Histol Histopathol (2014) 29: 33-44
DOI: 10.14670/HH-29.33

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Review

Hypoxia, hypoxia-inducible factors and fibrogenesis in chronic liver diseases

Stefania Cannito[1,3], Claudia Paternostro[1,3], Chiara Busletta[1,3], Claudia Bocca[1,2,3], Sebastiano Colombatto[2,3], Antonella Miglietta[1,3], Erica Novo[1,3] and Maurizio Parola[1,3]

[1]Department of Clinical and Biological Sciences, Unit of Experimental Medicine and Clinical Pathology, [2]Department of Oncology and [3]Interuniversity Centre of Hepatic Pathophysiology, University of Torino, Italy

Summary. Fibrogenic progression of chronic liver diseases (CLDs) towards the end-point of cirrhosis is currently regarded, whatever the aetiology, as a dynamic and highly integrated cellular response to chronic liver injury. Liver fibrogenesis (i.e., the process) is sustained by hepatic populations of highly proliferative, pro-fibrogenic and contractile myofibroblast-like cells (MFs) that mainly originate from hepatic stellate cells (HSC) or, to a less extent, from portal fibroblasts or bone marrow-derived cells. As is well known, liver fibrosis (i.e., the result) is accompanied by perpetuation of liver injury, chronic hepatitis and persisting activation of tissue repair mechanisms, leading eventually to excess deposition of extracellular matrix (ECM) components.

In this scenario, hypoxic areas represent a very common and major feature of fibrotic and cirrhotic liver during the progression of CLDs. Cells exposed to hypoxia respond by means of heterodimeric hypoxia-inducible factors (HIFs) that translocate into the nucleus and binds to a specific core sequence defined hypoxia-responsive element (HRE), present in the promoter on several genes which are considered as hypoxia-regulated target genes. HIFs transcription factors can activate a complex genetic program designed to sustain several changes necessary to efficiently counteract the decrease in oxygen tension. Accordingly, hypoxia, through up-regulation of angiogenesis, is currently believed to significantly contribute to fibrogenic progression of CLDs, mostly by affecting the pro-fibrogenic and pro-angiogenic behaviour of hepatic MFs. In addition, experimental and clinical evidence generated in the last decade also indicates that angiogenesis and fibrogenesis in CLDs may also be sustained by HIF-dependent but hypoxia-independent mediators.

Key words: Hypoxia, Hypoxia-inducible factors, Liver fibrogenesis, Chronic liver diseases, Liver angiogenesis

Offprint requests to: Prof. Maurizio Parola, Department of Clinical and Biological Sciences, Unit of Experimental Medicine and Clinical Pathology, School of Medicine - University of Torino, C.so Raffaello 30, 10125 Torino, Italy. e-mail: maurizio.parola@unito.it

Hypoxia and hypoxia-inducible factors (HIFs): introductory remarks

Availability of molecular oxygen (O_2) is critical for cells, tissues and the overall aerobic organism because it serves as the final electron acceptor in oxidative phosphorylation, being then crucial for respiratory activity. On the other hand, mitochondrial respiration can also potentially lead to intracellular generation of reactive oxygen species (ROS) and related reactive intermediates, all able to react with major cellular macromolecules, possibly resulting in redox changes or even oxidative stress, cell injury and death. For these reasons aerobic organisms have progressively developed and refined during evolution several cellular and systemic mechanisms able to carefully control oxygen homeostasis and sensing, as a critical issue in tuning and/or affecting either physiological or disease processes. Along these lines, hypoxia has been defined as a state of weakness of available oxygen in the blood and tissues and can result from either partial reduction of oxygen pressure, inadequate oxygen transport or

