Stem Cell Reports
Report

ISSCR

OPEN ACCESS

Functional Blood Progenitor Markers in Developing Human Liver Progenitors

Orit Goldman,[1] Idan Cohen,[1] and Valerie Gouon-Evans[1,*]
[1]Department of Developmental and Regenerative Biology, Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
*Correspondence: gouonevans@gmail.com
http://dx.doi.org/10.1016/j.stemcr.2016.07.008

SUMMARY

In the early fetal liver, hematopoietic progenitors expand and mature together with hepatoblasts, the liver progenitors of hepatocytes and cholangiocytes. Previous analyses of human fetal livers indicated that both progenitors support each other's lineage maturation and curiously share some cell surface markers including CD34 and CD133. Using the human embryonic stem cell (hESC) system, we demonstrate that virtually all hESC-derived hepatoblast-like cells (Hep cells) transition through a progenitor stage expressing CD34 and CD133 as well as GATA2, an additional hematopoietic marker that has not previously been associated with human hepatoblast development. Dynamic expression patterns for CD34, CD133, and GATA2 in hepatoblasts were validated in human fetal livers collected from the first and second trimesters of gestation. Knockdown experiments demonstrate that each gene also functions to regulate hepatic fate mostly in a cell-autonomous fashion, revealing unprecedented roles of fetal hematopoietic progenitor markers in human liver progenitors.

INTRODUCTION

The liver bud in the mouse embryo is formed from the foregut endoderm at around embryonic day 9.5 (E9.5) by the migration into the septum transversum of the fetal liver progenitors, hepatoblasts expressing the hepatic markers α-fetoprotein (AFP), and albumin (ALB). Hepatoblasts proliferate considerably to form the fetal liver mass and finally differentiate in midgestation into either hepatocytes or cholangiocytes based on their proximity to portal veins (Gordillo et al., 2015). In mammals, the fetal liver is also the major site of hematopoiesis (Golub and Cumano, 2013). Murine liver hematopoiesis is initiated at E10 with the colonization of the fetal liver by hematopoietic progenitors migrating from the yolk sac and the region of the aorta-gonad-mesonephros. The fetal liver hematopoietic activity decreases around E15 and disappears shortly after birth.

Surprisingly, few studies on human fetal livers have reported that the hematopoietic progenitor markers CD34, CD117, CD90, CD133, and CD44 are also expressed on a subset of human hepatoblasts and/or precursors of hepatoblasts depending on the embryonic stage examined (Table S1). Most of the EpCAM+ hepatoblasts express CD133 and CD44 in the second trimester of gestation (Schmelzer et al., 2007). Co-expressions of CD117 and AFP or CD117 and ALB in hepatoblasts are detected at around 14 weeks and represent about 2% and 1% of total cells, respectively (Nava et al., 2005). A subset of CD117+ cells that co-express CD34 can turn on the hepatic markers ALB and CK19 when further cultured in vitro suggesting the presence of CD177+CD34+ precursors of hepatoblasts (Nava et al., 2005; Nowak et al., 2005). Similarly, human fetal liver multipotent progenitor cells have been identified from fetal livers from first and second trimesters (Lazaro et al., 2003); they express CD34 and CD44 and differentiate into ALB+ glycogen+ hepatoblasts, CK7+GGT+CK19+ biliary cells, and mesenchymal cells (Dan et al., 2006). These studies indicate that some hematopoietic progenitor markers are surprisingly also expressed on developing hepatoblasts and/or precursors of hepatoblasts in human fetal livers.

Our previous work has established an efficient protocol to generate functional hepatoblast-like cells (referred to as hepatic cells or Hep cells) from human embryonic stem cell (hESC) differentiation cultures that express endoderm and hepatic markers including FOXA2, HNF4α, AFP, ALB, CK18, and EpCAM (Goldman et al., 2013). Here, we characterize expression kinetics of hematopoietic progenitor markers in Hep cells as they specify from the endoderm. We demonstrate dynamic expression patterns for the hematopoietic progenitor markers CD34, CD133, and GATA2 in developing Hep cells, and confirm these findings in vivo with analyses of human fetal livers collected in the first and second trimesters of gestation. Knockdown of CD34, CD133, and GATA2 revealed their impact on hepatic specification of Hep cells mostly in a cell-autonomous fashion. This study highlights the powerful utility of the hESC differentiation system to recapitulate early human hepatic specification and has uncovered the functional impact on hepatic specification and maturation of hematopoietic progenitor markers expressed in human hepatoblasts.

RESULTS AND DISCUSSION

Co-expression of Hematopoietic Progenitor Markers CD34, CD133, and GATA2 in Developing hESC-Derived Hep Cells

Endoderm-derived Hep cells were generated from hESC differentiation cultures as previously described (Goldman et al., 2013). Activin A-induced endoderm cells were

