Directed Differentiation of Functional Cholangiocytes from Human Mesenchymal Stem Cells

Wang, H.[1], Goh, S.[2], Yang, H.[2], Brooks, A.[2], Liang, X.[2], Doran, M.[3] and Roberts, M.[2]

[1] The University of Queensland Diamantina Institute, Australia, [2] The University of Queensland Diamantina Institute, Australia, [3] Queensland University of Technology, Australia

Cholangiocytes are the main site of dysfunction in bile duct disorders, including a diverse group of inherited diseases such as cystic fibrosis liver disease, developmental conditions such as Alagille syndrome, autoimmune diseases such as primary sclerosing cholangitis and primary biliary cirrhosis as well as drug- and toxin-induced conditions [1]. The development of effective pharmacological agents for bile duct disorders has been hampered by poor access to primary biliary tissue, difficulties in culturing primary cholangiocytes *in vitro* and inadequate disease models. Thus, there is a pressing need for technologies to generate functional cholangiocyte-like cells (CLCs) from other sources, such as stem cells, to provide an *in vitro* model for pharmacological studies of bile duct disorders and a foundation for developing tissue replacement strategies.

The mesenchymal stem/stromal cells (MSCs) are self-renewing cells of mesodermal origin that have the potential to differentiate into a variety of cell types [2]. These cells are excellent candidates for pharmaceutical studies and regenerative medicine. Despite recent advances toward guided differentiation of MSCs to hepatocytes [3], to the best of our knowledge, no study has been reported to differentiate MSCs to functional cholangiocytes, which are another principal type of cells in the liver and the main site of dysfunction in bile duct disorders.

In this study, we developed a new approach, which drives differentiation of MSCs toward the cholangiocytic lineage using small molecule-based feeder-free and defined culture conditions (**Fig. 1A**). This 27-day protocol started with the differentiation of human MSCs into endoderm, and followed by the generation of CLCs expressing biliary markers using small molecules such as epidermal growth factor, fibroblast growth factor, IL-6, and sodium taurocholate hydrate. The CLCs expressed biliary markers at levels similar to those in human cholangiocytes (**Fig. 1B**). Then we used a 3D culture system to assess the potential of CLCs to form organoids with cyst and duct-like structures. After 3 days of culture in Matrigel, CLCs displayed either a tubular, a cyst morphology (**Fig. 1A**) or a mixture of both (**Fig. 1C**), while MSCs cultured in 3D did not form these structures. To assess the functionality of our CLCs generated *in vitro*, we first used rhodamine 123, a fluorescent substrate for the cholangiocyte surface glycoprotein multidrug resistance protein-1 (MDR1). We found that rhodamine 123 was actively secreted in the lumen of CLC organoids, and luminal dye accumulation was prevented by the MDR1 inhibitor verapamil (**Fig. 1C**), confirming MDR1-dependent transfer of rhodamine 123. Then we analysed their response to adenosine triphosphate (ATP), known to induce a Ca^{2+} increase in biliary cells by way of P2Y1 receptors. There is a moderate differentiation efficiency with 46 % ± 6 % of responsive CLCs to ATP inducing an intracellular Ca^{2+} increase. In conclusion, we have developed a robust and efficient method for differentiating MSCs into CLCs, which display structural and functional similarities to normal human cholangiocytes. The capacity of our platform for generating large amounts of functional cholangiocytes will have broad applications for in vitro disease modelling, for screening pharmacological agents and as cell therapeutics.

This work was supported by grants from National Health and Medical Research Council (APP1126091 and APP1055176).

References

[1] K.N. Lazaridis, M. Strazzabosco, N.F. Larusso, The cholangiopathies: disorders of biliary epithelia, Gastroenterology 127(5) (2004) 1565-77.

[2] A. Brooks, K. Futrega, X. Liang, X. Hu, X. Liu, D.H.G. Crawford, M.R. Doran, M.S. Roberts, H. Wang, Concise Review: Quantitative Detection and Modeling the In Vivo Kinetics of Therapeutic Mesenchymal Stem/Stromal Cells, Stem Cells Transl Med 7(1) (2018) 78-86.

[3] K.D. Lee, T.K. Kuo, J. Whang-Peng, Y.F. Chung, C.T. Lin, S.H. Chou, J.R. Chen, Y.P. Chen, O.K. Lee, In vitro hepatic differentiation of human mesenchymal stem cells, Hepatology 40(6) (2004) 1275-84.

Figure

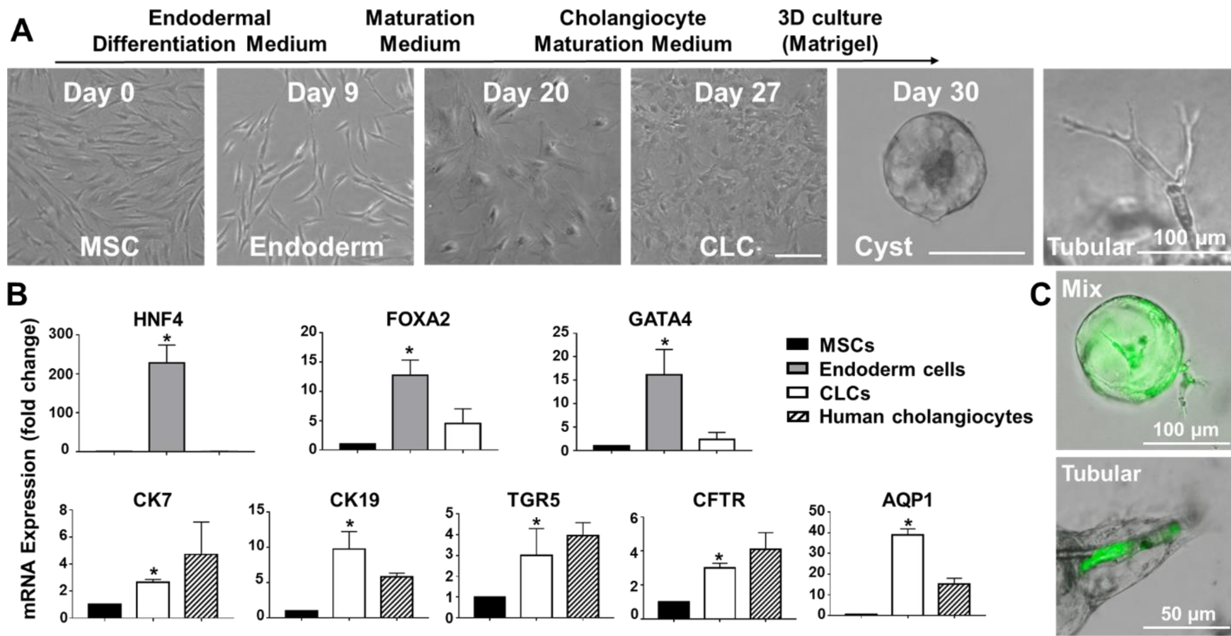

Figure 1 Generation of functional CLCs from human MSCs. (**A**) Overview of the protocols and the sequential morphological changes. (**B**) Real-time PCR analysis show the high expression of endoderm markers (HNF4, FOXA2 and GATA4) at day 6 and biliary markers (CK7, CK19, TGR5, CFTR and AQP1) at day 27 of MSC-derived cells. (**C**) Representative images demonstrating the MDR1 fluorescent substrate rhodamine 123 detected in the lumen of CLC organoids, confirming MDR1 functionality.