inability of tissue to use oxygen (Sharp and Bernaudin, 2004). If one has in mind a tissue scenario, hypoxia can be foreseen as an oxygenation state which is below the norm for a particular tissue, with average tissue partial pressure of O_2 (pO_2) being usually over 20 mm of Hg. As a reference value, hypoxia and hypoxia-dependent adaptive responses are usually switched on when average tissue pO_2 is below 10 mm of Hg. In the presence of low oxygen availability, all nucleated cells in humans as well as other higher organisms can sense and respond to hypoxic conditions by employing the members of a specific family of evolutionary conserved transcription factors defined as hypoxia - inducible factors (HIFs). HIFs are heterodimeric proteins composed of a constitutively expressed HIF-ß subunit and an oxygen-sensitive HIF-α subunit that can respond to significant changes in oxygen tension (Semenza, 2011, 2012), with at present three distinct HIF-α isoforms (i.e., HIF-1α, HIF-2α and HIF-3α), having been characterized. The best characterized HIF is by far hypoxia-inducible factor 1 (HIF-1) which is expressed by all metazoan species analyzed to date, even the simplest ones (Loenarz et al., 2011), and is formed by HIF-1α and HIF-1ß subunits that all contain so-called basic helix-loop-helix-PAS (bHLH-PAS) domains that are critical for heterodimerization and DNA binding (Wang et al., 1995; Jiang et al., 1996), with HIF-1α protein levels believed to represent the real determinant of HIF-1 transcriptional activity (Semenza et al., 1996).

Under normoxic conditions specific enzymes use O_2 as substrate (and α-ketoglutarate as co-substrate) to introduce molecular changes in HIF-1α, including prolyl-hydroxylase 2 (PHD2) and factor-inhibiting HIF-1 (FIH-1), which can result in the hydroxylation of the HIF-1α subunit on proline and asparagine residues, respectively. When HIF-1α is hydroxylated on proline residues by PHD2 it binds to the von Hippel-Lindau protein (pVHL) that recruits an ubiquitin ligase that, in turn, targets the HIF-1α subunit for ubiquitin-proteosomal degradation (Kaelin and Ratcliffe, 2008). Asparagine hydroxylation by FIH-1 blocks the association of HIF-1α with the p300 coactivator protein, then repressing HIF-1 - mediated transcription (Lando et al., 2002).

Under hypoxic conditions enzymes leading to HIF-1α modification are inhibited and then HIF-1α subunits can accumulate in the cytoplasm of hypoxic cells and, in turn, dimerize with HIF-1ß subunits to form the heterodimeric HIF-1 factor. HIF-1 can then translocate into the nucleus and bind to specific consensus core HRE (hypoxia-responsive element) RCGTG (where R is A or G) sequences, present in the promoter on several hypoxia - regulated target genes (Semenza, 2011, 2012). Binding of HIF-1 to HRE sequences is known to activate a complex genetic program designed to sustain several changes necessary to efficiently counteract the decrease in oxygen tension (Semenza, 2001, 2004; Sharp and Bernaudin 2004). Among HIF-1 transcriptional targets there are genes involved in cell survival, glucose metabolism, cell death, differentiation, invasiveness and angiogenesis (Talks et al., 2000; Semenza, 2001, 2004, 2011, 2012; Carmeliet, 2003). However, the real scenario is likely to be much more complex since, in addition to genes which are over-expressed in such a way, it has also been reported that expression of several other genes is decreased in response to hypoxia in a HIF-1-dependent manner, possibly through HIF-1-dependent expression of transcriptional repressors, including microRNAs, since binding of HIF-1 to these genes was not detected (see for more details Semenza, 2012).

