Embryological and Anatomical Considerations in Biliary Atresia

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Nobuyoshi Shiojiri, Ryosuke Yoshii, and Yotsuba Arai

Abstract

The developmental origins of intrahepatic bile ducts, and extrahepatic bile ducts with the gallbladder are different in mammalian embryos: the former is derived from the cranial part, and the latter from the caudal part of the liver primordium. Secondary joining of both extrahepatic and intrahepatic bile ducts occurs in a stochastic fashion during liver development. Embryologically their impaired development, including the failure of their joining, might result in biliary atresia (BA). Although portal mesenchyme induces adjacent hepatoblasts to give rise to epithelial cells of intrahepatic bile ducts during development, several signaling molecules may be involved in this inductive event, including Jag1, Wnt5a, and TGFβ. Maturation of hepatic tissue may be coupled to normal bile duct development. The development of an in vitro culture system is required to demonstrate molecular mechanisms of normal bile duct formation, and also the cause of BA.

Keywords

Bile ducts · Biliary atresia · C/EBPα · Jag1 · Notch2 · Organ culture

5.1 Introduction

Biliary atresia (BA) is a neonatal disease of the liver, in which extrahepatic bile ducts are abnormally narrow, blocked, or absent. The disease leads to fibrosis and end-stage liver disease by 2 years of age. Although the BA may be classified into congenital or acquired types, it still remains to be demonstrated what is the cause of the BA. To better understand the mechanism of BA and develop its medical treatment, it should be noted how bile ducts are formed at a molecular level in mammalian embryos. Several papers have been published on mouse models of BA [1–4]. They may shed a light on the cause of BA. In this chapter, important events of murine bile duct development will be reviewed with considering the possibility of BA development. Mutant mice with anomalous bile duct development will be introduced.

5.2 Normal Biliary Development

Liver primordium develops as a diverticulum in the ventral foregut of the anterior intestinal portal region at 8.5–9.0 days of gestation (E8.5-9.0) in mouse embryos, which corresponds to 3 weeks of gestation in human embryos [5]. The primordium becomes of two portions at E9.5; the cranial and caudal portions (Fig. 5.1a–c). The cranial portion gives rise to the liver parenchyma with intrahepatic bile ducts whereas the caudal part develops into the gallbladder and extrahepatic bile ducts in later development. At the early phase of development, the liver is a hemopoietic organ, and contains many hemopoietic cells. Hepatoblasts are scattered among hemopoietic cells as an endodermal component, but do not form intrahepatic bile ducts. From E13.5 on, which corresponds to 5 weeks of gestation in human embryos, periportal hepatoblasts are induced to form ductal plates (intrahepatic bile duct progenitors) by portal mesenchyme (Fig. 5.1d). The development of ductal plates proceeds from the hilum to the periphery region. When connections between each ductal plate are examined, they are first discontinuous and become confluent with development (Fig. 5.2b). Connections of intrahepatic ductal plates with extrahepatic bile ducts are also not confluent at the early phase. Various configurations of bile ducts are found in the hilum even in the syngeneic mouse liver (C3H/HeSlc strain) (Fig. 5.2a). Connection of intrahepatic bile ducts with extrahepatic bile ducts, and that between each ductal plate may occur in a stochastic fashion [6]. At E16.5 to

N. Shiojiri (✉) · R. Yoshii · Y. Arai
Department of Biology, Faculty of Science, Shizuoka University, Shizuoka, Japan
e-mail: shiojiri.nobuyoshi@shizuoka.ac.jp