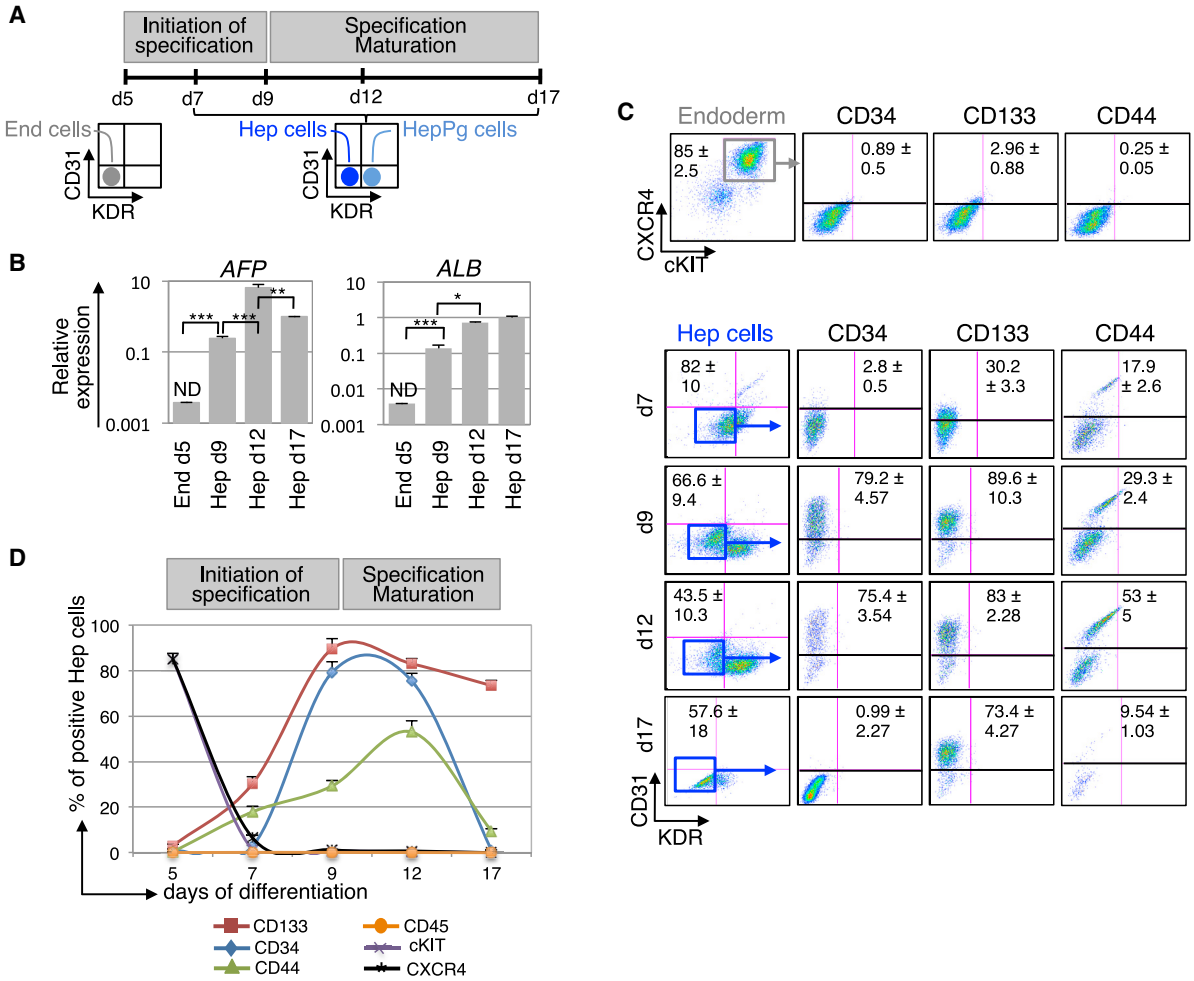

Figure 1. Expression of Cell Surface Hematopoietic Progenitor Markers in Hep Cells

(A) Timeline of generation of day 5 hESC-derived endoderm (End cells) and their progeny Hep and HepPg cells.

(B) Relative transcript levels in purified day 5 End cells and KDR−CD31− Hep cells purified at days 9, 12, and 17. All numbers reflect the mean ± SD for n independent differentiations (n = 3). Transcript levels from day 17 Hep cells were set to 1.

(C) Flow cytometry analysis of day 5 End cells and KDR−CD31− Hep cells from days 7 to 17 (n = 12 for CD31 and KDR and n = 3 for CD34, CD133, and CD44).

(D) Graph summarizing the flow cytometry data shown in (C) and Figure S1B.

*p < 0.05, **p < 0.01, and ***p < 0.001. ND, not detectable (cycle number above 40). See also Figure S1.

purified at day 5 of differentiation within the cell population positive for CXCR4 and cKIT and negative for the mesendoderm marker PDGFRα and the mesoderm marker KDR/VEGFR2/Flk-1. Purified endoderm cells were then cultured in hepatic medium that favors hepatic specification. We previously demonstrated that Hep cells develop concomitantly with an endoderm-derived hepatic progenitor population (referred to as HepPg cells) that surprisingly expresses KDR (Goldman et al., 2013). Hep and HepPg cells have a distinct cell-surface-marker profile with Hep cells being KDR−CD31− and HepPg cells being KDR+CD31− (Goldman et al., 2013) (Figure 1A). Both populations derive from day 5 KDR−CD31− endoderm cells (Figure 1A). At day 7 of differentiation, the KDR−CD31− cell population is heterogeneous and composed of Hep cells and some remaining unspecified endoderm cells. By day 9, new KDR+CD31− HepPg cells developed from the unspecified endoderm cells, and the ratio between KDR−CD31− Hep cells and KDR+CD31− HepPg cells reaches about 50% and remains in this range until day 17 (Goldman et al., 2013). Both Hep and HepPg cells express endoderm (FOXA2, GATA4), epithelial (CK18), and early hepatic commitment (HNF4α) markers, while specific hepatic genes (AFP, ALB, P450 enzymes, α1-antitrypsin) are only present in Hep cells (Goldman et al., 2013). Day 13–16 Hep cells are mature enough to functionally support hepatitis C virus replication