Although HIF-1α and HIF-2α share a high degree of sequence identity and regulation by prolyl and asparaginyl hydroxylation, as well as the ability to heterodimerize with HIF-1ß and to bind to HRE sequences in order to activate transcription of common genes, relevant differences have emerged for these two transcription factors (Patel and Simon, 2008; Keith et al., 2012). HIF-1α is expressed ubiquitously whereas HIF-2ß expression is restricted to vascular endothelial cells, neural crest-derived cells, type II pneumocytes, hepatocytes, cardiomyocytes and interstitial kidney cells (Talks et al., 2000; Keith et al., 2012). Moreover, HIF-2α (but not HIF-1α) up-regulates transforming growth factor-α and cyclin D1 genes as well as c-Myc activity, and its overexpression in human tumors has been described to correlate with a faster rate of neoplastic cell growth (Pouyssegur et al., 2006; Keith et al., 2012). HIF-2α has been also suggested, through up-regulation of KLF4, Sox2 and Octamer-4, to favor de-differentiation and confirmation of stem-cell properties of putative cancer stem cells and HIF-2α overexpression has been specifically described as a negative prognostic factor for malignant tumors (Keith et al., 2012). Less information is available concerning HIF-3α which might act even as an antagonist of the HIF system (Sharp and Bernaudin, 2004; Patel and Simon, 2008; Semenza, 2011).

The overall scenario of hypoxia-related cellular adaptive responses in physiological as well as pathological conditions, including those of malignant cancer cells, is extremely complex and includes a number of established events such as: i) regulation of glucose metabolism through up-regulation of genes codifying for glucose transporters and enzymes involved in anaerobic glycolysis; ii) up-regulation of genes codifying for enzymes, exchangers or transporters regulating intracellular pH; iii) vascular changes (for ex. through up-regulation of iNOS); iv) angiogenesis through up-regulation of relevant genes for either pro-angiogenic cytokines as well as of their related receptors; v) up-regulation of genes involved in the control of apoptosis; vi) modulation of cell cycle and proliferation; vii) modulation of migration and/or invasiveness as well as of the process of epithelial to mesenchymal transition.

A comprehensive list of ascertained or putative HIF targets as well as a deep analysis of the complex mechanisms regulating HIF-related signaling and

cellular responses would be well beyond the scope of this article and the interested reader may find more details in authoritative and exhaustive reviews (Pouyssegur et al., 2006; Kaelin and Ratcliffe, 2008; Semenza, 2011, 2012; Keith and Simon, 2012). In the present review we will instead focus attention on the role of hypoxia and HIFs in the progression of chronic liver diseases (CLDs), whatever the aetiology, towards the common end-point of cirrhosis and its complications, including hepatocellular carcinoma. In particular, according to the literature data that has emerged in the last decade, the main focus will be on the evidence that has unequivocally linked hypoxia and HIFs to angiogenesis and fibrogenic progression of CLDs.

Angiogenesis as a critical hypoxia-related response: general concepts

Angiogenesis is best defined as a dynamic, hypoxia-stimulated and growth factor-dependent process, leading to the formation of new blood vessels from pre-existing ones (Semenza, 2001, 2011, 2012; Carmeliet, 2003; Ferrara et al., 2003; Jain, 2003; Carmeliet and Jain, 2011). Angiogenesis should always be distinguished from other characteristic mechanisms of vessel growth. Vasculogenesis is the term which indicates a process leading to the formation of vascular structures through the recruitment of bone marrow-derived endothelial progenitor cells to be incorporated into nascent vessels or to stimulate new vessel growth by releasing pro-angiogenic factors. According to actual definitions, arteriogenesis is a process consisting of remodeling of existing blood vessels and subsequent stabilization of newly formed/nascent vessels via recruitment of smooth muscle cells. Collateral vessel growth is a process consisting of the expansive growth of pre-existing collateral vessels following occlusion of supply vessels. Along these lines, tumors can also employ an additional mode of vessel formation through co-option of pre-existing vessels or through formation of tumor vessels lined by tumor-derived cells (i.e., vascular mimicry) or by endothelial cells with cytogenetic abnormalities in their chromosomes and derived from putative cancer stem cells (Carmeliet and Jain, 2011).

