Loss-of-function mutation of *KIF3B* can cause a defective biliary development in Biliary Atresia: evidence from iPSC-derived biliary organoid

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Biliary Atresia (BA) is a poorly understood devastating fibro-obliterative biliary disease of newborns. Limited access to primary biliary tissue, difficulties in culturing primary biliary cells (cholangiocytes) and inadequate animal disease model have led to a slow advancement in unravelling the patho-mechanisms, diagnosis and treatment for BA. Human iPSC-derived biliary organoids provide us an unprecedented cellular model to study BA.

We have conducted whole exome sequencing on 85 BA trios, identified deleterious loss of function (LOF) mutations in cilia-related genes including *KIF3B* in 31.5% non-syndromic BA patients. *KIF3B* encodes Kinesin-like protein KIF3B that is a subunit of the anterograde intraflagellar transport (IFT) motor protein kinesin-II in cholangiocyte cilia. Functional analyses demonstrated absence of cilia in the BA livers with *KIF3B* mutation and knockdown of *KIF3B* in human fibroblasts resulted in reduced number of cilia. Additionally, CRISPR/Cas9-engineered zebrafish knockouts of *KIF3B* displayed reduced biliary flow. In this study, we generated *KIF3B+/-* & *KIF3B-/-* human iPSC cells and differentiated them into biliary organoids to investigate the impacts of the *KIF3B* LOF mutation in biliary development in BA.

Single-cell-RNA-seq analysis and immuno-staining showed that *KIF3B+/-* and *KIF3B-/-* iPSCs are less capable in the differentiation of hepatoblast and cholangiocyte progenitors (CPs). Individual cell AUC revealed down-regulation of Wnt, Notch and TGF-beta pathway activity, while cell-cell interaction analysis showed a defective cell-cell interaction mediated by TGAV and ITGB8 (integrin αvβ8) in the *KIF3B+/-* and *KIF3B-/-* CPs. Furthermore, *KIF3B+/-* & *KIF3B-/-* biliary organoids were few, tiny and with abnormal or no cilia. Bulk-RNA-seq and immunostaining analysis of biliary organoids revealed a shift from cholangiocyte to hepatocyte differentiation in *KIF3B+/-* & *KIF3B-/-* biliary organoids. Taken together, our data indicate that *KIF3B* plays a key role in

cholangiocyte differentiation, which demonstrates that the human iPSC-derived biliary organoid is a valuable disease model for patho-mechanistic study of BA.