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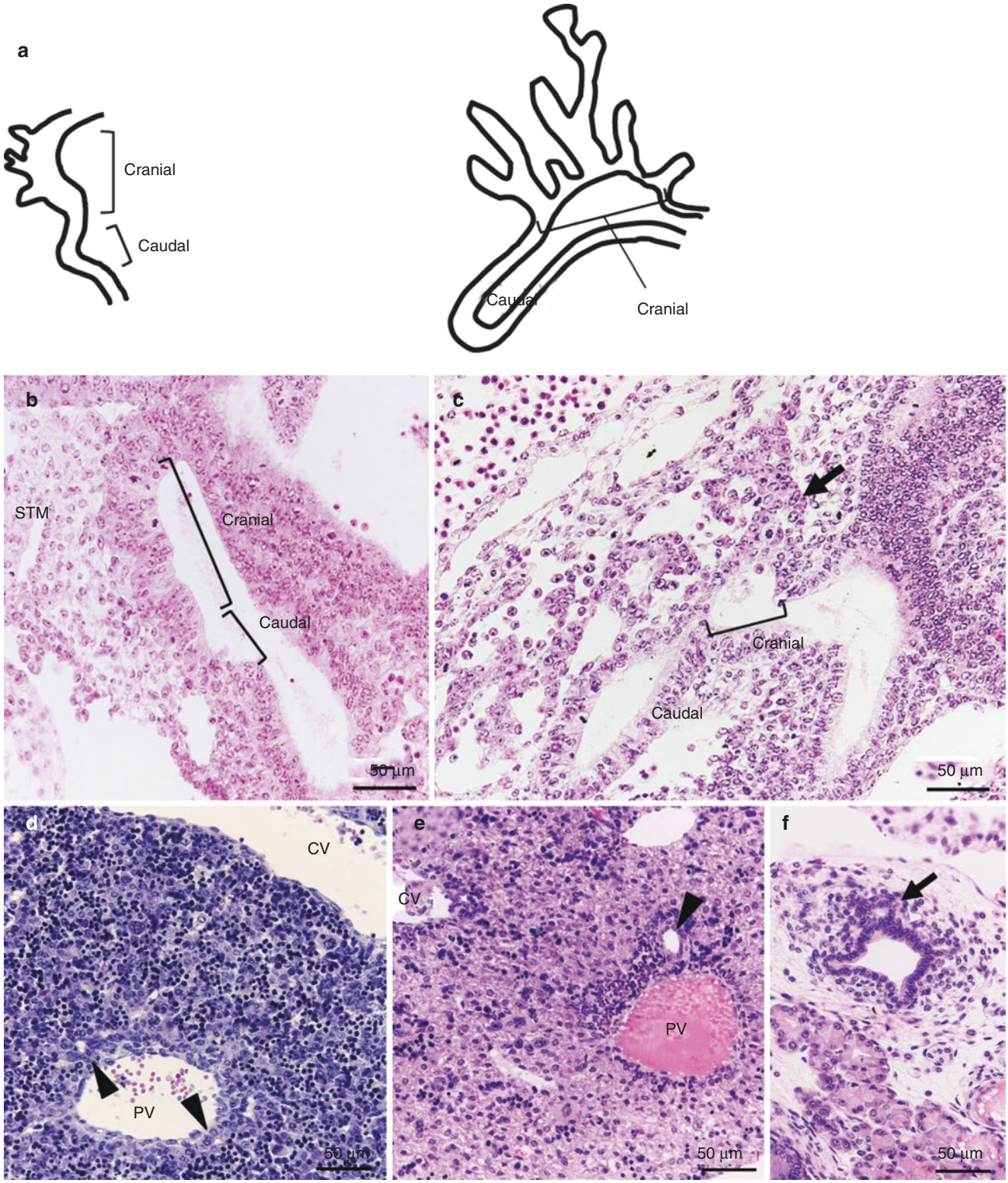

Fig. 5.1 Normal biliary development in mouse embryos. (**a**) Line drawing of liver primordium development. The liver primordium at E9.5 consists of cranial and caudal portions (left). The cranial portion extends hepatic cords of hepatoblasts into the septum transversum mesenchyme, and gives rise to the liver proper (right). Caudal portion develops into the gallbladder and extrahepatic bile ducts. (**b**) Liver primordium at E9.5. It consists of cranial and caudal portions. The cranial portion starts to extend hepatic cords into the septum transversum mesenchyme (STM). (**c**) Liver primordium at E10.5. It is well vascularized. Arrow indicates hepatic cords. (**d**) Ductal plates (arrowheads) around portal veins at E15.5. Hemopoietic cells with heavily blue nuclei are abundant. (**e**) Intrahepatic bile duct (arrowhead) in neonatal liver. (**f**) Extrahepatic bile duct having the biliary gland (arrow) at neonatal stage. b-f, hematoxylin-eosin staining. *CV* central vein, *PV* portal vein