As is well known, the main cells involved in angiogenesis are endothelial cells (ECs) and smooth muscle cells that, by constituting the first-line interface with blood, must necessarily have mechanisms to sense differences in the O_2 supply. This of course includes the already mentioned role of HIFs but also encompasses other classes of O_2-sensors that are expressed by these vascular cells, including O_2-sensitive NADPH oxidase, endothelial nitric oxide synthase (eNOS) and heme oxygenases (Ward, 2008). In the list should also be included the complex I and III of mitochondrial electron transport chain, resulting in H_2O_2 generation (Weir et al., 2005; Archer et al., 2008), which is a well known redox-mediator able to stabilize functional HIF-α subunits (Pouysségur and Metcha-Grigoriou, 2006).

Accumulating evidence indicates that hypoxia can control the fates of vascular cells already during embryo development by stimulating the differentiation of various progenitors, including those of early mesoderm, into hemangioblasts and then into endothelial cells in the process of vasculogenesis. Moreover, HIF-ß may act during the first steps of vascular development, through paracrine release of vascular endothelial growth factor (VEGF) and angiopoietin-1 (Ang-1) by hematopoietic cells (Ramirez-Bergeron et. al, 2006). However, hypoxia can also trigger differentiation of pulmonary artery endothelial cells into SMC-like cells (Zhu et al., 2006) and, even more relevant, has been reported to promote in the adult bone marrow (BM) the differentiation of endothelial progenitor cells (EPCs) into cells over-expressing the classic markers of mature vascular cells (Du et al., 2008; Tillmanns et al., 2008). Low oxygen tension has also been proposed to concur in the specification of EPCs toward an arterial rather than venous fate through Patel and Simon, 2008-mediated up-regulation of expression of Delta-like 4 (Dll4), a ligand of Notch receptor (Diez et al., 2007). The literature data also indicate that hypoxia, via HIF-1α-dependent up-regulation of stromal-derived factor 1 (SDF-1) by cells in hypoxic normal and tumor tissues, can recruit in these tissues various progenitors from bone marrow expressing SDF-1 receptor CXCR4, including circulating EPCs, pericyte progenitors and angio-competent CD45+ myeloid cells (Fraisl et al., 2009).

Interestingly, HIF-1α and HIF-2α seem to have different roles in vessel formation (Fraisl et al., 2009). HIF-1α is mostly involved in sustaining typical steps of angiogenesis, such as endothelial cell proliferation, migration and vessel sprouting, in addition to the mentioned role in recruiting EPCs and other progenitor cells, as well as of pericytes and smooth muscle cells and, more generally, in promoting formation of arteries. By contrast, HIF-2α, which is expressed in a subset of tissues and organs, including the liver, is actually believed to operate by regulating a distinct set of genes that, concerning angiogenesis, are likely to be mainly involved in controlling vascular morphogenesis, integrity and assembly.

One of the most impressive pro-angiogenic responses elicited by HIFs is the induction of VEGF expression with an increased transcription which is very rapid and highly significant (up to 30 fold, depending on the specific cell type). VEGF is a master pro-angiogenic cytokine that has been reported to sustain both physiological and pathological angiogenesis, whereas other members of the VEGF family, like for example placental growth factor (PlGF), may have a role only in pathological angiogenesis. Several other molecules/mediators are of course known to regulate angiogenesis but a detailed analysis of cellular and molecular mechanisms controlling angiogenesis is beyond the scope of the present study and the reader may refer to more exhaustive reviews for more details (Carmeliet, 2003; Ferrara et al., 2003; Pugh and

Ratcliffe, 2003; Medina et al., 2004; Dewhirst et al., 2008; Valfrè di Bonzo et al., 2009; Fernandez et al. 2009). In the present review we will focus only on those features that make unique the relationships between oxygen tension and angiogenesis in the liver. However, before leaving this section it is important to introduce the concept that HIF - related events, including angiogenesis, can also be regulated/modulated by a long list of hypoxia-independent factors and mediators which currently can include oncogenes, growth factors, cytokines, chemokines, adipokines, metabolic or mitochondrial stress and ROS which have been reported to lead to either increased HIF-1 mRNA transcription or, as for ROS following direct interaction, to HIF-1 increased stabilization (Dewhirst et al., 2008). This scenario, pertinent to the topic of this review, is likely to play a critical role either under conditions of CLDs or in the development of HCC.

