E-cadherin, guardian of liver physiology

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Summary

E-cadherin is a cell-cell adhesion molecule involved in epithelial cell behavior, tissue formation and cancer suppression. In the liver, E-cadherin is expressed by hepatocytes and biliary epithelial cells. The precise role of this protein in hepatic pathophysiology remains largely unknown. Recently, loss of E-cadherin in liver epithelial cell types has been shown to lead to periportal fibrosis and inflammation and to promote liver carcinogenesis.

Cell junctions, such as tight junctions and adherens junctions, organize and protect epithelia. E-cadherin, a major constituent of adherens junctions, establishes cell-cell interactions in epithelia but also participates in several cellular processes such as differentiation and cell signaling [1]. In liver, E-cadherin is expressed by hepatocytes and biliary epithelial cells, however its involvement in hepatic pathophysiology remains largely unknown [2]. Nakagawa *et al.* have recently shown that absence of E-cadherin leads to inflammatory biliary disease and liver cancer in mice.

Nakagawa *et al.* produced mice with a liver specific deletion of E-cadherin ($CDH1^{\Delta L}$) by crossing mice carrying floxed *CDH1* ($CDH1^{F/F}$) with mice expressing Cre recombinase under the control of the albumin promoter. In the $CDH1^{\Delta L}$ mice, E-cadherin expression was lost in hepatocytes and biliary epithelial cells of small ducts. Two month after birth, $CDH1^{\Delta L}$ mice spontaneously developed periportal inflammation. At 8-month of age, periportal fibrosis reminiscent of primary sclerosing cholangitis (PSC) was observed. Morphological analysis of cell junctions by electron microscopy showed no obvious abnormalities in liver epithelial cells. However, injection of fluorescent-labeled bile acid to $CDH1^{\Delta L}$ mice led to an abnormal canalicular staining and an absence of fluorescence in the bile duct lumen, suggesting that the cholestatic phenotype may be due to a dysfunctional intrahepatic biliary network.

In order to clarify the respective impact of hepatocytes and biliary epithelial cells in the phenotype of the $CDH1^{\Delta L}$ mice, the authors invalidated specifically E-cadherin in hepatocytes or biliary epithelial cells. E-cadherin expression was invalidated in 73.0 ± 4.2% of hepatocytes by injecting an adenovirus expressing Cre-recombinase to $CDH1^{F/F}$ mice. In these mice, no sign of periportal inflammation nor of portal fibrosis was observed. Conversely, when E-cadherin was deleted in 31.7 ± 7.2% of biliary epithelial cells by crossing $CDH1^{F/F}$ mice with mice carrying tamoxifen inducible Cre-ERT in the K19 locus, periportal inflammation was evidenced in half of the mice. Furthermore, patients with PSC

displayed abnormal expression of E-cadherin in biliary epithelial cells but not in hepatocytes. Taken together, these results suggest that abnormal expression of E-cadherin in biliary epithelial cells may lead to the development of a PSC-like phenotype.

In order to better define the molecular mechanisms leading to a PSC-like phenotype, the authors performed a cDNA microarray analysis of the *CDH1*$^{\Delta L}$ mouse liver. Expression of progenitor cell markers such as Sox9, CD44 and Epcam was increased in *CDH1*$^{\Delta L}$ mice compared to wild type mice. These progenitor cell markers were mainly expressed in primitive duct cells involved in the ductular reaction. Immuno-histological analysis of Ki67 expression indicated that in *CDH1*$^{\Delta L}$ mice primitive duct cells actively proliferate, while hepatocytes do not. In *CDH1*$^{\Delta L}$ mice, liver expression of proinflammatory mediators was also increased, while the number of infiltrated macrophages was higher. Interestingly, proinflammatory cytokines have been implicated in hepatic progenitor cell induction. Accordingly, macrophage depletion induced by liposomal clodronate reduced ductular reaction in *CDH1*$^{\Delta L}$ mice.