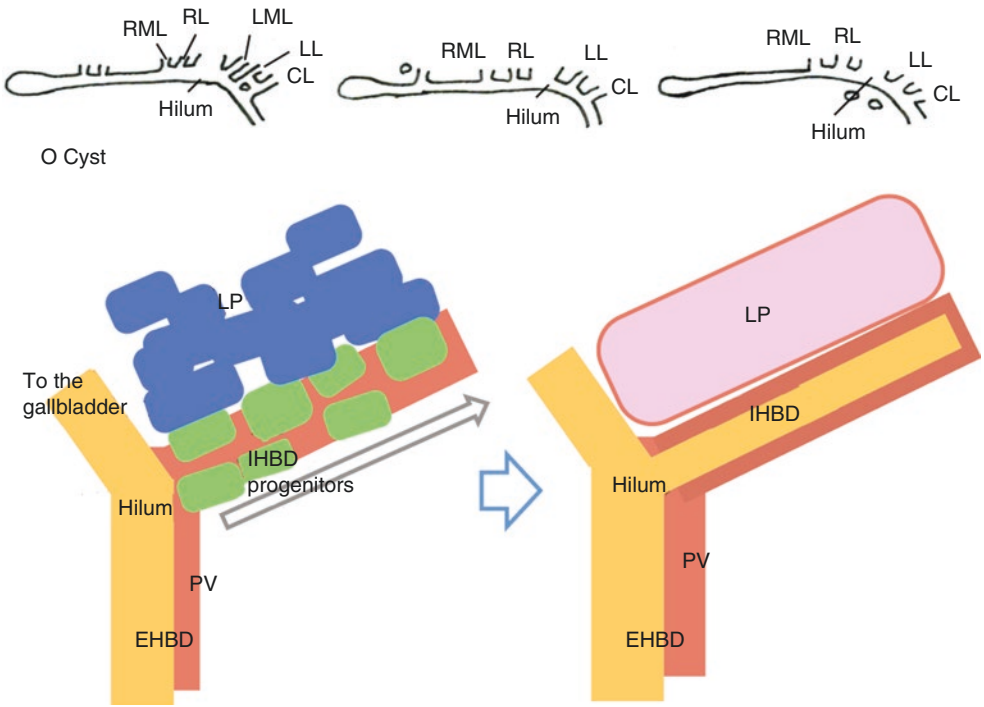

Fig. 5.2 Secondary joining of extrahepatic bile ducts and intrahepatic bile progenitors (ductal plates). (**a**) Various connections of extrahepatic bile ducts with intrahepatic bile duct progenitors at E15.5 in syngeneic C3H/HeSlc strain mice. Cyst development in the hilum is noted in one of syngeneic mice. *CL* caudal lobe, *LL* left lobe, *LML* left medial lobe, *RL* right lobe, *RML* right medial lobe. (**b**) Discontinuous bile duct progenitors appearing around portal veins. They connect with one another with development. Bile duct formation proceeds from the hilum to the periphery. *EHBD* Extrahepatic bile duct, *IHBD* intrahepatic bile duct, *LP* liver parenchyma, *PV* portal vein

17.5, intrahepatic bile ducts having squamous epithelial cells and supported by connective tissue cells develop around portal veins (Fig. 5.1e). Hepatocyte maturation starts at E13.5, and hepatocytes become larger at E16.5.

Extrahepatic bile ducts appear earlier than, and is independent of intrahepatic bile ducts. At perinatal stages, the biliary gland develops in the cystic duct and common bile duct (Fig. 5.1f). Smooth muscle is well developed around the gallbladder. Both extrahepatic and intrahepatic bile ducts do not have smooth muscle tissues.

5.3 Mechanisms of Bile Duct Development

Although portal mesenchyme induces adjacent hepatoblasts to give rise to epithelial cells of intrahepatic bile ducts during liver development, several signaling molecules may be involved in this induction, including Jag1, Wnt5a, and TGFβ1-3 (Fig. 5.3) [7–10]. In addition to biliary cell differentiation, portal mesenchyme may play a key role in the secondary connection of extrahepatic bile ducts with intrahepatic ductal plates, and of each ductal plate. It may also regulate bile duct morphogenesis from ductal plates. Jag1 may control this morphogenetic step [11]. However, it is still unknown how these histogenetic events are regulated at a molecular level.

When molecular characterization of portal veins is performed during mouse liver development, they have a molecular signature, including expression of connexin37, connexin40, and Jag1 in their endothelial cells from E10.5 at the latest (Fig. 5.4a–c) [12]. Expression of Hes1, one of target genes for Jag1-Notch2 signaling, is immunohistologically detected in intrahepatic biliary progenitor cells, and endothelial cells and mesenchyme cells of portal veins from E13.5 on. However, not all biliary progenitor cells are positive for Hes1 (Fig. 5.4d). Thus, molecular mechanisms of intrahepatic bile duct development may be more complicated than expected. Nerve cells, which are acetylated tubulin-positive, are detected only in portal veins near the hilum in E15.5 and E17.5 livers (Fig. 5.4e), and enter the liver along portal veins after birth, suggesting that nerve cells are not directly involved in development of ductal plates along portal veins.

Hes1 knockout mouse exhibits agenesis of extrahepatic bile ducts and their differentiation into the pancreatic tissue [13]. Notch signaling may suppress pancreatic development in extrahepatic bile ducts. Sox17 haploinsufficiency causes an impaired development of the smooth muscle tissue of the gallbladder, which results in embryonic cholecystitis [1].