Oxygen tension, hypoxia and angiogenesis in normal liver

The liver has a rather unique system of vascular supply with afferent blood flow deriving partly from highly oxygenated blood in the hepatic artery and mostly from oxygen-depleted blood in the hepatic portal vein. The directional flow of mixed oxygenated and deoxygenated blood from portal areas toward the central vein of the hepatic lobule creates a physiological oxygen gradient in which lower levels of oxygen tensions are found in perivenous portions of the liver parenchyma (Jungermann and Kietzmann, 2000). Indeed, the first rim of perivenular hepatocytes has been reported to positively stain for HIF-1α (i.e., in the nuclei) as well as for vascular endothelial growth factor A (VEGF-A), a typical HIF - related target gene (Bozova and Helpek, 2007).

Such a physiological gradient of oxygen tension across the hepatic lobule has been reported to display profound effects on the function of liver cell populations. Indeed, perivenous and periportal hepatocytes significantly differ for the expression of several enzymes of glucose metabolism or transport (Krones et al., 2001). This results in a scenario in which periportal hepatocytes, exposed to higher oxygen tension, have a major role in oxidative energy metabolism, glucose production, and synthesis of urea and bile, whereas perivenular hepatocytes are mainly specialized in glucose uptake, xenobiotic metabolism and glutamine formation (Jungermann and Kietzmann, 2000). Of interest, such a gradient of oxygen tension has potentially relevant consequences for the response of parenchymal cells to hypoxic stress, with perivenular hepatocytes suggested to be more sensitive to injury when exposed to conditions of low oxygen availability, such as liver ischemia and other pathological conditions (Krones et al., 2001).

These last concepts allow to introduce additional major evidence: although angiogenesis could be considered as beneficial for tissue growth and regeneration, as well as for growth and repair of injured tissues, it is well established that angiogenesis can also fuel inflammatory and fibro-proliferative diseases as well as malignancies, with newly formed vessels being also used by cancer cells to metastasize. Indeed, insufficient or altered normal vessel growth offer a major contribution to several diseases, including myocardial infarction, stroke, neurodegenerative and chronic inflammatory diseases, such as CLDs, pulmonary hypertension and cancer (Carmeliet, 2003; Ferrara et al., 2003; Pugh and Ratcliffe, 2003; Medina et al., 2004; Dewhirst et al., 2008; Valfrè di Bonzo et al., 2009; Fernandez et al. 2009; Carmeliet and Jain, 2011; Weis and Cheresh, 2011). In fact, liver angiogenesis has been detected either in liver regeneration (i.e., considered as a form of physiological angiogenesis) as well as in pathological conditions, including liver ischemia, chronic inflammatory and fibro-proliferative diseases, hepatocellular carcinoma (HCC) and metastatic liver cancers. In addition to what will be outlined in the next sections, it has been recently shown that mobilization and proliferation of EPCs are significantly enhanced in cirrhotic patients in comparison to controls; the Authors suggest that EPCs may play an important active paracrine role in liver angiogenesis by stimulating resident SECs in cirrhosis (Kaur et al., 2013).

Hepatic angiogenesis follows rules and steps which are common to other tissues and organs (see Medina et al., 2004) but, in addition, is affected by at least two relevant peculiarities of liver parenchyma. First, the existence in liver parenchyma of two different kinds of microvascular structures like portal vessels and liver sinusoids, which are lined by either continuous or fenestrated and discontinuous endothelium, respectively. Even more relevant, as we will see in the next sections, liver angiogenesis is influenced by the rather unique phenotypic profile and functional role of hepatic stellate cells (HSCs). HSCs, which are cells physiologically located in the space of Disse between the sinusoidal endothelium and hepatocytes, are at the same time currently regarded as the most relevant pro-fibrogenic cell lineage in CLDs but also as liver specific pericytes, at least in normal liver (Lee et al., 2007; Friedman, 2008; Parola et al., 2008; Dranoff and Wells, 2010, Forbes and Parola, 2011; Lee and Friedman, 2011).

