European Journal of Cell Biology 95 (2016) 574–584

Contents lists available at ScienceDirect

European Journal of Cell Biology

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

Research paper

Inactivation of p120 catenin in mice disturbs intrahepatic bile duct development and aggravates liver carcinogenesis

Jolanda van Hengel (Ph.D)[a,b,c,**], Celine Van den Broeke[a,b], Tim Pieters[a,b,d], Louis Libbrecht[e], Ilse Hofmann[f,g], Frans van Roy (Ph.D)[a,b,*]

[a] Molecular Cell Biology Unit, Inflammation Research Center, VIB, Ghent, Belgium
[b] Department of Biomedical Molecular Biology, Faculty of Sciences, Ghent University, Ghent, Belgium
[c] Department of Basic Medical Sciences, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium
[d] Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium
[e] Department of Pathology, Ghent University Hospital, Ghent, Belgium
[f] Division of Vascular Oncology and Metastasis, German Cancer Research Center, DKFZ-ZMBH Alliance, Heidelberg, Germany
[g] Department of Vascular Biology and Tumor Angiogenesis (CBTM), Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

ARTICLE INFO

Article history:
Received 25 August 2016
Received in revised form 6 October 2016
Accepted 9 October 2016

Keywords:
Cadherin
Armadillo
Hepatoblast
Hepato-biliary cells
Ductal plate malformation
Mouse model
Liver tumor

ABSTRACT

p120 catenin (p120ctn) is required for the stability of classic cadherins at the cell surface and is thought to play a central role in modulating cell–cell adhesion. Cytoplasmic p120ctn promotes cell motility, and probably other activities, by modulating the activities of RhoA, Rac and Cdc42. E-cadherin is expressed in periportal but not in perivenous hepatocytes. In contrast, all hepatocytes of normal mouse liver express N-cadherin. Cholangiocytes express exclusively E-cadherin. Mice with p120ctn ablation in hepatocytes and cholangiocytes (p120LiKO mice) were generated by Cre-*lox*P technology. Livers were examined by histological, immunohistochemical, ultrastructural and serum analysis to determine the effect of the p120ctn ablation on liver structure and function. Mouse hepatocyte differentiation and homeostasis were not impaired. However, hepatoblasts differentiated abnormally into hybrid hepato-biliary cells, ductal plate structures were irregular in p120LiKO newborns, and further development of intrahepatic bile ducts was severely impaired. In adults, enrichment of ductular structures was accompanied by portal inflammation and fibrosis. p120LiKO mice did not spontaneously develop hepatocellular carcinoma but initiation of hepatocarcinogenesis by diethylnitrosamine was accelerated. In summary: p120ctn has a critical role in biliary differentiation and is a potent suppressor of liver tumor growth.

1. Introduction

The liver consists of different cell populations including hepatocytes, which are the major parenchymal cells in the liver, and a variety of non-parenchymal resident cells, including Kupffer, stellate and endothelial cells, as well as cholangiocytes (bile duct epithelial cells, BECs). Hepatocytes and cholangiocytes differentiate from bipotential hepatoblasts. Mouse hepatoblasts start to differentiate into hepatocytes on E13.5 and into cholangiocytes on E14.5. By E17, hepatocytes adopt their characteristic cuboidal shape and prepare for transition from an hematopoietic supporting role to specialized metabolic functions. Intrahepatic bile duct (IHBD) development is initiated by alignment of biliary precursor cells around the branches of the portal vein to form a single-layered ring called the ductal plate. By E17.5, the ductal plate becomes bilayered at focal areas, which leads to the formation of bile ducts, while the remainder of the ductal plate disappears (Lemaigre, 2003).

Specialized junctional structures are critical for specific cell–cell adhesion during normal epithelial homeostasis. These structures include adherens junctions, desmosomes and tight junctions. Proper functioning of the adherens junctions requires interaction between the cytoplasmic tails of classic cadherins (*e.g.* E-cadherin) and β-catenin. Association of p120-catenin (p120ctn) with the jux-

Abbreviations: BECs, bile duct epithelial cells; DEN, diethylnitrosamine; DPM, ductal plate malformation; HCC, hepatocellular carcinoma; IHBD, intrahepatic bile duct; p120LiKO, with knockout of p120 catenin in liver-specific hepatoblasts and cholangiocytes.