5.4 Importance of Hepatocyte Maturation in Intrahepatic Bile Duct Morphogenesis

Inactivation of the gene coding for C/EBPα, which engages in transcriptional regulation of several liver-specific enzymes such as carbamoylphosphate synthase I and phosphoenolpyruvate carboxykinase, induces pseudoglandular

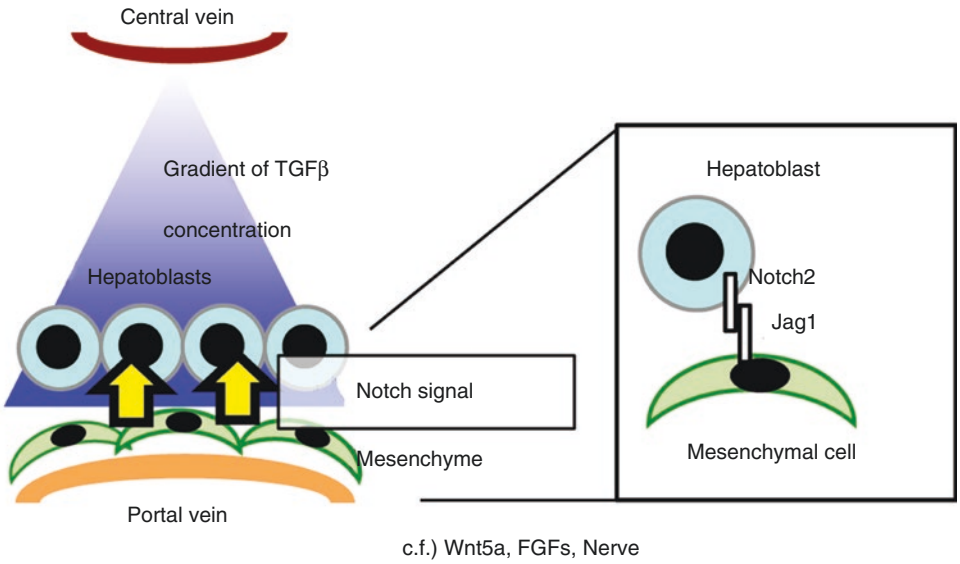

Fig. 5.3 Molecular mechanism of intrahepatic bile duct formation. Portal mesenchyme may induce adjacent hepatoblasts to give rise to biliary epithelial cells through Jag1-Notch2 signaling. TGFβ concentration in liver lobules may involve biliary development

tissues in addition to the suppression of hepatic maturation (Fig. 5.5a, b) [14]. In its knockout liver, occasional (stochastic) expression of biliary markers (Spp1, Sox9, and cytokeratins) in the parenchyma also occurs (Fig. 5.5c, d) [15]. These data indicate that the loss of C/EBPα expression may be a prerequisite for biliary cell differentiation. The knockout bile ducts around portal veins are abnormal for *Spp1* and Sox9 expressions and are not segregated from the parenchyma. From these data, the maturation of liver parenchyma through C/EBPα expression may be required for normal bile duct morphogenesis (Fig. 5.5e) [15]. Several signaling molecules, including Jag1, TGFβ1, and FGFs, were upregulated in microarray data of the knockout livers, and they may be involved in normal intrahepatic bile duct development.

5.5 Bile Duct Development In Vitro

An in vitro culture system, in which normal bile duct development proceeds, is required to demonstrate molecular mechanisms of bile duct formation or to screen the effect of various factors, including growth factors, toxic chemicals, and viruses possibly causing the BA, on bile duct development. A recent paper has indicated that Sox17 haploinsufficiency causes the dysfunction of smooth muscle tissue in the gallbladder using organ culture of fetal liver fragments with the gallbladder, which may lead to BA [1]. When murine liver fragments at E12.5, which do not have intrahepatic bile duct development, are cultured in vitro using Trowell's organ culture technique, intrahepatic bile ducts are not constructed irrespective of the presence of the hilum part and extrahepatic bile ducts (Fig. 5.6a) [16]. When both proximal liver fragments containing the hilum part and extrahepatic bile ducts, and distal liver fragments at E12.5 are transplanted into the testis for 2 months, they similarly generate mature liver tissue accompanied by intrahepatic bile ducts around portal veins [17, 18]. The mature tissue has a hepatic zonation of ammonia metabolizing enzymes.

The importance of Notch signaling in intrahepatic bile duct development has been indicated in vitro. When inhibitors for Notch signaling is added to the culture medium of primary hepatoblasts, they inhibit biliary cell differentiation [19]. By contrast, when Notch intracytoplasmic domain (NICD) is overexpressed in primary hepatoblasts, it stimulates biliary differentiation in vitro. Other signaling molecules, including Wnt5a and vasoactive intestinal peptide, also stimulate biliary development in three-dimensional cultures of hepatoblasts in collagen gels [9, 20]. However, it should be noted that cyst structures were formed but no bile ducts were formed in these cultures.

