論文の内容の要旨

Characterization of peribiliary gland-constituting cells based on expression of Trop2 in mouse biliary tract
（Trop2 の発現を指標としたマウス胆管周囲付属腺細胞の性状解析）

松井 理司

The bile duct, a tubular epithelial tissue, plays an important role in the drainage of bile from the liver into the small intestine. Based on histology and embryology, the bile duct is classified into the intrahepatic bile duct (IHBD) and the extrahepatic bile duct (EHBD). While IHBD forms an intricate tree-like network in the liver parenchyma, EHBD forms luminal structure that links IHBD to the duodenum. EHBD has many accessory glands, namely “peribiliary glands (PBGs)”. PBG is composed of heterogeneous cell populations such as mucus and pancreatic enzyme-producing epithelial cells, while it is known to constitute niches for multi-potential stem/progenitor cells, called “biliary tree stem/progenitor cells (BTSCs)”, in human EHBD. BTSC shows a similar gene expression profile to liver stem/progenitor cell (LPC) and pancreatic progenitor cell, having a potential for differentiating into hepatocytes, mature biliary epithelial cells (BECs) and pancreatic islets. However, there is no applicable method to isolate PBG-constituting cells from the EHBD.

Therefore, the role and nature of PBGs in the mouse EHBD remains unclear. The objective of this study is to establish the method for isolating and characterizing PBG-constituting cells in the mouse EHBD.

In the present study, I found that trophoblast cell surface protein 2 (Trop2) was expressed in the luminal epithelium of mouse EHBD exclusively, but not in the PBG. Based on the differential expression profile of Trop2, the lumen-forming biliary epithelial cells (LBECs) and PBG-constituting biliary epithelial cells (PBECs) were isolated for further characterization by gene expression analysis, immunostaining, and assays of colony and organoid formation.

Gene expression profiling revealed that the isolated mouse PBECs expressed several genes characteristic of human PBGs, fetal pancreatic progenitor and intestinal tuft cells. In the colony formation assay, PBECs showed significantly higher colony formation capacity than LBECs. The expanded PBECs showed up-regulation of Trop2 expression and down-regulation of human PBG-related genes in the 2D culture condition. In the 3D organoid formation assay, PBECs gave rise to a cysts structure with epithelial polarity, showing the gene expression patterns similar to LBECs.

Finally, I examined the expression pattern of Trop2 during EHBD regeneration after bile duct ligation (BDL), a severe cholestasis model. After BDL, the luminal epithelium was severely injured and damaged LBECs were peeled off from the lumen. On the other hand, PBECs proliferated and re-expressed Trop2 in PBGs upon EHBD injury. Next, I compared the colony formation capacity between $Trop2^{+}$ and $Trop2^{-}$ BECs after BDL and showed that the colony formation capacity of $Trop2^{+}$ BECs was dramatically increased after EHBD injury.

Taking these *in vitro* and *in vivo* data together, PBGs contain progenitor-like cells with high capacity for proliferation, which supply new LBECs during biliary regeneration. Thus, Trop2 is a useful marker to investigate the pathophysiological roles and characteristics of PBGs in biliary diseases.

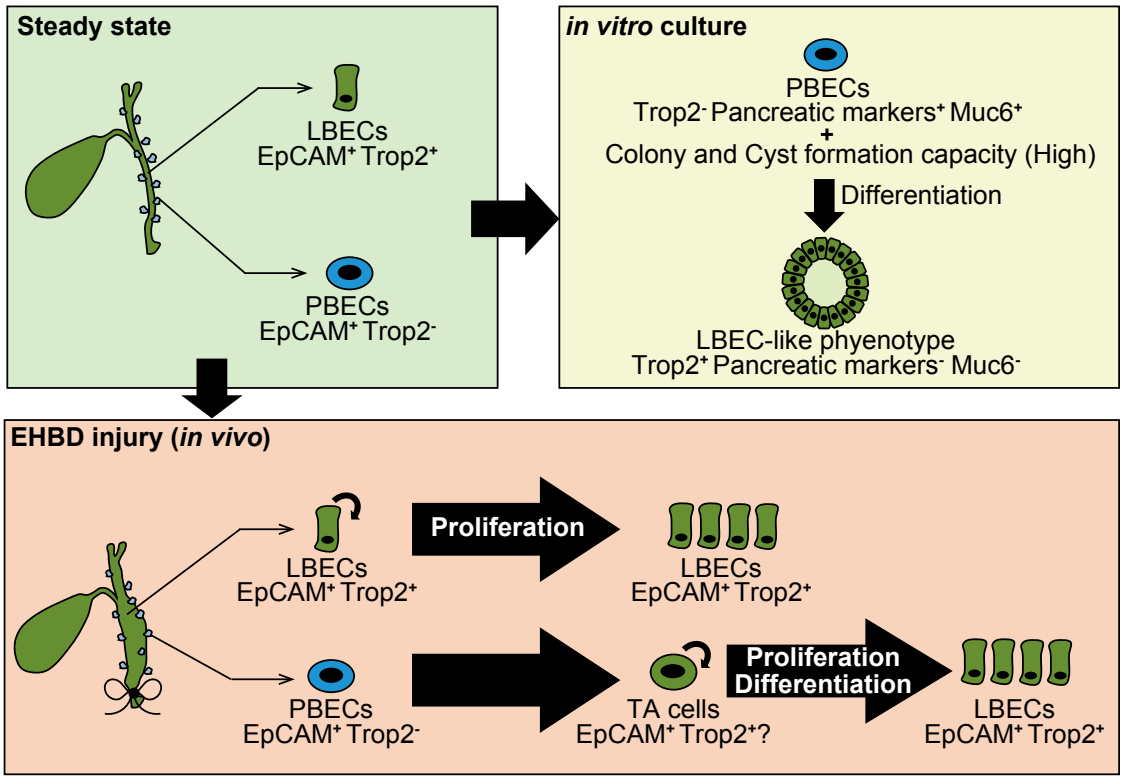

Figure 1. Schematic summary of the present study.

(Upper left panel) The characteristics of the LBECs and PBECs at steady state. The differential expression profiles of Trop2 in EHBD makes it possible to discriminate between LBECs and PBECs by FACS. (Upper right panel) The isolated PBECs show higher colony formation capacity than LBECs *in vitro*. The expanded PBECs have a potential for forming cysts composed of LBEC-like Trop2^{+} cells with luminal epithelial polarity in the 3D organoid culture. (Lower panel) Model of biliary regeneration by PBECs after injury. By BDL, Trop2 expression is rapidly induced in PBGs *in vivo* and PBECs proliferated, giving rise to transit-amplifying (TA) cells for the luminal epithelium regeneration.