HUMAN BILIARY ORGANOID MODEL TO EVALUATE ADD3 GENE PREDISPOSITION IN BILIARY ATRESIA

Ha Jerry Long Hei[1], Liu Leo Hailong[1], Lui Vincent Chi Hang[1,2], Tam Paul Kwong Hang[1,3]

[1]Department of Surgery, School of Clinical Medicine, the University of Hong Kong, Hong Kong.

[2]Dr. Li Dak-Sum Research Centre, the University of Hong Kong, Hong Kong.

[3]Faculty of Medicine, Macau University of Science and Technology, Macau SAR, China.

Abstract: Biliary atresia (BA) is a neonatal fibroinflammatory disease, resulting in the obstruction of biliary trees. To date, one of the putative risk factors – ADD3 gene was discovered in genome-wide association studies although the mechanisms of ADD3 have not yet been delineated in human specimens. In this study, we aimed to unearth the roles of ADD3 in biliary development, as well as the underlying pathogenesis in BA. We combined the state-of-the-art techniques of human induced-pluripotent stem cell (hPSC) and CRISPR-Cas9 to generate control, $ADD3^{+/-}$ and $ADD3^{-/-}$ stem cells. Organoids were derived in a stepwise manner to recapitulate key biliary development, which started with the differentiation of hPSCs into endoderms, foregut progenitors, hepatoblasts, cholangiocyte progenitors and eventually mature cholangiocytes. Knock out of ADD3 perturbed biliary development, given that markers of hepatoblasts (alpha fetoprotein and SOX9) and those of cholangiocyte progenitors (cytokeratin19 and SOX9) were downregulated. $ADD3^{+/-}$ and $ADD3^{-/-}$ biliary organoids also demonstrated aberrant morphology and retarded growth, which expressed fewer differentiation markers (hepatocyte nuclear factor 1 homeobox B [HNF1B], cytokeratin 19, SOX9, and γ glutamyl transferase [GGT]) and proliferation marker (Ki67). Interestingly, loss of ADD3 disrupted apical – basal organization through impairing the localizations of F-actin, tight junction protein zonula occludens-1 (ZO-1), claudin, radixin, β catenin and cystic fibrosis transmembrane conductance regulator (CFTR), which indicated the disruption of cell polarity. Last but not least, loss-of-function mutation of ADD3 gene can inactivate Notch, Wnt and YAP/TAZ pathways which governed biliary development. Together, these results suggest that downregulation of ADD3 hinders biliary development and disrupts apical–basal organization, recapitulating the phenotype of BA patient liver organoids.

Keywords: hPSC – derived biliary organoids, ADD3, Biliary Atresia