MECHANISMS OF DEVELOPMENT 126 (2009) 665–676

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journal homepage: www.elsevier.com/locate/modo

MECHANISMS OF DEVELOPMENT

Mouse hepatoblasts at distinct developmental stages are characterized by expression of EpCAM and DLK1: Drastic change of EpCAM expression during liver development

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ARTICLE INFO

Article history:
Received 27 September 2008
Received in revised form
29 May 2009
Accepted 6 June 2009
Available online 13 June 2009

Keywords:
Mouse
Hepatoblasts
EpCAM
DLK1
Hepatocyte
Cholangiocyte
Bile duct
Flow cytometry

ABSTRACT

Hepatoblasts are hepatic progenitor cells that expand and give rise to either hepatocyte or cholangiocytes during liver development. We previously reported that delta-like 1 homolog (DLK1) is expressed in the mouse liver primordium at embryonic day (E) 10.5 and that DLK1$^+$ cells in E14.5 liver contain high proliferative and bipotential hepatoblasts. While the expression of epithelial cell adhesion molecule (EpCAM) in hepatic stem/progenitor cells has been reported, its expression profile at an early stage of liver development remains unknown. In this study, we show that EpCAM is expressed in mouse liver bud at E9.5 and that EpCAM$^+$DLK1$^+$ hepatoblasts form hepatic cords at the early stage of hepatogenesis. DLK1$^+$ cells of E11.5 liver were fractionated into EpCAM$^+$ and EpCAM$^-$ cells; one forth of EpCAM$^+$DLK1$^+$ cells formed a colony in vitro whereas EpCAM$^-$DLK1$^+$ cells rarely did it. Moreover, EpCAM$^+$DLK1$^+$ cells contained cells capable of forming a large colony, indicating that EpCAM$^+$DLK1$^+$ cells in E11.5 liver contain early hepatoblasts with high proliferation potential. Interestingly, EpCAM expression in hepatoblasts was dramatically reduced along with liver development and the colony-forming capacities of both EpCAM$^+$DLK1$^+$ and EpCAM$^-$DLK1$^+$ cells were comparable in E14.5 liver. It strongly suggested that most of mouse hepatoblasts are losing EpCAM expression at this stage. Moreover, we provide evidence that EpCAM$^+$DLK1$^+$ cells in E11.5 liver contain extrahepatic bile duct cells as well as hepatoblasts, while EpCAM$^-$DLK1$^+$ cells contain mesothelial cell precursors. Thus, the expression of EpCAM and DLK1 suggests the developmental pathways of mouse liver progenitors.

1. Introduction

Liver development begins at embryonic day (E) 8.5 in the mouse from the foregut endoderm. The ventral wall of the foregut endoderm faces the developing heart by approximately E8 and receives inductive signals for the hepatic fate, such as fibroblast growth factor (FGF), from the heart (Douarin, 1975; Gualdi et al., 1996; Jung et al., 1999). Septum transversum mesenchyme (STM) also contributes to liver development from the foregut endoderm by providing bone

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0925-4773/$ - see front matter
doi:10.1016/j.mod.2009.06.939

morphogenetic protein (BMP), another factor involved in the liver specification (Rossi et al., 2001). By these signals, pre-hepatic cells become proliferative and begin to form the liver bud. Following the formation of primary liver bud from E8.5 to E9.0, the basement membrane surrounding the liver bud is lost by E9.5, and pre-hepatic cells migrate as cords into the surrounding STM (Matsumoto et al., 2001; Sosa-Pineda et al., 2000; Zaret, 1998). These pre-hepatic cells are also designated as hepatoblasts and a number of studies have indicated that hepatoblasts give rise to both hepatocytes and cholangiocytes, biliary epithelial cells (Germain et al., 1988; Rogler, 1997; Shiojiri, 1994; Shiojiri et al., 1991; Spagnoli et al., 1998). As cell sorting using antibodies (Abs) is a powerful means to isolate and characterize a specific cell type, efforts have been made to search for specific cell-surface antigens on hepatoblasts. We previously reported that DLK1, also known as Pref-1, was strongly expressed in the E10.5 mouse liver bud and that DLK1$^+$ cells isolated from E14.5 livers expressed albumin and formed colonies composed of hepatocyte and cholangiocyte lineages when cultured in the presence of hepatocyte growth factor (HGF) and epidermal growth factor (EGF) (Tanimizu et al., 2003). Thus, DLK1$^+$ cells contain hepatoblasts and DLK1 is a useful marker to enrich highly proliferative hepatoblasts from fetal liver. Kubota et al. showed that the RT1A1$^-$ OX18^{low} ICAM-1$^+$ fraction of E13 rat fetal liver contained hepatoblasts (Kubota and Reid, 2000). Suzuki et al. developed a single cell-based assay designated the hepatic colony-forming unit in culture (H-CFU-C) and showed that the CD45$^-$ TER119$^-$ c-Kit$^-$ CD29$^+$ CD49f$^+$ and CD45$^-$ TER119$^-$ c-Kit$^-$ c-Met$^+$ CD49f$^{+/low}$ fraction of E13.5 mouse liver contained hepatic progenitor/stem cells (Suzuki et al., 2000). Thus, the colony-forming capacity is a criterion to evaluate hepatoblasts. They also showed that CD45$^-$ TER119$^-$ c-Kit$^-$ c-Met$^+$ CD49f$^{+/low}$ cells of E11.5 mouse liver had high H-CFU-C potential. On the other hand, Minguet et al. reported that CD45$^-$ TER119$^-$ c-Kit^{low} cells in E11 mouse liver contained the earliest hepatic progenitors, also displaying features of liver-repopulating stem cells (Minguet et al., 2003); however, despite finding these markers, negative selection markers are less informative for the localization of hepatoblasts. Thus, cell-surface antigens expressed on hepatoblasts are valuable for localization and prospective