Along these lines, revascularization after experimental partial hepatectomy (PH) can be regarded as the most appropriate model to investigate physiological hepatic angiogenesis (Medina et al., 2004). Two/third PH is followed by an immediate and strong proliferative response of hepatocytes that usually occurs as two peaks of DNA synthesis at 24 and 48 hrs. This proliferative response is mainly sustained by transforming growth factor α (TGFα) released by hepatocytes and HGF released by non-parenchymal cells. This early proliferative response involves mainly hepatocytes in the periportal areas and leads to the formation in the same areas of avascular (i.e., hypoxic)

clusters of hepatocytes which respond to hypoxia by up-regulating VEGF transcription within 48-72 hrs after PH. These hepatocytes then release VEGF in a paracrine fashion that can act on arteriolar and sinusoidal endothelial cells as well as on HSCs, which are the cells expressing VEGF receptors type 1 and 2, starting from 72 hrs after PH and persisting for several days. As a consequence, reconstitution of sinusoids by endothelial cells initiates in the same periportal areas, and VEGF has been suggested to also concur in sustaining hepatocyte proliferation and to prime ECs to express growth factors that may contribute to prevent hepatocyte injury (Medina et al., 2004).

At this point, a number of concepts that will be critical for the scenario of angiogenesis in CLDs (described in more details in the next sections), should be anticipated here: i) VEGF can exert a number of relevant effects on either HSCs, which can behave as liver-specific pericytes in physiological angiogenesis, due to their strategic location in the space of Disse and intimate contact with sinusoidal endothelial cells, as well as, in CLDs, on activated HSC/MFs; ii) as we will see, HSCs and HSC/MFs can produce and release VEGF when exposed to hypoxic conditions, then contributing to modulate angiogenesis in a way that differs from the one usually attributed to conventional pericytes. Actually, the current feeling is that the overall scenario in CLDs is even more complex considering that hepatic MFs represent a heterogeneous population of pro-fibrogenic, highly proliferative and contractile cells that have been reported to also originate from portal fibroblasts and bone marrow-derived stem cells recruited into the injured liver (Dranoff and Wells, 2010; Forbes and Parola, 2011; Lee and Friedman, 2011).

Hypoxia, angiogenesis and liver fibrogenesis in CLDs

Angiogenesis, from an historical point of view, has been mainly implicated in cancer, arthritis and psoriasis, as well as in many other diseases. However, the literature data of the last decade have outlined the occurrence of pathological angiogenesis in chronic liver diseases characterized by persisting inflammation, wound healing and fibrogenesis. Along these lines, hypoxia, through up-regulation of angiogenesis, is currently believed to exert a significant pro-fibrogenic role by affecting the responses of hepatic myofibroblasts (Friedman, 2008; Parola et al., 2008; Dranoff and Wells, 2010; Lee and Friedman, 2011; Forbes and Parola, 2011), including up-regulation of pro-angiogenic cytokines and increased proliferation, deposition of extracellular matrix (ECM) and migration/chemotaxis (Aleffi et al., 2005; Novo et al., 2007, 2011; Fernandez et al., 2009). The pro-fibrogenic and pro-angiogenic role of MFs is currently believed to be critical for the progression of CLDs towards cirrhosis and HCC, the latter being considered as a highly angiogenic tumor.