When endodermal cells of the liver primordium are recombined and cultured with chick lung mesenchyme in vitro, they construct typical duct epithelial cells with mature hepatocytes [21]. Because these endodermal cells contain progenitors for epithelial cells of extrahepatic bile ducts and gallbladder, they may respond to the stimuli of lung mesenchyme to make duct structures. When hepatoblasts isolated from E12.5 mouse livers with a temperature-reversible gelation polymer were recombined and cultured with avian lung mesenchyme in vitro, they never formed ductular structures as seen in cultures of liver endodermal cells (Fig. 5.6b, c). Addition of Jag1 peptides promoted isolated hepatoblasts to express biliary markers (Fig. 5.6d).

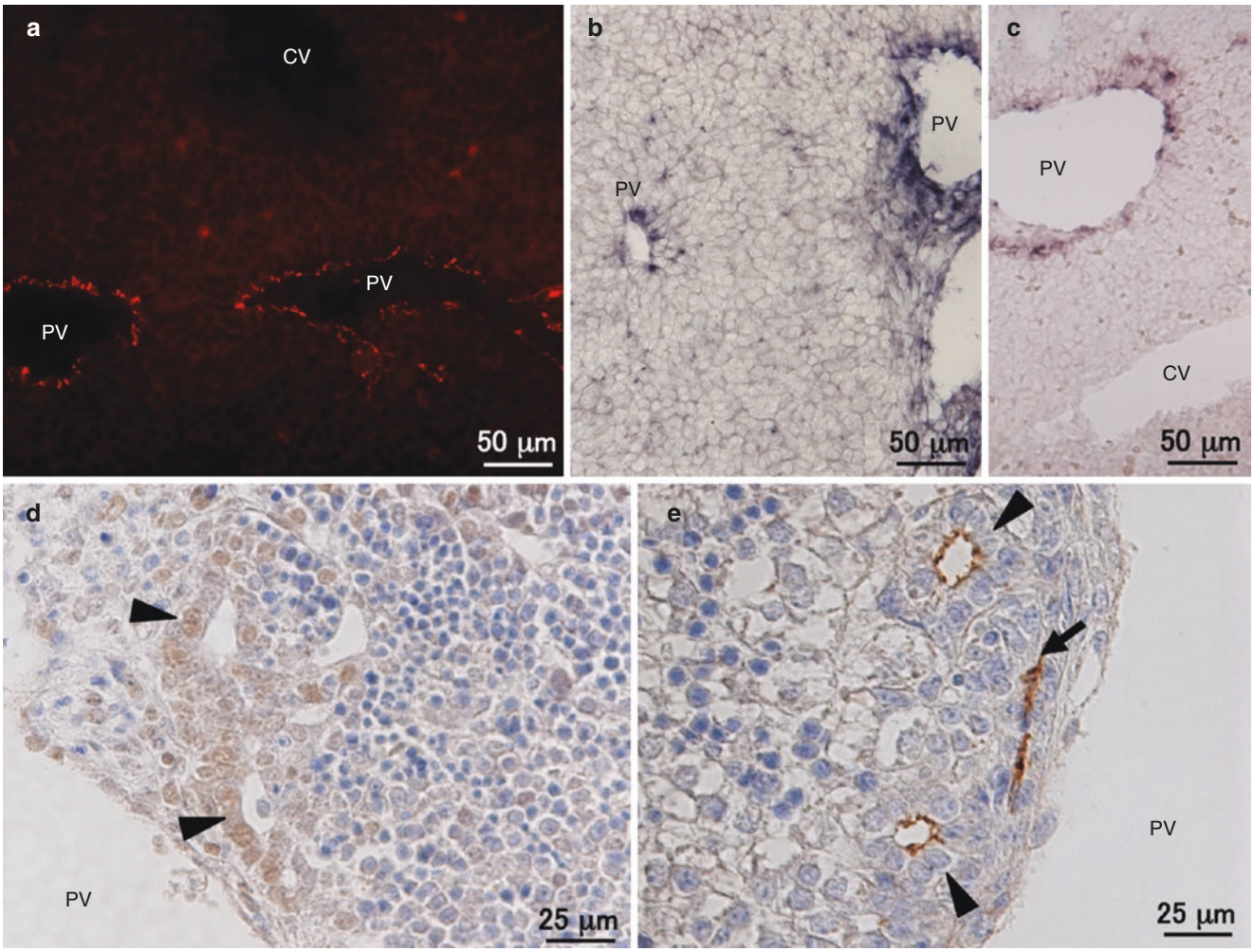

Fig. 5.4 Immunohistochemical characterization of portal vein tissues in fetal mouse livers. (**a**) Immunohistochemical detection of connexin40 in endothelial cells of portal veins at E12.5. Those of central veins do not express connexin40. (**b**, **c**) in situ hybridization of *Jag1* mRNA in E12.5 and E15.5 mouse liver, respectively. Portal endothelial cells and mesenchyme cells express *Jag1*. (**d**) Hes1 expression in biliary progenitors, and endothelial cells and mesenchyme cells of portal veins at E15.5. Not all biliary progenitors (arrowheads) are positive. (**e**) Immunodetection of nerve cells with anti-acetylated tubulin. Nerve cells (arrow) are detected only in portal veins near the hilum in E17.5 liver. Apical staining of bile progenitors is derived from acetylated tubulin-positive cilia. Arrowheads indicate ductal plates. *CV* central vein, *PV* portal vein

Special mesenchyme expressing Jag1 in portal veins may be important for bile duct formation.

