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LIVER AND PANCREAS DEVELOPMENT

The group studies the molecular and cellular mechanisms that govern differentiation and morphogenesis of the liver and pancreas, two organs which derive from the endoderm (primitive gut of the embryo). The fundamental knowledge gained by this work is essential for understanding the pathophysiology of organ malformations, such as polycystic diseases, and for improving cell therapy of liver and pancreatic diseases, such as hepatic deficiencies (e.g. metabolic deficiencies and cirrhosis) and diabetes.

LIVER DEVELOPMENT

A. Antoniou, R. Carpentier, I. Laudadio, S. Margagliotti, P. Raynaud

The main cell types of the liver are the hepatocytes, which exert the metabolic functions of the organ, and the biliary cells which delineate the bile ducts. We study how the hepatocytes and biliary cells differentiate and how bile ducts are formed in the embryo. Our preferred model organism to investigate liver develop-

ment is the mouse, and this includes generation and analysis of transgenic mouse lines.

The biliary tract consists of intrahepatic bile ducts which collect bile produced by the hepatocytes, and of extrahepatic ducts which drain bile from the liver to the intestine. Biliary cells, also called cholangiocytes, delineate the lumen of the bile ducts and modify the composition of bile. These cells, like hepatocytes, derive from liver progenitor cells called hepatoblasts. Our discovery of the Onecut transcription factors Onecut-1 (OC-1/HNF-6), OC-2 and OC-

3, and the subsequent phenotypic characterization of HNF-6 and OC-2 knockout mice led to the identification of the first transcriptional network regulating bile duct development [1, 2]. Current efforts are devoted to the characterization of the transcription factors and signal transduction pathways that control bile duct development.

We have recently identified the transcription factor Sox9 as the earliest and most specific biliary cell marker in liver development. Using Sox9 in combination with hepatoblast markers, we analyzed the morphogenesis of the bile ducts and found that it occurs according to a new mode of tubulogenesis [3, 4]. Biliary tubulogenesis starts with formation of asymmetrical ductal structures, lined on one side (adjacent to the portal vein) by cholangiocytes and on the other side (adjacent to the liver parenchyma) by hepatoblasts. When the ducts grow from the hilum to the periphery of the liver, the hepatoblasts lining the asymmetrical structures differentiate to cholangiocytes, thereby allowing formation of symmetrical ducts lined only by cholangiocytes. This mode of tubulogenesis is unique as it is to our knowledge the only one characterized by transient asymmetry (Figure 1). We are currently investigating

how this new knowledge impacts on the interpretation of congenital malformations of the bile ducts.

The transcription factor network that drives cholangiocyte morphogenesis and bile duct formation has been further investigated. By means of a liver-specific gene inactivation strategy we found that Sox9 controls the timing of bile duct development. Within the biliary transcriptional network Sox9 is located downstream of HNF-6 and upstream of C/EBP-alpha, two factors whose dysfunction is associated with biliary cyst development. In addition, the function of Sox9 was found to be tightly linked with that of the Notch signaling pathway [5]. The latter is deficient in liver of patients affected with Alagille syndrome, a disease characterized by bile duct paucity and severe cholestasis. We pursue this research by evaluating the role of other members of the Sox family.

Our work also addresses the mechanisms of hepatocyte differentiation. We found that the Onecut factors HNF-6 and OC-2 are required for liver expansion at the onset of liver development [6]. They are also critical for normal differentiation of hepatic precursor cells to hepatocytes or cholangiocytes [2], and their

