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EpCAM expression in normal, non-pathological tissues

Eva Schmelzer[1], Lola M. Reid[2]

[1] Leipzig University, Biotechnological-Biomedical Center, Department of Cell Culture and Stem Cell Biology, Deutscher Platz 5, 04103 Leipzig, Germany, [2] Departments of Cell and Molecular Physiology and Biomedical Engineering, Program in Molecular Biology and Biotechnology, Cancer Center and Center for Gastrointestinal and Biliary Disease Biology, UNC School of Medicine, Campus Box 7038, Glaxo Building Rms 32-35, Chapel Hill, NC 27599, USA

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1. ABSTRACT

Epithelial Cell Adhesion Molecule (EpCAM) is a transmembrane glycoprotein that is associated with various cancers. Most normal, non-pathological epithelial tissue is EpCAM positive with the exception of epidermal keratinocytes, gastric parietal cells, myoepithelial cells, thymic cortical epithelial, and hepatocytes. However, during early liver development EpCAM expression is also observed. In our studies, we have demonstrated that EpCAM is expressed in non-pathological human livers on hepatic progenitors in livers of all donor ages, from 16 weeks gestation fetal livers to adult. Hepatic progenitors of the liver consist of the stem cells and their descendants, the hepatoblasts, that give rise to the hepatocytic and biliary lineages. Both hepatic stem cells and most hepatoblasts express EpCAM, but only hepatoblasts are alpha-fetoprotein positive. The percentage of EpCAM positive progenitors in human livers varies with donor age and is about 2.5% in the adult and 12.1% in fetuses. *In vivo*, hepatic stem cells have been found associated with the canals of Hering. Xeno-transplantation experiments with EpCAM positive human liver cells have revealed their potential for proliferation and differentiation to mature liver parenchymal cells.

2. INTRODUCTION

Epithelial Cell Adhesion Molecule or EpCAM (also called GA733-2, KSA, CD326, ESA, or 17-1A antigen) is a transmembrane glycoprotein identified initially as associated with various epithelial cancers (e.g. carcinomas of colon and rectum, stomach, pancreas, liver, lung, mammary gland, ovary, thyroid, kidney, urinary bladder, and prostate). EpCAM positive (+) cells are found also in normal, non-pathological epithelial tissue with the exception of epidermal keratinocytes, gastric parietal cells, mature liver cells (hepatocytes), thymic cortical epithelial, and myoepithelial cells (1). In general, cell-cell adhesion through EpCAM is calcium independent (2, 3).

3. EPCAM EXPRESSION IN NORMAL, NON-PATHOLOGICAL TISSUES

In liver, EpCAM expression is observed during early organ development (4-6). In our studies, we have demonstrated that EpCAM is expressed in non-pathological human livers on hepatic progenitors but not hepatocytes in livers of all donor ages; our studies comprised assays on livers from 16 weeks gestation fetal livers to geriatric adults (7-9). Hepatic progenitors of the liver consist of the

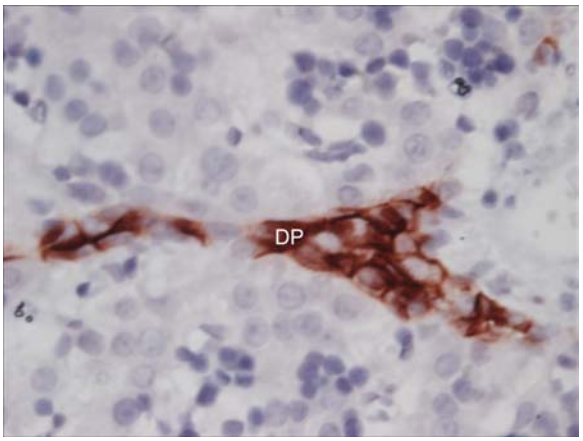

Figure 1. EpCAM staining on human fetal liver section, red: anti-EpCAM stain with NovaRed substrate, blue: nuclear stain with hematoxylin. DP: Ductal plate.