Our preliminary data indicated that several growth factors, which are upregulated in C/EBPα knockout livers having pseudoglandular structures in place of mature hepatic tissue, stimulated bile duct development in organ cultures of fetal liver fragments, especially of the proximal fragments containing the hilum part, but not the distal liver fragments. The data suggest that the hilum part may have a decisive center for bile duct formation.

5.6 Conclusion

Bile duct development may be a stochastic event for secondary joining of extrahepatic bile ducts and intrahepatic bile ducts. Several signaling molecules may be involved in bile duct development, including Jag1, Wnt5a, and TGFβ. An in vitro culture system having normal bile duct development should be developed to reveal molecular mechanisms of BA as well as those of bile duct development.

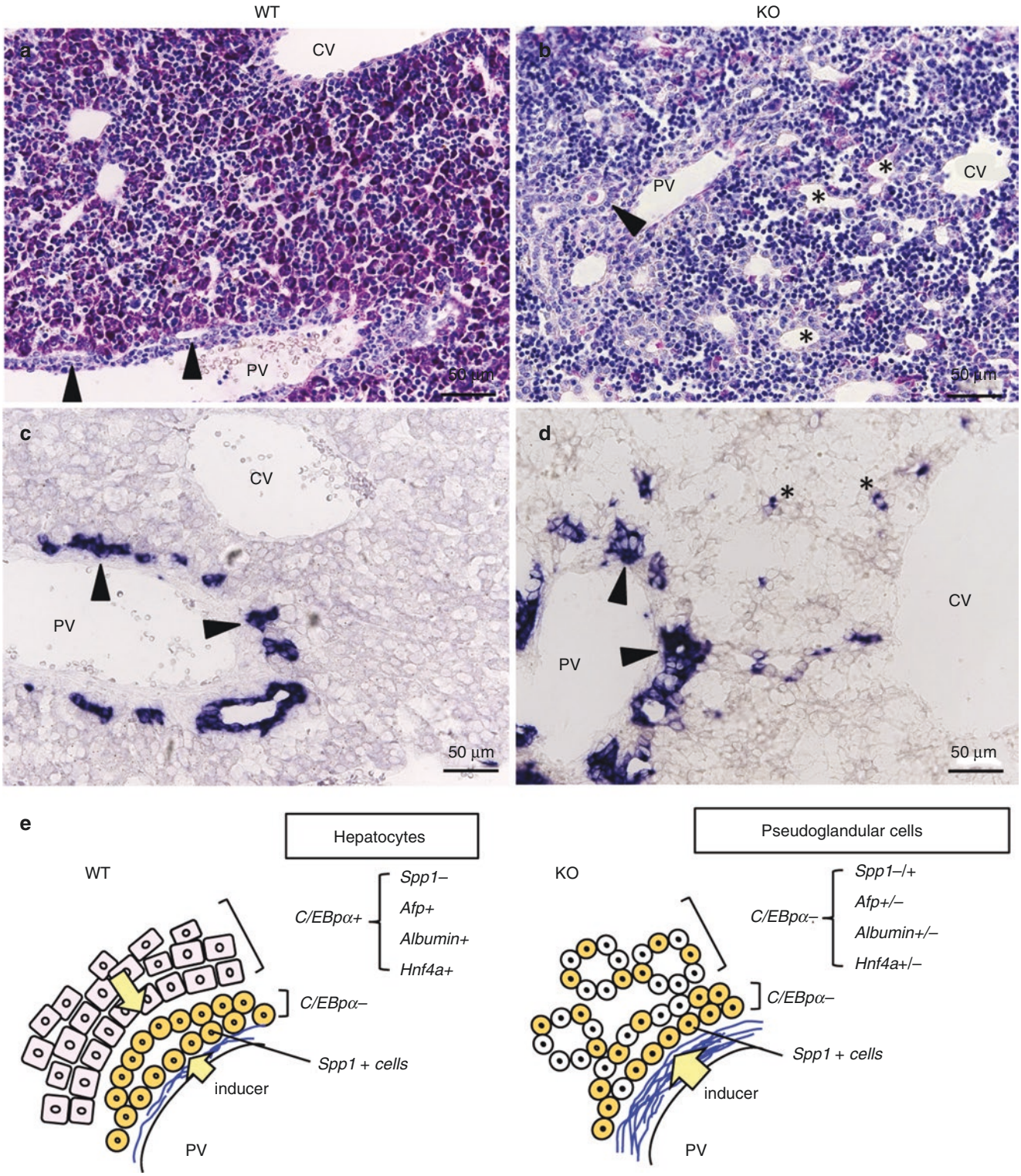

Fig. 5.5 Impairment of hepatocyte maturation and abnormal biliary development in *C/EBPa*-knockout mouse liver at E17.5. (**a**, **b**) Periodic acid-Schiff (PAS)-hematoxylin staining of wild-type and knockout livers, respectively. Glycogen storage is impaired in the knockout liver, compared with that of the wild-type liver. Pseudoglandular cells (asterisks) are noted in the liver parenchyma, including those of pericentral regions. (**c**, **d**) in situ hybridization of *Spp1* in wild-type and knockout livers, respectively. *Spp1* expression in the wild-type liver is highly specific for periportal biliary epithelial cells. By contrast, *Spp1* expression in the knockout liver is heterogeneous and shows a mosaic pattern of positive cells and negative cells even in periportal bile duct progenitors. *Spp1*-positive cells are also detectable in the parenchymal regions. Arrowheads and asterisks indicate periportal biliary structures and parenchymal cells, respectively. (**e**) C/EBPα expression and bile duct formation. Normal biliary development may need maturation of the hepatic parenchyma. *CV* central vein, *PV* portal vein