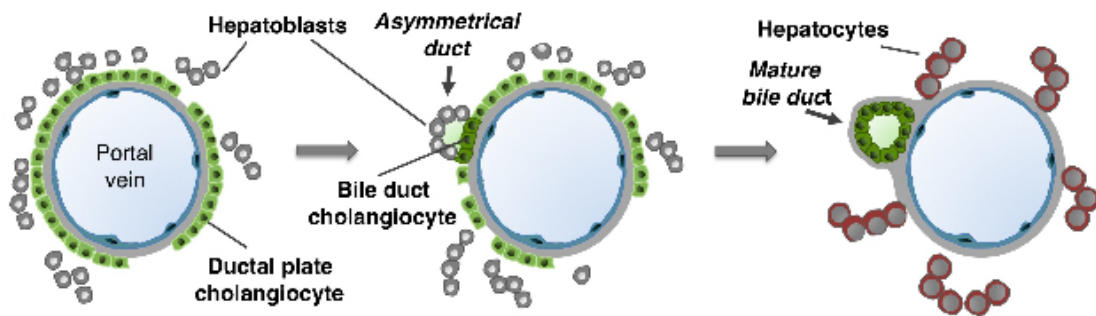


Fig. 1. Development of bile ducts. Left panel: In embryos, hepatoblasts (grey) differentiate to ductal plate cholangiocytes (green) which form a ring of cells around the branches of the portal vein (blue). Middle panel: hepatoblasts become apposed to the ductal plate cells and delineate a developing duct. The latter is asymmetrical as it is lined on the portal side by cholangiocytes (dark green) and on the parenchymal side by hepatoblasts. Right panel: when the ducts have matured, they are entirely lined by cholangiocytes, while parenchymal hepatoblasts have differentiated to hepatocytes (brown) which line up as cords (from Si-Tayeb K, Lemaigre FP, Duncan SA. Organogenesis and development of the liver. *Dev. Cell*, 18, 175-189, 2010).

level of expression during hepatocyte differentiation determines time-specific gene activation in the liver [7]. Current work focusses on the molecular mechanisms by which HNF-6 and OC-2 fine-tune gene expression at several stages of hepatocyte differentiation. This involves work on the function of microRNAs that repress or are transcriptionally regulated by HNF-6 and OC-2 [8].

PANCREAS DEVELOPMENT

M. Colletti, A. Grimont, E. Heinen, P.-P. Prévot, A. Simion

In the embryo, the pancreas develops as an outgrowth of the endoderm which is the cell layer that delineates the primitive gut. Pancreatic progenitors derived from the endoderm form two buds (dorsal and ventral) which later fuse to form a single organ. Within these buds the progenitor cells give rise, through a stepwise process, to endocrine, acinar and duct cells. Our group investigates the molecular mechanisms that control development of the various pancreatic cell types.

The Onecut transcription factor HNF-6 is required for endocrine cell development [9]. In HNF-6 knockout mice, endocrine cells fail to develop in the embryo and this results from the lack of HNF6 in pancreatic progenitor cells. In the latter, HNF-6 is required to activate the gene coding for Ngn3, a pro-endocrine transcription factor. We have now looked for microRNAs that are downstream of HNF-6 in pancreatic progenitor cells. We identified several whose expression is stimulated by HNF-6, and we are in the process of characterizing their function. We also found that miR-495 and miR-218 repress translation of HNF-6 and OC-2, respectively, and we characterized the microRNA population that is expressed at the earliest stage of pancreas development [8].

Interestingly, in mice deficient in HNF-6, the endocrine cells and islets of Langerhans

have not developed at birth. However, five weeks later, islets of Langerhans have formed. We investigated the source of these neogenic endocrine cells and obtained evidence, including from genetic lineage tracing studies, that Hnf6^{-/-} duct cells can give rise to the endocrine cells (Figure 2). This suggests that in normal conditions, HNF-6 maintains the identity of duct cells, but that its absence in these cells favours their transdifferentiation towards endocrine cells. We are currently investigating how HNF-6 maintains pancreatic duct cell identity.

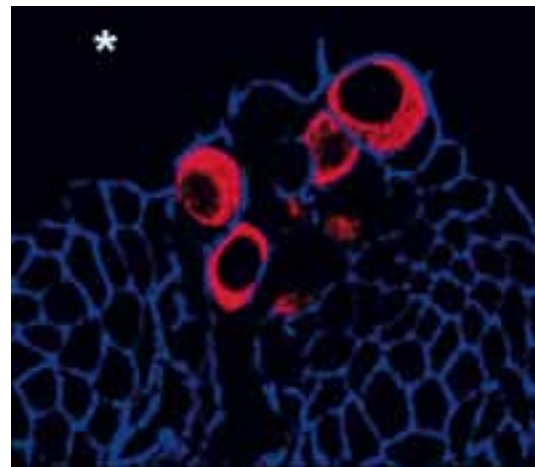


Fig. 2. Endocrine cells differentiate from duct cells in postnatal Hnf6^{-/-} pancreas. Endocrine cells expressing insulin (red) become detectable in the epithelium (e-cadherin staining, blue) lining the lumen (star) of the pancreatic ducts.

CONCLUSIONS

Our work on the signaling pathways and transcription factors in developing liver and pancreas opens perspectives for understanding the pathophysiology of liver and pancreatic congenital diseases. The application of our findings to the programmed differentiation of cultured stem cells should help developing cell therapy of hepatic deficiencies and of pancreatic diseases such as diabetes.

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