stem cells, multipotent progenitors, and their descendants, the hepatoblasts, bipotent progenitors that give rise to the hepatocytic and biliary lineages and have been shown to co-express hepatocytic and billiary proteins (7,10). We were able to show that both hepatic stem cells and most of the hepatoblasts express EpCAM (see Figure 1 for an immunohistochemical staining of EpCAM in fetal human liver) and CD44 (hyaluronan receptor) (11), but only hepatoblasts are alpha-fetoprotein positive. The percentage of EpCAM+ progenitors in human livers varies with donor age and is about 0.5-1.5% in the adult (up to 3% in livers subjected to ischemia) and 12.1% in fetuses (9). The higher percentage in fetal livers correlates with the fact that the majority (>80%) of parenchymal cells in fetal livers are hepatoblasts, whereas in adult livers hepatoblasts have declined in number to constitute less than 0.01% of the parenchymal cells. *In vivo*, hepatic stem cells have been found in pediatric and adult human livers associated with ductal plates in fetal and neonatal livers and in the canals of Hering in pediatric and adult livers (9, 12, 13) (Zhang et al., submitted). The ductal plate, a band of tissue encircling the portal triads like a sleeve (see Figure 2), has proven to be liver's stem cell compartment or niche and transitions in postnatal life to become the canals of Hering (Zhang et al., submitted). The canals of Hering are 1 – 2 μm wide anatomical structures clustered around the portal triads and consisting of small ductules connecting the biliary epithelia system with the hepatocytes. Bile, produced by hepatocytes, is released from bile canaliculi, found at the apical borders of the hepatocytes, and from there is transported to the bile duct epithelium; the bile ducts transport the bile and releases it into the gut. Xeno-transplantation experiments with EpCAM+ human liver cells and with long-term cultures of human hepatic stem cells, also EpCAM+, have revealed their potential for proliferation and for differentiation to mature liver parenchymal cells (9).

Studies by Dan et al. (14) on progenitors culture selected from human fetal livers of gestational days 74-108 similarly demonstrated EpCAM expression. These progenitors were judged by the authors as mesendoderm-derived, mesenchymal-epithelial transitional cells and could give rise to both liver and to tissues of mesodermal fates such as cartilage. However, the culture conditions potentially had co-selected a mesenchymal progenitor along with the hepatic progenitor. Their findings are distinct from our own in which hepatic progenitors do not express mesenchymal, endothelial, or hematopoietic markers (9).

Interestingly, EpCAM expression has been correlated to many populations of progenitors in the development of other organs, too. Adhesion molecules in general have been shown to play the major role in morphogenesis and cell movement. This has been demonstrated for EpCAM in the assembly of germ line cells in development, where EpCAM is expressed during formation and early gonad assembly (15). Neonatal male and female germ cells remain EpCAM+, whereas in adult testis EpCAM is absent with the exception of spermatogonia.

Research by Cirulli et al. (16, 17) indicates a regulatory role of morphogenesis of EpCAM during pancreas development. The inhibition of cell-cell interactions mediated by EpCAM in human fetal pancreas progenitor cell suspensions was achieved using an EpCAM antibody, and cells were cultured with constant inhibition of cell-cell interactions. This blocking resulted in increased insulin and glucagon gene transcription, i.e. the blocking induced differentiation towards pancreatic island cells by imitating either a functional down-regulation or inactivation of the EpCAM receptor from the cell surface. In addition, transplantation experiments of fetal pancreatic EpCAM+ cells demonstrated that down regulation of EpCAM expression is associated with endocrine differentiation. Analyses of human EpCAM expression *in vivo* revealed highest levels of islet-like cells outgrowing from the ductal epithelium in fetal pancreas. However, islet cells of the adult pancreas showed low EpCAM expression and the adult ductal epithelium high levels.

In human mammary tissue, three types of normal progenitor populations of the breast epithelium could be identified (18, 19): 1) a luminal-restricted, 2) a myoepithelial-restricted, and 3) a bipotent progenitor that gives rise to both restricted progenitors. Only bipotent and luminal restricted progenitors expressed EpCAM. Recent advances in breast cancer research (for review, see (20)) demonstrated the existence of an individual cancer stem cell population within the heterogeneous cell populations of solid breast tumors (21). This distinct, minor cell population was exclusively able to form new tumors, i.e. to be tumorigenic, throughout serial passaging. It showed self-renewal as well as generation of the non-tumorigenic phenotypes of the initial tumor. The phenotype of this proposed cancer stem cell or "tumor initiating cell" was $CD44^+/CD24^-/ESA^+$(EpCAM), bearing strikingly similarities in surface molecule expression to normal hepatic progenitors.

