed the expression of hepatic developmental and functional genes through immunostaining, quantitative reverse transcription-polymerase chain reaction (qRT-PCR), and Western blotting. RNA-sequencing was conducted on iPSC-derived HP and MH, and functional assays were performed on iPSC-derived MH. Furthermore, we introduced FOXA2 overexpression in FOXA2-deficient cells during the HP stage and evaluated its impact during the maturation stage.

Results: Our study revealed that FOXA2 expression initiated during the definitive endoderm stage

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and exhibited a gradual increase in expression levels until it reached its peak at day 8, corresponding to HPs. Subsequently, after day 8, FOXA2 levels began to decrease, ultimately showing minimal to no expression by the end of the differentiation process at the mature stage. The absence of FOXA2 led to significant alterations in the expression of crucial developmental and functional genes in HP and MH, along with an upregulation of markers for endoplasmic reticulum (ER) stress. Functional assays demonstrated increased lipid accumulation, increased bile acid synthesis, and glycerol production, alongside reduced glucose uptake, glycogen storage, and albumin secretion. RNA-sequencing analysis substantiated these findings, indicating a marked increase in genes related to lipid metabolism and bile acid secretion, as well as suggesting the activation of hepatic stellate cells and the onset of hepatic fibrosis in FOXA2-deficient MH. The introduction of FOXA2 overexpression in HP reversed these defective phenotypes and enhanced hepatocyte functionality in iPSC-derived MH lacking FOXA2.

Conclusion: Our findings underscore the critical importance of proper FOXA2 expression in human hepatocytes for normal hepatocyte development, safeguarding against ER stress, hepatic steatosis, and bile acid toxicity. Also, the human hepatocyte model presented in this study offers potential for identifying novel therapeutic targets related to FOXA2-associated liver disorders.