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ABSTRACT – PISA 2024

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Title: **HNF1B Controls the Cell's Commitment to Hepatic Fate.**

Abstract (2,500 Character limit):

Introduction/Background

Gene expression during embryonic development determines cell differentiation and organ morphogenesis. The transcription factor Hepatocyte Nuclear Factor 1 homeobox beta (*HNF1B*) is required for liver development. Heterozygous mutations in *HNF1B* result in a multi-system disorder in humans. In mice, deletion of *Hnf1b* causes embryonic lethality by embryonic day (e)6.5-7.0 due to defective visceral endoderm differentiation. When the defect in the visceral endoderm was rescued, *HNF1B* was found to be required for liver bud formation. Although HNF1B is thought to be critical for the developmental transition of the endoderm to a hepatic fate, the molecular mechanisms through which *HNF1B* mediates hepatic cell fate in humans are poorly defined.

Methods

Using iPSCS as a model, we are studying the mechanism through which *HNF1B* controls the transition of the endoderm to form hepatic progenitor cells. We generated an *HNF1B* knockout, a dox-inducible *HNF1B* rescue, and dox-inducible HNF4A rescue human iPSC lines using CRISPR-Cas9 gene editing. We confirmed the integrity of the cell lines by DNA sequencing, immunofluorescence staining, and western blot analyses. We characterized the differentiation of our *HNF1B* -/- iPSCs by defining the changes in the expression of characteristic hepatic markers. We also performed RNA sequencing, ATACseq, and ChIPseq to understand the molecular mechanisms by which HNF1B controls the transition from the endoderm to a hepatic fate.

Results

We established the expression pattern of important transcription factors, including HNF1B, in hepatic differentiation, showing that *HNF1B* precedes the expression of key hepatic genes such as *HNF4A*. We confirmed the knockout and rescue cell lines. Importantly, *HNF4A,* which is a central regulator of hepatocyte fate, was markedly downregulated in the *HNF1B* -/- cells. We observed that the gene expression signature of the *HNF1B* -/- cells was indicative of a complete block in the transition of the endoderm to a hepatic fate. In the absence of *HNF1B*, transcription factors known to be essential for hepatic differentiation such as *HNF4A* and *HNF1A* were significantly downregulated, while genes characteristic of the endoderm such as SOX17 and FOXA2 were significantly upregulated. We determined the direct binding of HNF1B to important TFs during liver development which concided with the accessibility of chromatin.

Conclusion

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We conclude that HNF1B is essential to establish hepatic fate by controlling the expression of the master regulator of hepatic gene expression, HNF4A.