

Hepatocyte HIF signaling is dispensable for β -catenin-dependent liver zonation

Prerna Chakkingal^{1,2,3}, Chang Kyung (Joanna) Kim^{1,2,3}, Minakshi Poddar^{1,2,3}, Sucha Singh^{1,2,3}, Satdarshan P Monga^{1,2,3}

¹Organ Pathobiology and Therapeutics Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.

²Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.

³Pittsburgh Liver Research Center, University of Pittsburgh and University of Pittsburgh Medical Center, Pittsburgh, PA, USA.

Background: The adult liver is divided into three zones along the porto-central axis, where hepatocytes located in each zone play distinguished metabolic functions to maximize functional efficiency. Zone 1 hepatocytes near the portal vein, where blood flows into the liver, are exposed to the highest oxygen concentration, whereas Zone 3 hepatocytes near the central vein, where blood leaves the liver, are exposed to the lowest oxygen concentration. We have previously shown WNT/ β -catenin signaling is a main regulator of Zone 3 identity. What remains unclear is the upstream regulator of Zone 3 WNT activity. Since liver metabolic functions are dictated by the environmental factors where they reside, we hypothesized that hypoxia-inducible factor (HIF) signaling in hepatocytes driven by relative hypoxia regulates Zone 3 identity.

Methods: The transcriptional activity of HIF requires both HIF α (either 1 α or 2 α) and HIF1 β subunits to dimerize. To test our hypothesis, we generated transgenic mouse models to delete *Hif1a* and *Epas1* (HIF2 α) (HC-Hif1a-Hif2a-DKO) or *Arnt* (HIF1 β) (HC-Hif1b-KO) from hepatocytes. Mice were intraperitoneally injected with AAV8-TBG-eGFP (control) or AAV8-TBG-Cre at 8 weeks of age, and livers were resected after 14 days post-injection. Liver tissues were stained with H&E and Sirius Red to assess for liver morphology and fibrosis, respectively. Immunohistochemistry (IHC) was performed to stain for glutamine synthetase (GS) and CYP2E1 as zone 3 markers and CYP2F2 as a zone 1 marker, to determine the changes in liver zonation.

Results: All control, HC-Hif1a-Hif2a-DKO, and HC-Hif1b-KO mice did not show difference in liver weight-to-body weight ratio after *Hif1a/Epas1* or *Arnt* deletion compared to control mice. We also did not see any morphological differences in livers from three mouse groups, as well as no changes in the degree of fibrosis. There was no difference in serum AST, ALT, total bilirubin, and alkaline phosphatase to suggest hepatocyte injury or cholestasis. We also did not see differences in serum glucose, triglyceride, and cholesterol level. More importantly, we did not see any difference in the liver zonation in all three mouse livers based on IHC of GS and CYP2E1 (Zone 3 markers) and CYP2F2 (Zone 1 marker) based on %area quantifications.

Conclusion: Overall, these data demonstrated that HIF signaling in adult hepatocytes does not have a direct effect on liver homeostasis or liver zonation. Our data is meaningful in that it addresses the long-standing question in the field of whether relative hypoxia has a direct effect on β -catenin activity in hepatocytes to regulate liver zonation.