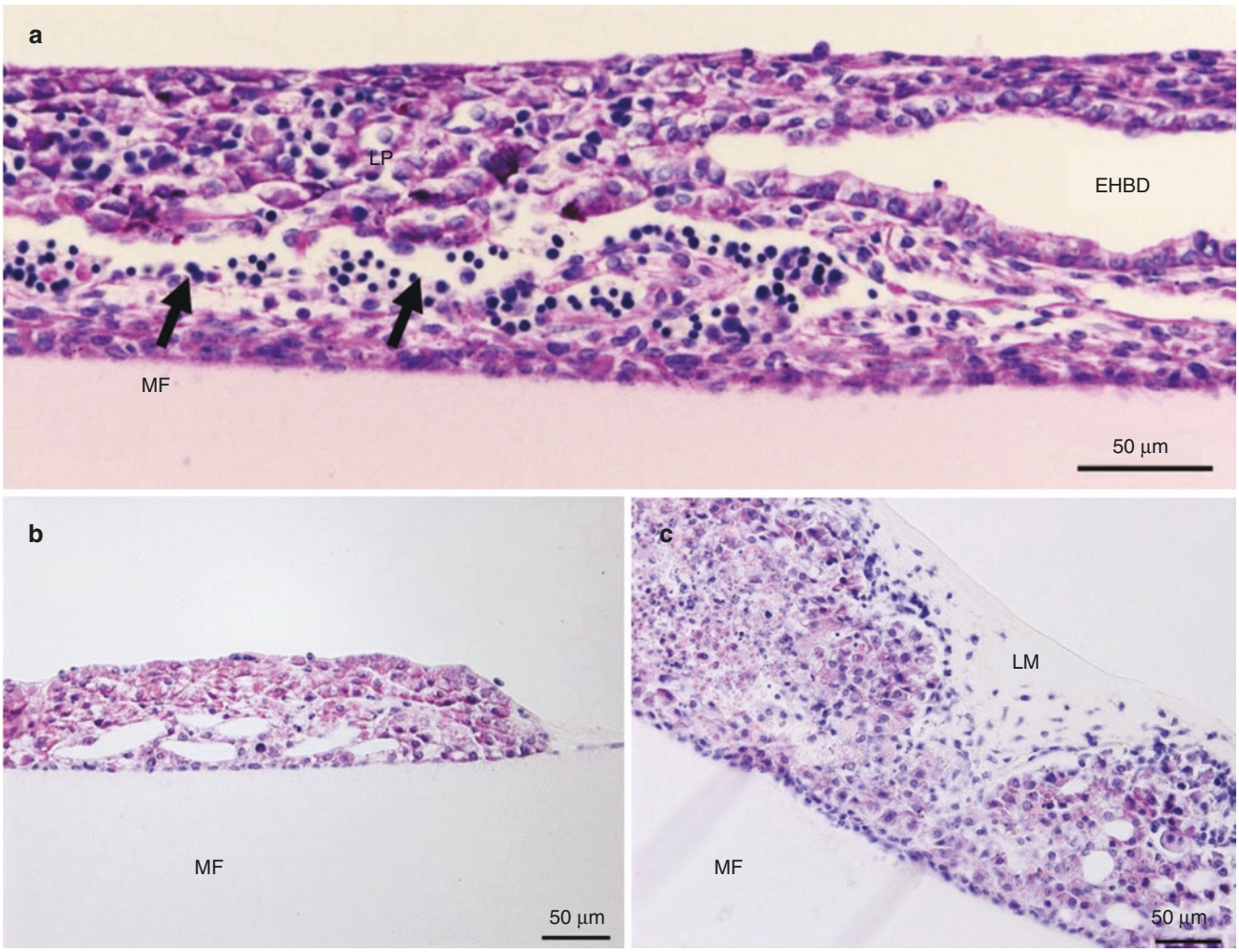

Fig. 5.6 Bile duct development in organ culture of liver fragments of fetal mice. (**a**) Proximal liver fragments with extrahepatic bile duct in vitro for 5 days (PAS-hematoxylin staining). No intrahepatic bile duct develops around a portal vein-like structure (arrows). (**b**) Culture of E12.5 hepatoblasts isolated with a temperature-responsive gelation polymer for 5 days (hematoxylin-eosin staining). (**c**) Co-culture of purified E12.5 hepatoblasts with quail lung mesenchyme for 5 days (hematoxylin-eosin staining). The mesenchyme