At 11-month of age, liver tumors were evidenced in 16.7% of *CDH1*$^{\Delta L}$ mice, suggesting that the absence of E-cadherin can directly lead to the development of tumors. To investigate the mechanisms leading to liver cancer in these mice, the authors generated *CDH1*$^{\Delta L}$ mice expressing active hepatic *Kras* (*Kras*/*CDH1*$^{\Delta L}$ mice). E-cadherin loss significantly accelerated tumorigenesis induced by Ras activation in mice, as shown by tumor number and size evaluation. All *Kras*/*CDH1*$^{\Delta L}$ mice developed hepatocellular carcinoma (HCC) tumors. Cholangiocellular carcinoma (CCC) and mixed type HCC/CCC tumors were also seen in 10% and 40% of the mice, respectively. The enhanced carcinogenesis observed in *Kras*/*CDH1*$^{\Delta L}$ mice was ascribed to an increase in the phosphorylation of ERK and of the epithelial growth factor receptor, EGFR. Because EGFR is a potent inducer of epithelial-mesenchymal transition (EMT) in liver tumor cells, the authors next evaluated EMT features in the hepatic

tumors of *Kras*/*CDH1*$^{\Delta L}$ mice. Loss of E-cadherin was accompanied by an increased expression of both mesenchymal (*i.e.* vimentin) and stem cell markers (*i.e.* CD44 and Sox9) in these tumors and in human HCC derived cell lines. Furthermore, small nodules near tumors expressed the same types of markers suggesting intrahepatic metastatic processes. Thus, these results suggest that loss of E-cadherin has a causal role in EMT and the invasive phenotype of liver cancer.

In summary, Nakagawa *et al.* show that loss of E-cadherin in biliary epithelial cells leads to inflammation and subsequent periductal fibrosis reminiscent of PSC. Furthermore, the work of Nakagawa *et al.* indicates that loss of E-cadherin in hepatocytes and biliary epithelial cells accelerates liver carcinogenesis and favors tumor invasiveness.

The establishment of cell junctions between epithelial cells is crucial for the normal organization of liver functions, such as bile secretion [1]. Consistently, alterations in tight junctions have been related to cholestatic liver diseases, such as the neonatal ichthyosis and sclerosing cholangitis (NISCH) syndrome [3], primary biliary cirrhosis (PBC) and PSC [4]. Furthermore, expression of E-cadherin may also be altered in the liver of patients with arthrogryposis, renal dysfunction and cholestasis (ARC) [5], a syndrome for which liver features include paucity of bile ducts, cholestasis and mild inflammation [6]. We have recently shown that, during experimental bile duct obstruction, adherens junctions were altered by the expression of a truncated form of E-cadherin. Furthermore, our data indicate that liver injury was exacerbated when the truncated E-cadherin expression was increased [7]. Here, Nakagawa *et al.* show that the absence of hepatic E-cadherin leads to a cholestatic phenotype evocative of PSC. Adherens junctions in hepatocytes and biliary epithelial cells were not altered in 2-month old *CDH1*$^{\Delta L}$ mice, as shown by electronic microscopy analysis. However, bile acid secretion into bile ducts was impaired in these mice. The latter observation could reflect adaptive mechanisms able to compensate for adherens junctions organization but

not for E-cadherin signaling functions. In this context, the analysis of the expression and localization of transporters in hepatocytes and biliary epithelial cells would be valuable. In the adult mouse liver, E-cadherin expression is restricted to peri-portal hepatocytes, while in peri-venous hepatocytes N-cadherin is expressed [8]. Because E-cadherin and N-cadherin zonation takes place after birth [8], N-cadherin could compensate for the hepatic absence of E-cadherin. Furthermore, adherens junctions morphological analysis was performed in 2-month old animals while the cholestatic phenotype was evident at 8-month of age (ALP levels, histology). Thus, altered adherens junctions morphology may appear later in the *CDH1*$^{\Delta L}$ mice as we have previously shown that E-cadherin alterations were increased when cholestasis was established in *Vdr*$^{-/-}$ mice [7].