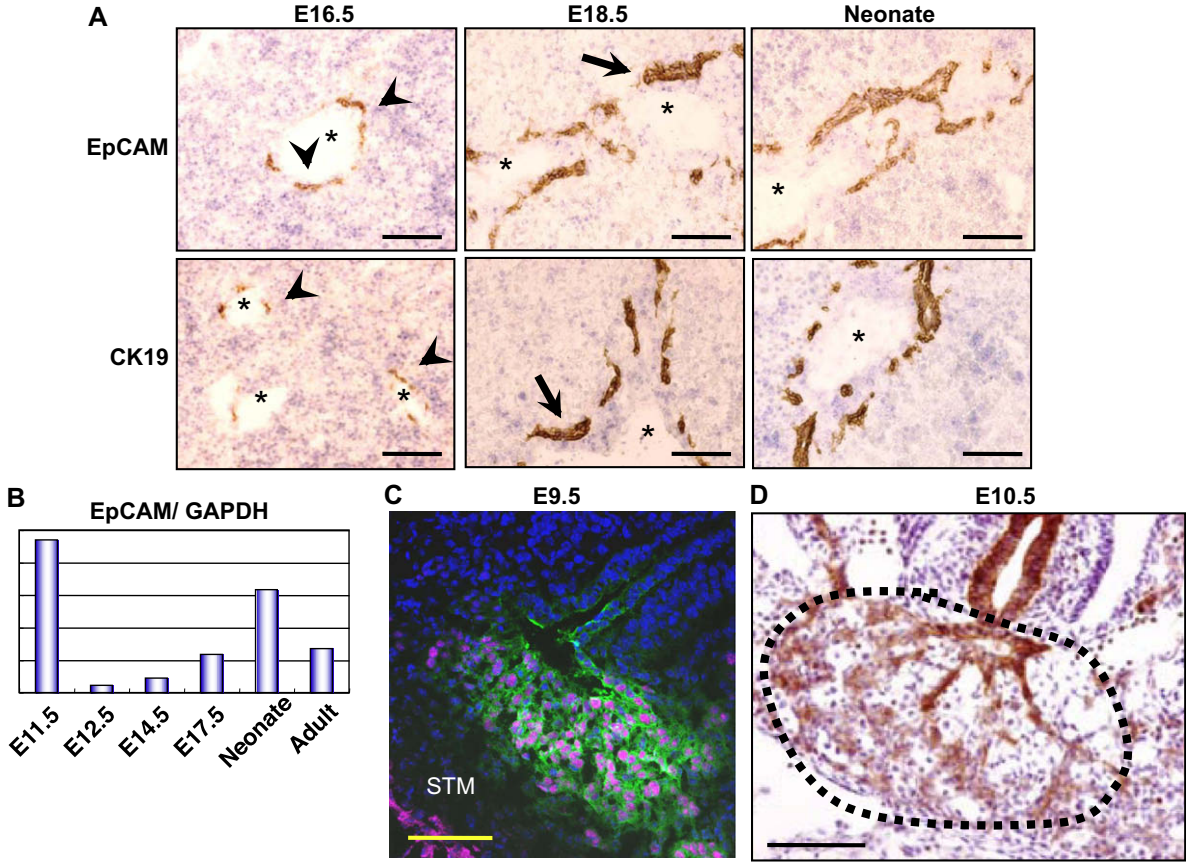

Fig. 1 – Expression profiles of EpCAM during liver development. (A) Immunostaining of E16.5, E18.5 and neonatal liver sections with either anti-EpCAM or anti-CK19 Ab. In E16.5 liver, arrowheads point to mono-layered bile duct progenitor cells. (Arrows) In E18.5 liver, arrows point to bi-layered ductal plates strikingly stained with both Abs. Asterisk: portal veins. (B) Expression of EpCAM in liver is analyzed by real-time RT-PCR during liver development (E11.5, E12.5, E14.5, E17.5, neonate and adult liver). The expression level of EpCAM is normalized by GAPDH (C) IHC of an E9.5 liver section with anti-EpCAM (green) and anti-HNF4α (magenta) Abs. Both gut endoderm cells and liver precursor cells spreading into STM are stained with anti-EpCAM Ab. (D) IHC of an E10.5 liver section with anti-EpCAM Ab by DAB staining. Dotted line encircles liver primordium. Scale bars: 100 μm.