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## LIVER AND PANCREAS DEVELOPMENT

*The group studies the molecular and cellular mechanisms that govern development of the liver and pancreas, two organs which play essential metabolic roles and which derive from the endoderm (primitive gut of the embryo). The fundamental knowledge gained by this work is essential for improving cell therapy of liver and pancreas diseases (metabolic disease, acute hepatitis, cirrhosis, diabetes), and for understanding the pathophysiology of organ malformations (e.g. polycystic liver diseases). Identifying developmental mechanisms also impacts on understanding abnormal differentiation of liver and pancreatic cancer cells..*

### LIVER DEVELOPMENT

*A. Antonion, J.-B. Beaudry, R. Carpentier, I. Laudadio, A. Poncy, 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 development is the mouse, and this includes genera-

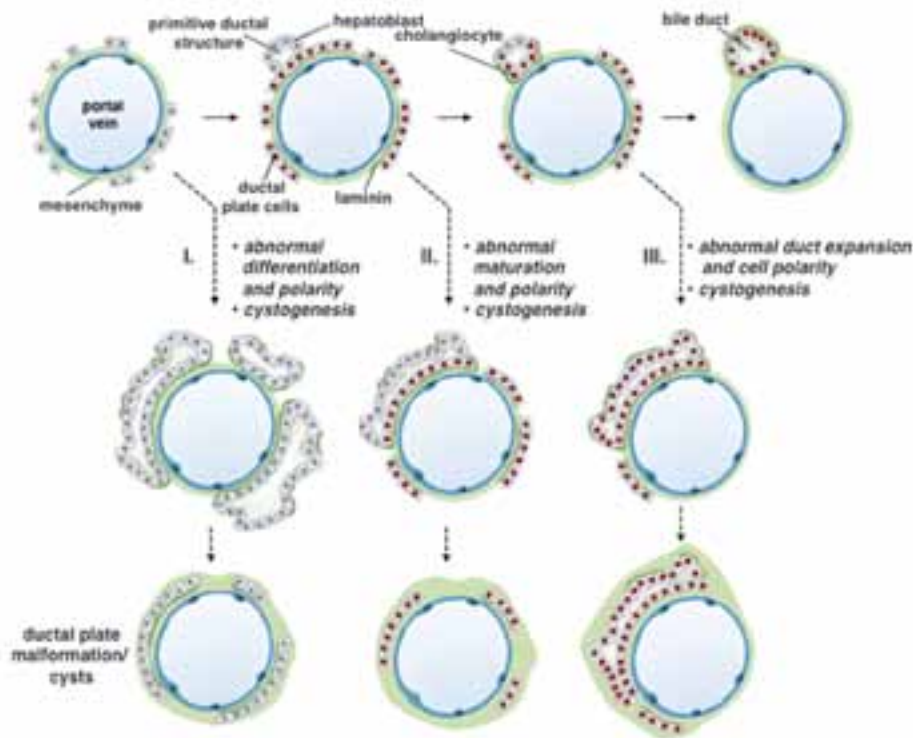
tion and analysis of transgenic mouse lines. In collaboration, we also use the Zebrafish as a model organism.

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 fac-

tors 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 in health and disease.

We have recently identified novel molecular markers that enabled us to revisit the morphogenesis of the intrahepatic bile ducts. We found that biliary morphogenesis 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 chol-

angiocytes 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 further investigated how this new knowledge impacts on the interpretation of congenital malformations of the bile ducts. To this end we studied several mouse models and samples from human liver fetuses. This work allowed us to propose a new pathogenic classification of biliary malformations (Figure 1) [5].



**Fig. 1. Classification of biliary malformations based on distinct pathogenic mechanisms.** Normal bile duct morphogenesis is illustrated at the top. Malformations can arise by three mechanisms: (I) differentiation of hepatoblasts to ductal plate cells is abnormal and associated with perturbed polarization and cyst formation; (II) asymmetrical ducts are formed but fail to mature to bile ducts; this is associated with formation of cysts and abnormal polarity; (III) differentiation of ductal plate cells and maturation of asymmetrical ducts proceeds but duct expansion is perturbed; this is associated with defects in cell polarity.

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. 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. HNF-6 and OC-2 are critical for normal differentiation of hepatic precursor cells to hepatocytes or cholangiocytes : in the absence of HNF6 and OC2, the precursor cells generate hybrid hepato-biliary cells instead of distinct hepatocyte and biliary cell populations [2]. Moreover, we found that the hepatic concentration of HNF6 rises during liver development. Specific levels of HNF6 determine recruitment of co-activators at specific stages of development, thereby inducing time-specific expression of HNF6-target genes [6]. Current research focuses on the molecular mechanisms by which HNF-6 and OC-2 fine-tune gene expression at several stages of hepatocyte differentiation.

