Editorial

Angiogenic factors in chronic liver diseases: the effects on hepatic progenitor cells

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The biliary tree is a complex network of interconnected ducts, lined by epithelial cells called cholangiocytes, that drains the bile produced by the liver into the duodenum (1). At the periphery of the biliary tree, the interlobular bile ducts continue with the bile ductules, which are entirely constituted by cholangiocytes, may traverse the limiting plate, and are connected with the canal of Hering (2). The latter represents the physical link between the bile canaliculus (formed by the apical membranes of hepatocytes) and the biliary tree, and is thought to be an intrahepatic stem cell niche, harboring the multipotent hepatic stem/progenitor cells (HPCs) (3). To this end, the canal of Hering acts as the centre from which the maturation of both hepatocytes and cholangiocytes arises and proceeds in opposite directions. Indeed, HPCs can give rise to hepatoblasts that are also present as single cells or small aggregates in the canal of Hering and are bipotent cells that can further differentiate into committed progenitors of both the hepatocytic and cholangiocytic lineages. While committed hepatocytic progenitors proceed towards to central vein of the lobule to fully differentiate into mature hepatocytes, cholangiocytic progenitors, also known as "small cholangiocytes", are thought to move towards the periphery of the biliary tree (4). Recent studies suggest that small cholangiocytes, which can be found up to the bile ductules, are the precursors of large cholangiocytes that, on the other hand, line the interlobular (or larger) bile ducts (5). However, peribiliary glands, which are located in the wall of extrahepatic and large intrahepatic bile ducts, also contain progenitor-like cells and might contribute to or drive the maturation of cholangiocytes (6).

In normal condition, HPCs are quiescent cells with a low proliferation rate. Also in case of liver injury, the contribution of the stem cell niche to the regeneration of hepatocytes or cholangiocytes does not become evident until the intrinsic proliferating capacity of mature epithelial cells is overcome. In several human liver diseases a prominent activation of the stem cell niche has been described and the activation of HPCs and progenitor cells is characterized by the appearance of the so-called "ductular reaction", a proliferation of different cells which is characterized by a ductular phenotype. The proliferation of cholangiocytes in the context of the ductular reaction has been classically divided into 4 subclasses (7). However, a certain degree of overlap exists between them and some authors question the validity of the distinction (2).

Research of the recent years has also clarified that, in the course of damage, proliferating cholangiocytes undergo substantial modifications of their phenotype and develop neuroendocrine-like features (7). Activated cholangiocytes have been indeed showed to acquire neuroendocrine granules and markers such as chromogranin A, to express the parathyroid hormone-related peptide, and to synthesize, secrete and respond to a variety of hormones, growth factors and cytokines (7). The neuroendocrine-like transdifferentiation of the activated cholangiocytes in the course of liver injury places these cells in the middle of complex and still not completely defined interactions with all the other cells that populate the liver, such as hepatocytes, endothelial cells, HPC and also with immune cells that are recruited in the liver in case of damage (7).

The release of vascular endothelial growth factors (VEGFs) is a well-known characteristic of proliferating cholangiocytes. The family of VEGFs includes the cytokines VEGF-A, -B, -C, -D, -E, angiopoietin-1 (Ang-1), and the placenta growth factor. VEGFs are mitogen for endothelial cells and regulate many aspects of their biology, including permeability, vascular dilatation, migration, and survival, and their effects are mediated by three different tyrosine kinase receptors, called VEGFR-1, -2, and -3. The expression of the VEGFs and their receptors is not restricted to endothelial cells but has been found in a number of different tissues. Among them, cholangiocytes have been recently demonstrated to secrete VEGF-A and VEGF-C and to express VEGFR-2 and VEGFR-3 (8). VEGF isoforms regulate in an autocrine fashion the proliferative response of biliary cells via activation of the inositol 1,4,5-triphosphate/Ca^{2+}/PKC-α pathway and via phosphorylation of Src and ERK1/2. The administration of anti-VEGF antibodies to BDL rats significantly decreased the proliferation of cholangiocytes and increased the number of apoptotic cells, whereas the administration of recombinant VEGF induced proliferation of cholangiocytes in normal rats. Interestingly, the administration of anti-VEGF antibodies could also prevent the adaptive modifications that occur in the microvascular network that supply the intrahepatic biliary tree during the course of BDL (8).

The blood supply of the intrahepatic biliary tree is provided by a specialized capillary network, called peribiliary plexus (PBP), which runs along the biliary tree. The PBP originates from branches of the hepatic artery and nourishes cholangiocytes up to the interlobular bile ducts, whereas bile ductules and the canal of Hering lack a direct vascularization. Several reports indicate that the changes in cholangiocytes biology occurring during animal models of injury or in human liver diseases are associated with adaptive modifications of the PBP. Previous scanning electron microscopy studies of vascular corrosion casts indicated that the PBP of rats proliferates after BDL but only after the expansion of the biliary epithelial cells, thus providing nutritional support to the increased metabolic activity of the reactive cholangiocytes (9). To this extent, the VEGF secreted by cholangiocytes is thought to be of major importance in driving the adaptive changes of the PBP. The interrelations between the two compartments are even more complex. The ligation of the hepatic artery in BDL rats has been shown to blunt the proliferative response of the PBP after BDL and, interestingly, to decrease the expression of VEGF-A in cholangiocytes, despite the usual induction of VEGF production by hypoxia. The ligation of the hepatic artery was also associated with increased apoptosis and impaired expansion of cholangiocytes after BDL and the chronic administration of recombinant VEGF could prevent both phenomena (10). These findings suggest a complex and bidirectional interaction between the biliary cells of the ductular reaction and the endothelial cells of the PBP.

