Generation of Cholangiocyte Organoids Using Human Primary and Immortalized Intrahepatic Cholangiocytes

Emily H Chestnut[1], Minh-Uyen T. Le[1], Barry Wei[1], Ping Li[1], Burcin Ekser[1*], Wenjun Zhang[1*]

[1]Transplant Surgery, Department of Surgery, Indiana University School of Medicine. (*) shared senior authors.

Background: Developing cellular models for studying cholangiopathies has been challenging. Cholangiocyte (CHOL) organoids offer promise in recapitulating structure and function of *in vivo* biliary tree. To enhance expansion, isolated CHOLs were immortalized using lentiviral vectors expressing human papillomavirus (HPV) E6/E7. It remains unclear whether immortalization alters other cellular properties. This study aims to determine whether immortalized CHOLs can self-organize into bile duct-like structures ex vivo, providing an avenue for modeling cholangiopathies and biliary tree development.

Methods: Human cholangiocytes were isolated from explanted liver tissue (Figure 1A). 5,000 primary or immortalized CHOLs were seeded into a 24-well plate at 50ml/well in a mixture of Matrigel and supplemented media (Figure 1B). At 10 days of growth, diameter and number of organoids from each condition were quantified. Expression of the key markers for CHOLs such as CK19, EpCAM, and others were measured by qPCR. Rhodamine123 transport assay was used to assess functionality of CHOL organoids.

Results: Fluorescent microscopy confirmed that cholangiocyte organoids generated with immortalized CHOLs express cholangiocyte-specific cell markers CK19 and CK7 and maintain p-glycoprotein function (Figure 1D). However, organoids derived from immortalized CHOLs showed a significant reduction in diameter (12.68 ± 2.4 vs 20.61 ± 2.8, $p<0.01$) and number per well (17.3 ± 4.9 vs 27.0 ± 3.2, $p<0.02$) compared to organoids derived from primary CHOL. qPCR analysis revealed elevated expression of progenitor cell marker EpCAM and reduced expression of mature CHOL marker CK19 in organoids derived from immortalized cell, suggesting immortalization impaired differentiation of CHOLs (Figure 1C).

Conclusion: Although establishing immortalized CHOL cell lines from human livers provides cellular models for studying cholangiopathies, caution should be exercised when using them for studies requiring functional, mature CHOL behavior/3D organization. Future work should focus on developing alternative immortalization methods that better preserve the functionality and differentiation of primary cholangiocytes in 3D culture.

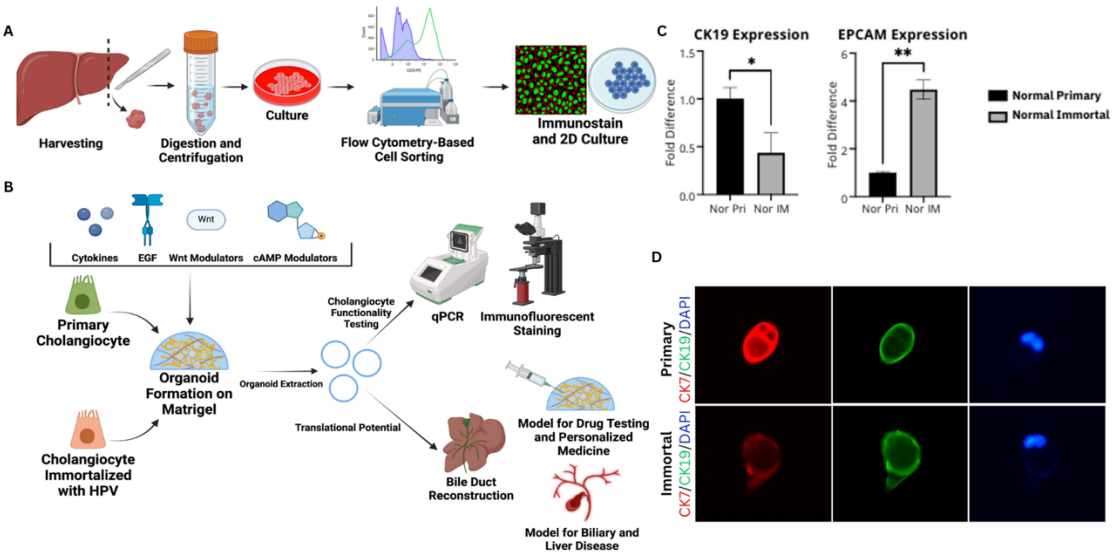

Figure 1A.Schematic illustration of human liver tissue procurement and cell dissociation.
Figure 1B.Illustration of cholangiocyte organoid generation and maintenance, and functional tests performed.
Figure 1C.qPCR results for EpCAM and cholangiocyte marker CK19 expression.
Figure 1D.Immunofluorescent staining for cholangiocyte markers CK19 and CK7 in immortalized and primary cell cholangiocyte organoids.