CDH1$^{\Delta L}$ mice show signs of ductular reaction and portal inflammation at 2-month of age. The latter observations may account for the inflammatory and proliferative effects of bile acids on liver cells [9, 10]. However, eight weeks after E-cadherin expression was specifically suppressed in hepatocytes, no sign of portal inflammation or ductular reaction could be evidenced. In contrast, specific invalidation of E-cadherin in biliary epithelial cells was associated with portal inflammation and ductular reaction. Consistently, mice invalidated for hepatic E-cadherin that maintain E-cadherin expression in non-hepatocyte cells do not show ductular proliferation and portal fibrosis [11]. Thus, accumulation of bile acids in the portal space may be the first trigger inducing inflammation and proliferation of biliary epithelial cells. Proliferating biliary epithelial cells devoid of E-cadherin may be unable to assemble new adherens junctions, as previously described in other epithelial cells [12], leading to the formation of leaky bile ducts. Leaky bile ducts would then favor the release of toxic compounds, such as bile acids and endotoxins, from bile. The latter would lead to the recruitment of macrophages that are major inducers of the ductular reaction through the

release of inflammatory cytokines (*i.e.* IL-6). Thus, a vicious circle of inflammation and proliferation is set by the absence of E-cadherin in the portal space.

The ductular reaction observed in *CDH1*$^{\Delta L}$ mice was associated with an increased expression of K19. The over–expression of K19 is indicative of biliary epithelial cell proliferation, but also of the presence of bi-potential hepatic progenitor cells. Bi-potential hepatic progenitor cells have the ability to differentiate either into hepatocytes or biliary epithelial cells [13] and may be involved in the development of HCC and CCC [14]. Consistently, a few *CDH1*$^{\Delta L}$ mice spontaneously developed liver tumors. Furthermore, all *CDH1*$^{\Delta L}$ mice in which hepatic Ras signaling is active (*Kras*/*CDH1*$^{\Delta L}$ mice) developed tumors (*i.e.* HCC, CCC or HCC/CCC tumors). The fact that the number of tumor rises when carcinogenesis is primed in mice deficient for liver E-cadherin, indicates that E-cadherin is mainly involved in the progression of the primary tumors. Consistently DEN-induced HCC tumor growth is accelerated in liver of E-cadherin deficient mice [11].

Analysis of cell signaling indicates that EGFR activation is central in liver tumorigenesis of *Kras*/*CDH1*$^{\Delta L}$ mice. E-cadherin and EGFR have well documented interactions in the context of cancer [15-17]. In CCC cells, E-cadherin colocalizes with EGFR [18], while E-cadherin displays inhibitory activities towards EGFR [17]. Thus, absence of E-cadherin may favor cell proliferation, migration and invasion by activating EGFR pathways [17, 19, 20]. Furthermore, we have recently shown that CCC cells produce TGF-β1 that in turn activates myofibroblasts from the tumor stroma. These myofibroblasts will produce HB-EGF that in a paracrine loop activates EGFR on CCC cells [21]. Thus, the absence of E-cadherin in biliary epithelial cells should activate the EGFR pathway leading to TGF-β1 production. TGF-β1 will increase HB-EGF production by mesenchymal cells of the portal space that will in turn favor biliary epithelial cell proliferation and EMT through EGFR signaling [22-24]. Interestingly, the observation of CCC tumors in mice lacking hepatic E-cadherin recapitulates the

pathophysiological continuum of PSC, a well-known pathology associated with CCC development [25].

In conclusion, Nakagawa *et al.* show that dysregulation of E-cadherin expression in epithelial liver cells is sufficient to induce portal fibrosis and liver cancer. Even though it is still unclear how the specific absence of E-cadherin leads to a PSC-like phenotype, the demonstration that PSC may arise solely from biliary epithelial cells is a disruptive concept. Finally, the authors also demonstrate that the absence of E-cadherin in liver epithelial cells favors carcinogenesis and intrahepatic metastasis. The origin of these tumors needs however further clarifications. Taken together, the observations of Nakagawa *et al.* suggest that stabilization of adherens junctions in epithelial liver cells may prevent inflammatory biliary disease and liver cancer.

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