* Corresponding author at: Molecular Cell Biology Unit, Inflammation Research Center, VIB, Ghent, Belgium
** Corresponding author at: Department of Basic Medical Sciences, Faculty of Medicine and Health Sciences, Ghent University, De Pintelaan 185, Building B, B-9000 Ghent, Belgium.
E-mail addresses: jolanda.vanhengel@ugent.be (J. van Hengel), frans.vanroy@ugent.be (F. van Roy).

http://dx.doi.org/10.1016/j.ejcb.2016.10.003
0171-9335/

tamembrane domain of E-cadherin stabilizes E-cadherin at cell–cell contacts (Ireton et al., 2002). All hepatocytes of normal mouse liver express N-cadherin. Hepatocytes in the periphery of the lobules coexpress E-cadherin, but hepatocytes around the central veins do not (Doi et al., 2007; Kozyraki et al., 1996; Straub et al., 2011). Moreover, E-cadherin is present in the lateral membranes of mouse BECs, while N-cadherin is not.

Mice lacking E-cadherin in both hepatocytes and BECs develop periportal inflammation via an impaired intrahepatic biliary network (Nakagawa et al., 2014). The roles of β-catenin and plakoglobin in liver morphogenesis, regeneration and carcinogenesis have been examined using conditional β-catenin– or plakoglobin- knockout (KO) mice. Specific deletion of β-catenin in the foregut endoderm and in mouse liver as early as E8 to E8.5 (utilizing Foxa3-Cre transgenic mice) was lethal for E17 embryos, which were overall deficient in parenchymal hepatocytes (Tan et al., 2008). However, when β-catenin was conditionally knocked out in hepatoblasts (E10) using the same Alb-Cre transgene as in our present study, liver mass was reduced by only 10–20% (Sekine et al., 2006; Tan et al., 2006). Loss of plakoglobin in the liver using the same Alb-Cre transgene results in a lack of an overt phenotype (Zhou 2015). The hepatic role of the related p120ctn has not been reported, though it is important in the embryogenesis of several tissues (Davis and Reynolds, 2006; Elia et al., 2006; Oas et al., 2010; Perez-Moreno et al., 2006; Smalley-Freed et al., 2010).

The p120ctn family consists of four proteins: p120ctn, ARVCF, and the more distantly related δ-catenin and p0071 (McCrea and Park, 2007). *In vitro*, both δ-catenin and ARVCF can functionally substitute for p120ctn in stabilizing cell surface E-cadherin (Davis et al., 2003). p120ctn can stabilize many cadherins, including E- and N-cadherin (Reynolds, 2007). Numerous studies demonstrate that p120ctn can also intersect with a range of signal transduction molecules, including Rho-family GTPases and the transcriptional repressor Kaiso (Anastasiadis et al., 2000; Daniel and Reynolds, 1999).

Phenotypes associated with p120ctn ablation *in vivo* are largely tissue-dependent and therefore poorly predictable (Bartlett et al., 2010; Davis and Reynolds, 2006; Elia et al., 2006; Hendley et al., 2015; Kurley et al., 2012; Marciano et al., 2011; Oas et al., 2010; Perez-Moreno et al., 2006; Schackmann et al., 2013; Smalley-Freed et al., 2010, 2011; Stairs et al., 2011; Tian et al., 2012). We analyzed the functions of p120ctn in hepatocytes and BECs *in vivo* by ablating p120ctn early in development using AlbCre in $p120^{fl/fl}$ mice. These mice exhibited ductal plate malformation (DPM) and were more sensitive to development of diethylnitrosamine (DEN)-induced hepatocellular carcinoma (HCC). This is the first description of the phenotype of p120LiKO mice.