Throughout human lung development, EpCAM can be detected in the epithelia of the primary pulmonary

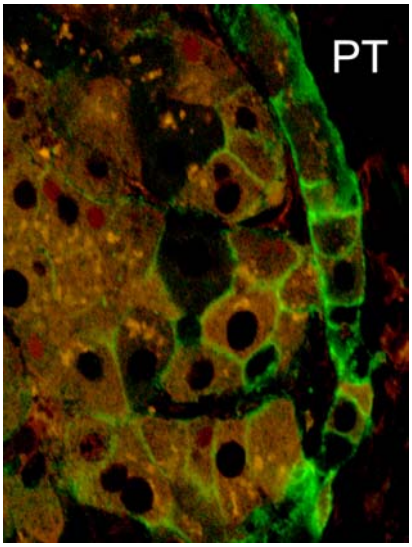

Figure 2. EpCAM expression in the ductal plate of fetal liver. The section of fetal liver is also stained for alpha-fetoprotein. Hepatoblasts are observed outside the ductal plate and are pale orange due to the combination of the fluoroprobe marking alpha-fetoprotein (red) with that for EpCAM (green) (liver section, staining and photography were done by Dr. Lili Zhang (Zhang et al., submitted). PT: portal triad.

primordium, secondary bronchi and bronchial epithelium (22). In the adult lung, EpCAM is expressed in type I and II alveolar epithelial cells.

The murine homologue of EpCAM, gp40, has been detected on outgrowths of dendritic cells from epidermis, skin-associated lymph nodes, keratinocytes, and spleen (23). The gp40 protein is also expressed by thymic epithelium (24). Fetal thymocytes express high levels of gp40, but its expression declines during development and has been suggested to play a role in thymocyte development, interactions between epithelium and thymocytes or dendritic cells, and the formation of a stromal network within the thymus (25). Immature erythroids of the bone marrow (erythroblasts) have been shown to be EpCAM+ (26), but the expression remains restricted to progenitors as it is strongly diminished with erythroid maturation (27).

The apparent connection of EpCAM to highly proliferating cell types, i.e. its expression in progenitors of diverse tissues and its reduced expression during differentiation as well as the upregulation (1) or induction (28) during carcinogenesis, raises the question whether EpCAM might be involved in cell proliferation. Interestingly, a direct role for EpCAM in cell proliferation and cell cycle could be demonstrated by its capability for upregulation of c-myc and cyclin A/E, with its intracellular part sufficient and necessary for the upregulation (29). In addition, the epidermal fatty acid binding protein, a target of c-myc, has been demonstrated to be a target of EpCAM (30). In primary and metastatic breast cancer EpCAM gene expression is increased up to 1000fold; silencing of EpCAM gene expression in breast cancer cell lines caused a decrease in cell proliferation, migration, and invasion, associated with an increase in the detergent-insoluble protein fractions of E-cadherin, alpha-, and beta-catenin (31). Similarly, primary squamous carcinoma of the tongue show elevated EpCAM gene expression and its expression was associated with tumor size, metastasis and invasion; RNAi mediated knockdown of EpCAM expressions likewise decreased the invasive potential and proliferation (32). Two possible intrinsic cellular signaling mechanisms relating EpCAM to cell migration and proliferation have been suggested (31): induced down-regulation of EpCAM increases the fractions of E-cadherin, alpha-, and beta-catenin anchored to the cytoskeleton; cadherins are known to be fundamental for maintenance of cell polarity and proliferation. Additionally, beta-catenin is the major transducer in the Wnt-pathway which has been demonstrated to be an important regulator in cell proliferation and tumor development (see also (33) for an overview of the role of EpCAM in cancer therapy and the possible association of EpCAM with the Wnt pathway).

In summary, EpCAM has been demonstrated to identify many types of normal and malignantly transformed epithelial progenitor populations and potentially plays regulatory roles in proliferation of these cells. Its expression fades and its regulatory effects become muted as the progenitor cells differentiate through their respective maturational lineages to adult, differentiated fates. The potential roles of EpCAM in regulating proliferation of normal and transformed progenitor cells and the mechanisms of action have yet to be elucidated and are likely to be the focus of many future studies.

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Abbreviations: EpCAM: epithelial cell adhesion molecule

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Send correspondence to: Dr Lola M. Reid, Departments of Cell and Molecular Physiology and Biomedical Engineering, Program in Molecular Biology and Biotechnology, Cancer Center and Center for Gastrointestinal and Biliary Disease Biology, UNC School of Medicine, Campus Box 7038, Glaxo Building Rms 32-35, Chapel Hill, NC 27599, USA, Tel: 919-966-0347, Fax: 919-966-6112; E-mail: stemcell@med.unc.edu

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