does not induce bile duct development. (**d**) Immunofluorescent analyses of hepatic cell aggregate cultured in a temperature-responsive gelation polymer for 5 days. Addition of Jag1 peptide (10 µg/ml) in the culture medium increases cytokeratin19-positive cells (yellow arrowheads), and decreases carbamoylphosphate synthase I-positive cells (white arrowheads). *EHBD* extrahepatic bile duct, *LM* lung mesenchyme, *LP* liver parenchyma, *MF* membrane filter

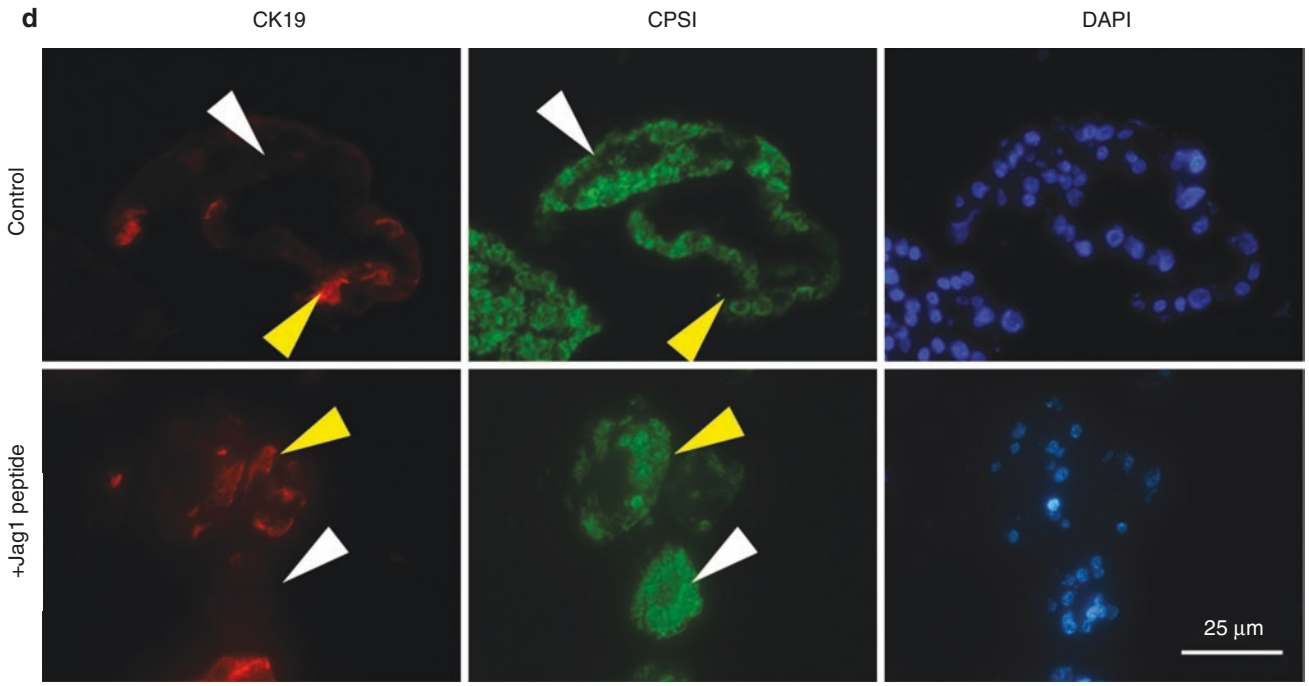

Fig. 5.6 (continued)

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