Review

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Development of the liver: Insights into organ and tissue morphogenesis

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Summary

Recent development of improved tools and methods to analyse tissues at the three-dimensional level has expanded our capacity to investigate morphogenesis of foetal liver. Here, we review the key morphogenetic steps during liver development, from the prehepatic endoderm stage to the postnatal period, and consider several model organisms while focussing on the mammalian liver. We first discuss how the liver buds out of the endoderm and gives rise to an asymmetric liver. We next outline the mechanisms driving liver and lobe growth, and review morphogenesis of the intra- and extrahepatic bile ducts; morphogenetic responses of the biliary tract to liver injury are discussed. Finally, we describe the mechanisms driving formation of the vasculature, namely venous and arterial vessels, as well as sinusoids.

Keywords: Liver; Development; Hepatocyte; Cholangiocyte; Sinusoidal endothelial cell; Bile duct; Organ asymmetry; Morphogenesis; Liver growth; Tissue architecture.

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Introduction

In the last two decades most efforts to decipher the mechanisms of liver development were focussed on identifying regulators of cell fate specification and differentiation. As a result, a number of developmental disease mechanisms are now better understood and hepatocyte-like cells can be produced *in vitro* by recapitulating developmental processes.[1,2] However, the way in which the three-dimensional tissular architecture of the liver develops in the embryo remains much less explored. Yet, gaining new knowledge in this area has become key to enabling *in vitro* production of stem cell-derived hepatic tissue, in which hepatocyte cords, bile ducts and vasculature are reliably connected. This review focusses on new insights into the morphogenesis of the embryonic liver.

Initiation of liver morphogenesis

Origin of liver progenitor cells

The liver is largely composed of hepatocytes and cholangiocytes, which differentiate from bipotent liver progenitors, the hepatoblasts. During development, hepatoblasts are specified in the ventral foregut endoderm by signals released from adjacent mesodermal tissues, including ligands of the Bone morphogenic protein (Bmp), Wnt and Fibroblast growth factor (Fgf) families, depending on the species.[3,4] At around embryonic day (E)8.5 in mice, hepatoblasts start expressing liver specific proteins, such as transcription factors Hhex and Prox1, followed soon after by α-fetoprotein (Afp), hepatocyte nuclear factor (Hnf)4α and albumin (Alb). Single-cell dye labelling has identified the origin of hepatoblasts within the foregut prior to the onset of fate specific gene expression.[5,6] Liver progenitors arise from bilateral populations of lateral endoderm, which merge in the process of gut tube formation at the ventral midline (Fig. 1). A second smaller progenitor population, positioned slightly more anteriorly in chicken and mice, was identified in the ventral midline endodermal lip (VMEL), which moves caudally and contributes to large parts of the liver and other organ fates.[5,7,8] The bilateral and VMEL progenitors initially respond to Fgf and Bmp respectively,[9] appearing to give rise to different parts of the liver. The latter was revealed when blocking early Fgf signalling elicited a differential survival response in the forming liver bud, with high apoptosis in the anterior bud, and low apoptosis posteriorly.[10] This indicates that the Fgf-responsive bilateral cells give rise to the posterior part of the liver bud, while the VMEL cells mostly contribute to the anterior population. It is unclear whether the two different progenitor sources correlate with distinct metabolic functions or spatial distribution within the adult liver and whether they exist in all vertebrates. Deleting *Sfrp5* in frogs revealed that repression of non-canonical Wnt signalling is essential for epithelial integrity of the foregut endoderm prior to liver induction.[11]

Organ bud morphogenesis

Newly specified hepatoblasts form the organ bud by undergoing a combination of morphogenetic processes, including cell shape changes, cell proliferation and migration. The first sign of liver bud morphogenesis is the thickening of the ventral foregut endoderm, which coincides with the start of hepatoblast gene expression and formation of the gut tube at around E8.5. Following inductive signalling from the adjacent mesoderm, early liver bud morphogenesis occurs via distinct steps: firstly, the cuboidal foregut epithelium changes into a thickened columnar epithelium of hepatoblasts, which subsequently transitions into a pseudostratified epithelium until it finally breaks down, at which point hepatoblasts delaminate and migrate into the adjacent mesenchyme. In

Key point

Three neighboring regions of the endoderm give rise to bipotent liver progenitors which eventually differentiate into hepatocytes and cholangiocytes.

