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Identification of a candidate stem cell in human gallbladder

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Abstract
There are currently no reports of identification of stem cells in human gallbladder. The differences between human gallbladder and intrahepatic bile duct (IHBD) cells have also not been explored. The goals of this study were to evaluate if human fetal gallbladder contains a candidate stem cell population and if fetal gallbladder cells are distinct from fetal IHBD cells. We found that EpCAM+CD44+CD13+ cells represent the cell population most enriched for clonal self-renewal from primary gallbladder. Primary EpCAM+CD44+CD13+ cells gave rise to EpCAM+CD44+CD13+ and EpCAM+CD44+CD13− cells *in vitro*, and gallbladder cells expanded *in vitro* exhibited short-term engraftment *in vivo*. Last, we found that CD13, CD227, CD66, CD26 and CD49b were differentially expressed between gallbladder and IHBD cells cultured *in vitro* indicating clear phenotypic differences between the two cell populations. Microarray analyses of expanded cultures confirmed that both cell types have unique transcriptional profiles with predicted functional differences in lipid, carbohydrate, nucleic acid and drug metabolism. In conclusion, we have isolated a distinct clonogenic population of epithelial cells from primary human fetal gallbladder with stem cell characteristics and found it to be unique compared to IHBD cells.

Abbreviations: IHBD, intrahepatic bile duct; EHBD, extrahepatic bile duct; CBD, common bile duct; LDA, Limiting Dilution Analysis; CFU, colony forming unit; EGF, epidermal growth factor; MACS, magnetic activated cell sorting; PCA, principal component analysis; CEACAM, carcinoembryonic antigen-related cell adhesion molecule; AQP, aquaporin; CYP, cytochrome; SLC, solute carrier; Ihh, Indian hedgehog; IFN, interferon.

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Introduction

Understanding the resident stem cell populations of the bile duct system is important for both basic biology and developing therapeutic strategies to treat bile duct diseases. The bile duct system is divided into IHBD and extrahepatic bile duct (EHBD) systems. The EHBD system consists of the common hepatic duct, gallbladder, cystic duct and the common bile duct (CBD) (Hand, 1973; Cardinale et al., 2010). The gallbladder stores bile while modifying its content and concentration (Nakanuma et al., 1997; Frizzell and Heintze, 1980).

There is a paucity of data characterizing stem cells in both adult and fetal human gallbladders. In addition, the differences between IHBD and EHBD cells are not well understood. The EHBD system, the liver and the ventral pancreas develop from the posterior ventral foregut endoderm (Shiojiri, 1997; Zaret and Grompe, 2008; Tremblay and Zaret, 2005). However, the cell-intrinsic factors responsible for IHBD and EHBD system specification are unclear. Recently, Spence et al. (2009) by using a PDX1-Cre transgenic mouse showed that hepatocytes and IHBD cells descend from Pdx1− cells while the EHBD cells including the ventral pancreas derive from Pdx1+ cells. These data were corroborated by a study in our lab, where we found that adult mouse gallbladder stem cells have a distinct phenotypic and expression profile compared to adult mouse IHBD cells (Manohar et al., 2011). However, the differences between human IHBD and EHBD cells have not yet been explored.

The goals of this study were to evaluate if human fetal gallbladder contains a clonogenic candidate stem cell population and compare its phenotypic and expression profiles to those of fetal IHBD cells. The evaluation of human fetal gallbladder stem cells would have important ramifications for the study of congenital diseases such as biliary atresia (Bassett and Murray, 2008) and gallbladder carcinoma, the most common malignancy of the bile duct system (Miller and Jarnagin, 2008), because of the importance of stem cells in development, tissue regeneration and cancer (Lagasse, 2008). In addition, a comparison of human fetal gallbladder and IHBD cells would further elucidate the ontogeny of bile duct cells and represent the first time that the developmental differences between the human IHBD and EHBD cells have been explored.

Stem cells are defined by their ability for single cell self-renewal and lineage commitment (Wagers and Weissman, 2004). We have previously used colony forming assays along with single cell and morphogenesis assays to characterize a resident stem cell population in adult mouse gallbladder (Manohar et al., 2011). In this report, we adapt these assays to the study of human cells. We identify an EpCAM+CD13+CD44+ epithelial subpopulation from primary human fetal gallbladder that can expand *in vitro* through seven passages, exhibits single-cell self-renewal and engrafts in the subcutaneous space of immunodeficient mice. Last, we found that expanded human IHBD cells and gallbladder cells had distinct phenotypic and expression profiles with many of the predicted functional differences between both cell types mirroring those from our previous report (Manohar et al., 2011). To our knowledge, this is the first report to prospectively isolate a clonogenic epithelial population from human fetal gallbladder and evaluate its genealogy relative to IHBD cells.

Methods

Gallbladder and IHBD cell isolation and culture

Fetal liver and gallbladder tissues were obtained from the Tissue Bank at the Magee Women's Hospital of UPMC. All samples were between 19 and 23 weeks of gestation and none of the fetal gallbladders were obtained from therapeutic abortions. (Supplementary Table 1). The research protocol was reviewed and approved by the Institutional Review Board for Human Research Studies at the University of Pittsburgh. Gallbladders were cut and opened along the middle in order to expose the mucosa and placed in HBSS. Bile was washed off by gently scraping the mucosal surface with blunt-ended forceps. Liver samples were minced into small pieces. Gallbladder and liver samples were incubated with EBSS/10 mM EGTA/1% HEPES for 15 min at 37 °C and treated with 1 mg/ml Collagenase II (Invitrogen, CA) + 1 mg/ml Hyaluronidase (Sigma) + 100 μg/ml of DNase I (Roche, IN) for 1–1.5 h followed by 0.25% Trypsin /0.1% EDTA (Fisher Scientific, MA) for 30 min to obtain a cell suspension. Cell suspensions were plated on irradiated rat feeder cells as described previously (Manohar et al., 2011).

FACS analysis

FACS analysis and sorting and subsequent data analysis were performed as previously described (Manohar et al., 2011). LDAs were performed by sorting 1, 10, 25, 50, 100, 200, and 500 cells/well into respective (≥4) columns of 96-well plates (Corning, NY) seeded with irradiated feeders. Colonies were scored after 4–6 weeks post-plating and candidate stem cell frequencies of sorted sub-populations determined in L-Calc™ (StemCell Technologies, Vancouver). In experiments involving expanded cell populations, primary identification of sorted populations involved gating of human (HLA+) cells followed by epithelial (EpCAM+) cells.

Results

EpCAM is a human gallbladder epithelial cell marker

EpCAM is a cell surface marker that was first described in colorectal cancer (Koprowski et al., 1979). Its expression has since been found on a wide variety of epithelial cells such as keratinocytes, thymic epithelial cells and IHBD cells (Balzar et al., 1999; de Boer et al., 1999). Previously, we have determined that mouse gallbladder epithelial cells were EpCAM+, and subsequently used EpCAM to label these cells by flow cytometry (Manohar et al., 2011). EpCAM expression has also been observed on adult human gallbladder epithelial cells (Momburg et al., 1987; Auth et al., 1993) but no evidence exists for its expression in fetal gallbladder. We co-stained EpCAM and CK19, a pan biliary marker (Moll et al., 1982) on cross sections of fetal gallbladders and found that most CK19+ cells were EpCAM+ (Fig. 1A). We subsequently used EpCAM