Since fine-tuning of gene expression is in part exerted by microRNAs (miRNA), we addressed the function of the hepatocyte-specific miR-122 in development. In collaboration with the Katholieke Universiteit Leuven, we found that the expression of enzymes synthesizing ketone bodies rises during development, while that of an enzyme catabolising ketone bodies (2-oxoacid CoA transferase) decreases. We showed that the decrease in 2-oxoacid CoA transferase is in part due to repression mediated by miR-122. Therefore, miR-122 promotes hepatocyte maturation, in part by regulating

ketone body metabolism [7].

## 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, 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.

In collaboration with the CELL research group, we investigated the role of blood vessels in pancreas development, and uncovered the existence of a molecular cross-talk between the blood vessels and the pancreatic epithelium which determines acinar differentiation of epithelial cells. This is illustrated in the report of the CELL research group.

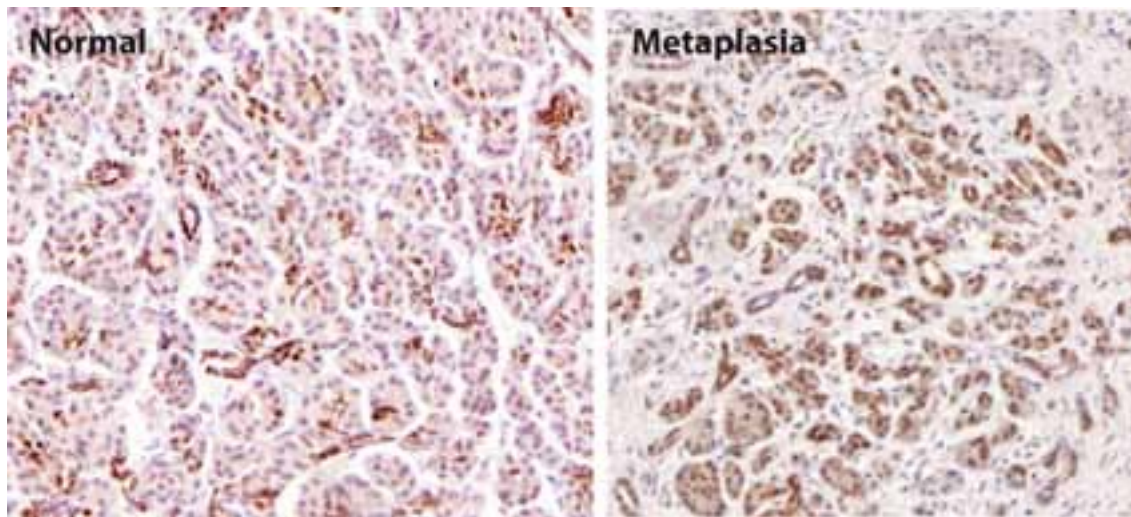
The role of the Onecut transcription factor HNF-6 in pancreas development is being studied since several years. We showed that HNF6 is required for development of endocrine cells and pancreatic ducts [8,9]. When further addressing how HNF6 is controlled and how it exerts its effects, we identified two miRNAs that control HNF6 expression [10], and eighteen miRNAs which are controlled by HNF6. Current research is focussed on the determination of the function of those miRNAs that are downstream of HNF6.

After birth, HNF6 is expressed in the pancreas exclusively in the duct cells where it most likely maintains duct cell identity. Interestingly, there is evidence from other laboratories that pancreatic ductal adenocarcinoma may derive from acinar cells which switch their phenotype from acinar to ductal during progression

to cancer. This process is called acinar-to-ductal metaplasia, and constitutes a preneoplastic state. We hypothesized that the switch in cell identity may depend on the ectopic expression of ductal transcription factors and tested if HNF6 is ectopically induced in acinar cells undergoing metaplasia. This was the case in human pancreas (Figure 2). In addition, we recently collected evidence from mouse models that induction of HNF6 in acinar cells promotes acinar-to-ductal metaplasia, suggesting that HNF6 is a key inducer of preneoplastic lesions.

## CONCLUSIONS

Our findings on the role of transcription factors that regulate liver and pancreas development contribute to a better understanding of the diseases affecting these organs. In liver, our work opens perspectives for understanding the pathophysiology of congenital diseases of the liver. Applying our findings to the programmed differentiation of cultured stem cells should also help in developing cell therapy of hepatic deficiencies. In pancreas, our observations on the expression and function of HNF6 in preneoplastic lesions are expected to improve diagnosis and to help preventing progression towards pancreatic ductal adenocarcinoma.



**Fig. 2. Sections from human pancreas immunostained to detect HNF6.** In normal pancreas, HNF6 is detected in the ducts, whereas in pancreas with acinar-to-ductal metaplasia, HNF6 is detected in ducts as well as in acinar cells acquiring a duct-like morphology.

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