The role of VEGF has been further analysed by different groups in different contexts. Fabris *et al*. explored the relationship between intrahepatic bile duct and PBP during the development of the liver in fetal tissues at different gestational ages and in a model of defective biliary morphogenesis in mice (11). Developing bile ducts and hepatocytes of the ductal plate highly express VEGF (and VEGFR-1) and angiopoietin-1, respectively, while the specific cognate receptors for the two growth factors are differentially expressed by vascular cells, according to their maturational state. The authors suggested that the VEGF secreted by cholangiocytes is likely to create a gradient that attracts and stimulates vascular cells, while the angiopoietin-1 secreted by hepatocytes contributes to the remodelling of the vascular structures (11). A similar upregulated expression of VEGF, VEGFR-1 and VEGFR-2 has also been described in cholangiocytes of patients affected by the autosomal dominant polycystic kidney disease (ADPKD), in which the involvement of the liver consists in the development of biliary cyst (12).

Given the pro-proliferative effects of VEGF on reactive cholangiocytes, an obvious interest for this angiogenic factor has also been raised in cholangiocarcinoma. In this context, VEGF has been found to be overexpressed in different cholangiocarcinoma cell lines (13) and an interaction between TGF-beta 1 and VEGF has been proposed as a possible factor determining a malignant phenotype of cholangiocarcinoma (14). Furthermore, estrogens have been shown to stimulate the expression of VEGF and thereby to induce the proliferation of human cholangiocarcinoma cell lines (15).

In the present issue of *Hepatobiliary Surgery and Nutrition*, Franchitto *et al*. explored an additional role of angiogenic factors in the complex scenario of ductular reaction, and investigated the expression of VEGFs and their receptors in HPCs during chronic liver diseases in human. The authors first evaluated by a semi-quantitative analysis the immonohystochemical staining for the epithelial cell adhesion molecule (EpCAM) within the reactive bile ductules, as a marker for the HPC compartment, in controls and in patients affected by liver cirrhosis due to hepatitis C

virus (HCV) infection or primary biliary cirrhosis (PBC). As expected, in the diseased liver the authors found an extensive expansion of the EpCAM positive cells. Interestingly such an increase was significantly higher in PBC, where a positive staining could also be found in the cirrhotic nodules, compared to HCV samples. In this context, EpCAM positive hepatocytes have been lately shown to be the recent progeny of HPCs via intermediates of the cells of the ductular reaction (16). In line with previous studies, the number of proliferating biliary cells was much higher in the two pathological conditions than in control livers and the reactive ductules were situated in the fibrous septa of the cirrhotic livers and at the interface with cirrhotic nodules. In parallel, the authors could demonstrate, by immunohistochemistry for the Von Willebrand factor, that the extent of vascular proliferation was significantly increased in pathological conditions, with a more prominent angiogenesis in PBC samples. In normal livers, HPCs were virtually negative for VEGF-A, VEGF-C, and VEGFRs. However, in pathological conditions the number of VEGF-expressing HPCs increased, in particular in PBC patients where, significantly, they correlated with the extension of both ductular reaction and angiogenesis. VEGFR-1 and VEGFR-3 (but not VEGFR-2) were also found to be expressed by HPCs. Taken together these data provide evidence that the stem cell compartment of the liver may take part to a similar cross-talk to the one between reactive cholangiocytes and endothelial cells in the course of liver damage.

The exact sequence of events that are elicited in the course of chronic liver injury is yet to be elucidated. This is particularly true in chronic cholestatic conditions, in which the activation of the stem cell compartment is often not clearly distinguishable from the proliferation of already formed cholangiocytes. Such a concept is brought forward by the current study by Franchitto *et al*.: HPCs express both VEGFs and their receptors, in a fashion similar to what was previously shown for the biliary epithelium. Studies on the role of HPC in the pathophysiology of chronic liver diseases are indeed limited by the absence of a precise molecular marker that may unequivocally identify those cells. As an example, cholangiocytes of normal livers but also intermediate cells generated in course of ductular reaction have been shown to have a positive cytoplasmatic staining for EpCAM (16).

The understanding of the mechanisms that regulate the proliferation of cholangiocytes and their blood supply has important implications in different liver diseases. In cholangiopathies such as PBC the balance between the reactive proliferation and the damage of the biliary cells is believed to tend towards a predominance of the apoptotic processes during the course of the disease and therefore sustaining the proliferation of cholangiocytes via VEGF analogues seems an attractive possibility. On the other hand, the uncontrolled expansion of biliary cells that occurs in cholangiocarcinoma might be counteracted via the use of neutralizing anti-VEGF antibodies.

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