2. Materials and methods

2.1. Generation of p120ctnfl/fl;AlbCre mice

Transgenic $p120ctn^{fl/fl}$ mice (Davis and Reynolds, 2006) (courtesy of Albert Reynolds, Vanderbilt University, Nashville) were crossed with AlbCre mice expressing Cre recombinase under control of a rat albumin promoter (Postic et al., 1999). All the mice analyzed had a genetically mixed background of C57BL6, 129, and Swiss strainsMice were maintained in standard SPF housing according to the European rules on animal welfare.

2.2. DEN model of hepatocellular carcinoma

A single injection of DEN (20 μg/g) was administered to 15-day old p120LiKO mice and control littermates. Mice were killed at 26–50 weeks of age to determine tumor incidence and size. Hepatic tissue was prepared for histology.

2.3. RNA isolation and quantitative RT-PCR

Total RNA was extracted from whole livers using the RNeasy Mini Kit (Qiagen, Venlo, the Netherlands), and 400 ng were used to synthesize cDNA using the iScript Kit (Bio-rad, Hercules, CA). To detect ARVCF, p0071 and δ-catenin transcripts, primer pairs were designed so that amplicons were intron spanning. The resulting RT-PCR products were cloned in pGEMTeasy (Promega, Madison, WI) and sequenced to confirm their specificity. RT-PCR was performed with SYBR Green Master Mix Reagent (Applied Biosystems, Foster City, CA) using an ABI Light Cycler 480. Transcript quantities were determined using the method of ΔCt and values were normalized to *Hprt*.

2.4. Histology and immunohistochemistry

Paraffin sections were contrasted with hematoxylin and eosin. The following antibodies were used: anti-E-cadherin (BD Transduction Labs, San Jose, CA), anti-p120ctn and FITC-conjugated anti-p120ctn (BD Transduction Labs), anti-β-catenin (BD Transduction Labs and Sigma, St Louis, MO), anti-ZO-1 (Zymed, St Francisco, CA), anti-N-cadherin (Zymed), anti-connexin 32 (Zymed), anti-Dsp (R&D systems, Minneapolis, MN), anti-glutamine synthetase (GS; BD, Transduction Labs); anti-BrdU-POD (Roche, Basel, Switzerland), anti-p0071 (Hofmann et al., 2009), anti-ARVCF (Walter et al., 2008), anti-δ-catenin (BD Transduction Labs) and Troma-3, an anti-CK19 antibody (generously provided by Rolf Kemler, Freiburg, Germany). For detection of cadherins and catenins, rehydrated paraffin sections were pretreated with H_2O_2 (0.3% in methanol) for 45 min and with citrate buffer in a 2100 Retriever (PickCell Laboratories, Amsterdam, The Netherlands). For detection of CK19 the rehydrated paraffin sections were pretreated with H_2O_2 followed by incubation for 30 min in EDTA-buffer (pH 9) at 98 °C. Labeling with 5-bromo-2′-deoxyuridine (BrdU, Roche) was by injecting mice two hours before sacrificing. BrdU-positive cells were detected with anti-BrdU-peroxidase (Roche). For electron microscopy analysis small fragments of liver tissue (6 mice of five days old and 2 mice of 17 weeks old) were immediately fixed in 2.5% glutaraldehyde and further prepared for routine electron microscopy. Ultrathin sections were examined in a Zeiss EM 900 electron microscope (Jena, Germany).

2.5. Immunofluorescence analysis

Livers were frozen in cryo-embedding compound. Sections were air-dried for 1 h and fixed in 4% paraformaldehyde. Cells were permeabilized with 0.2% Triton X-100 in PBS. The detection of the primary antibodies was done with Alexa coupled secondary antibodies (Molecular probes, Eugene, OR). Sections were embedded in Vectashield with DAPI (Vector, Burlingame, CA) and examined with a Zeiss Axiophot or a confocal microscope (Leica, Mannheim, Germany).

2.6. Biochemical analysis

Serum samples of knockout and age matched control mice were analyzed at the clinical-chemical and hematological laboratory of the German Mouse Clinic (GMC) using an AU400 autoanalyzer (Olympus) and adapted reagents (Olympus) (van Hengel et al., 2008a).