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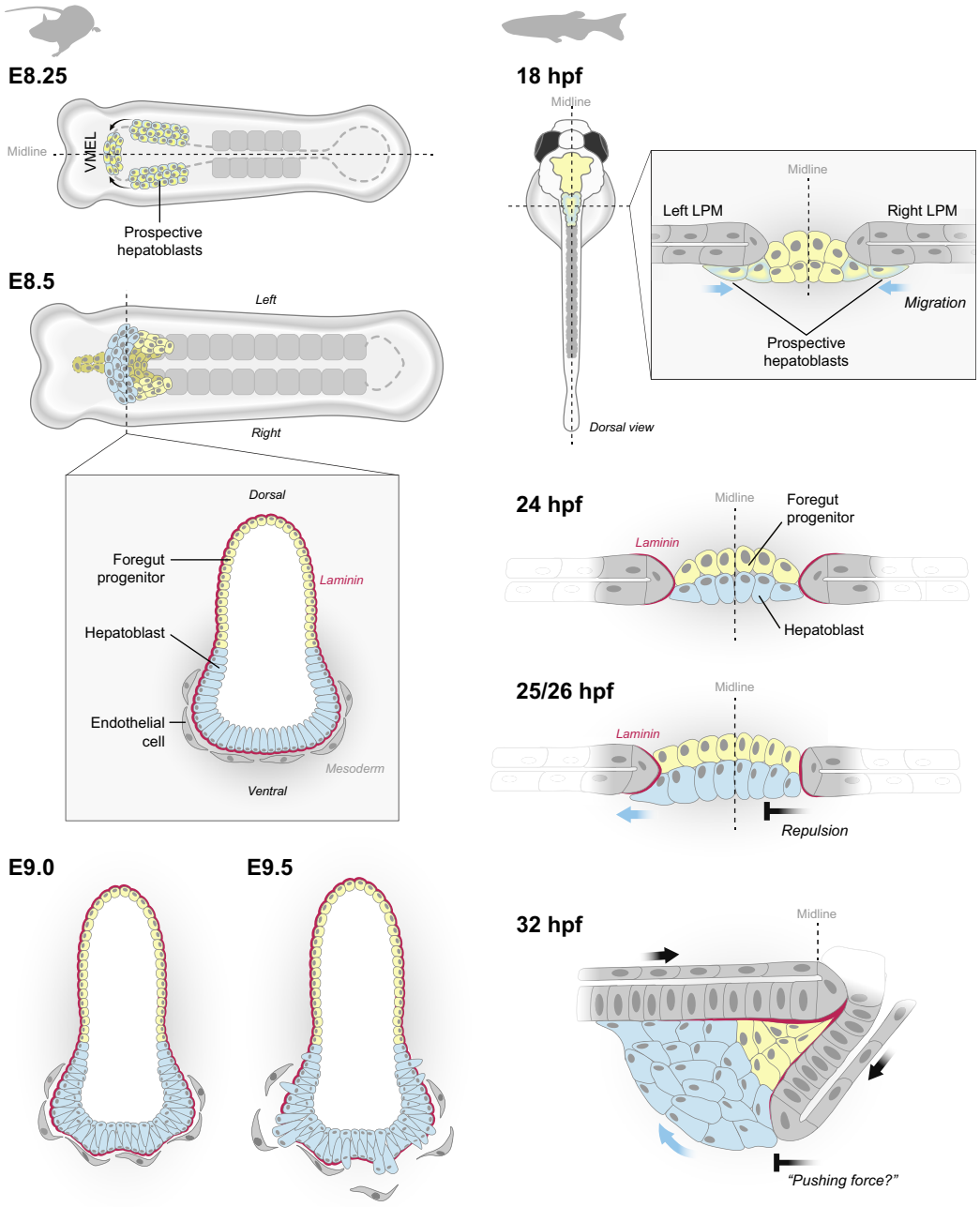

Fig. 1. Origin of hepatoblasts and early stages of liver budding. (A) Mouse and (B) zebrafish.

the pseudostratified state, hepatoblasts undergo internuclear migration, with nuclei entering S-phase in the basal region and mitosis when positioned apically.[12] The transcription factor Hhex is a critical regulator of the switch from columnar to pseudostratified epithelial morphology. *Hhex* mutants fail to form a thickened epithelium, while exhibiting impaired proliferation and a loss of hepatic gene expression by E10.5.[12] Hepatoblast migration is promoted by nascent endothelial cells, which are in close contact with the basement membrane, outlining the early liver bud before differentiating to form functional blood vessels.[13] Ablation of endothelial cells by inactivation of Vascular endothelial growth factor receptor 2 (*Vegfr2*) blocks liver outgrowth at E9.5 and causes cessation of hepatic gene expression. In contrast, in zebrafish, blood vessels are only essential for supporting later stages of liver growth.[14,15] The onset of liver outgrowth coincides with a loss of contacts between hepatoblasts resulting from downregulation of E-cadherin. This allows hepatoblasts to migrate into the surrounding mesenchyme. Concomitantly, matrix metalloproteinases (MMPs) breakdown the laminin, collagen IV and fibronectin containing basal membrane, facilitating hepatoblast migration.[16] This extracellular matrix (ECM) remodelling is controlled most prominently by mesenchymally expressed Mmp2 and hepatoblast-expressed Mmp14.[17,18]