Chronic liver diseases and liver fibrogenesis: general concepts

CLDs are currently envisaged as conditions characterized by the reiteration of hepatocyte injury due to various aetiological conditions, including chronic infection by hepatotropic viruses (mainly hepatitis B and C viruses), autoimmune injury, as well as by metabolic and toxic/drug - induced causes, with chronic alcohol consumption being predominant in western countries. Reiterated liver injury results in persisting inflammatory response and in chronic activation of wound healing response: these two main events, together with oxidative stress and derangement of epithelial-mesenchymal interactions, represent the main "driving force" sustaining persistent liver fibrogenesis and then liver fibrosis (Friedman, 2008; Parola et al., 2008; Dranoff and Wells, 2010; Povero et al., 2010; Lee and Friedman, 2011; Forbes and Parola, 2011).

Hepatic fibrogenesis (i.e. the process leading to fibrosis, then to excess deposition of extracellular matrix components) should be regarded as a dynamic and highly integrated molecular, tissue and cellular process that leads to the replacement of low-density basement membrane of sub-endothelial space of Disse with fibril-forming matrix (Friedman, 2008; Parola et al., 2008). This progressive accumulation of ECM components results from a disequilibrium between excess deposition of fibrillar collagens and reduced/altered degradation/remodeling of fibrotic ECM. Persistent fibrogenesis is considered as responsible for CLDs progression to the end-points of liver cirrhosis and hepatic failure. Cirrhosis is currently defined as an advanced stage of fibrosis, characterized by the formation of regenerative nodules of parenchyma surrounded and separated by fibrotic septa, and associated with significant changes in organ vascular architecture, development of portal hypertension and related complications (variceal bleeding, hepatic encephalopathy, ascites and hepato-renal syndrome) (Friedman, 2008; Parola et al., 2008).

As previously proposed (see Parola et al., 2008) persistent fibrogenesis and cirrhosis have a major clinical impact because of the following considerations: i) approx. 170 million patients world-wide (28-30 million in Europe) are affected by a form of CLDs, of which 25-30% are expected to progress to cirrhosis; ii) cirrhosis is, among diseases of the GI tract, the most common non-neoplastic cause of death and represents the 7th most common cause of death in western countries; iii) mortality is even higher because HCC, a very aggressive cancer that almost invariably develops on the background of a cirrhotic liver, is the 5th most common cancer and the 3rd most common cause of cancer mortality worldwide (Llovet et al., 2003; El-Serag and Rudolph, 2007; Dufour and Johnson, 2010); iv) epidemiologists predict a peak for end-stage CLDs (and HCC) for the next decade (Llovet et al., 2003; El-

Serag and Rudolph, 2007; Dufour and Johnson, 2010), in parallel with a shortage of donor organs for ortothopic liver transplantation (OLT).

Hypoxia, liver angiogenesis and fibrogenic progression of CLDs

The literature data indicate that detection of hypoxic areas in liver parenchyma is a very common finding in the scenario of active CLDs and hypoxia is the most obvious (although not the only) stimulus able to switch on the transcription of pro-angiogenic genes through the action of HIFs (Medina et al., 2004; Fernandez et al., 2009; Valfrè di Bonzo et al., 2009). This statement seems rather obvious since angiogenesis is a common feature of any kind of wound healing and, as previously suggested, chronic activation of wound healing response represents the major driving force for progressive accumulation of ECM components, leading eventually to cirrhosis and hepatic failure.

CLD progression to cirrhosis is by itself an event favoring the development of hypoxia, being characterized by the formation of regenerative nodules of parenchyma, surrounded and separated by fibrotic septa, and associated with significant changes in angio-architecture (vascular remodeling). In this scenario the progressive increase of tissue hypoxia is then likely strictly linked to the anatomical modifications following the establishment of periportal fibrosis, with an increased contribution of the hepatic artery to the formation of sinusoidal blood. Sinusoidal blood flow becomes increasingly arterialized with hepatocytes adjusting to an abnormally high oxygen concentration. Subsequently, the progressive capillarization of sinusoids leads to an impairment of oxygen diffusion from the sinusoids to hepatocytes with the consequent up-regulation of pro-angiogenic pathways (Medina et al., 2004; Fernandez et al., 2009; Valfrè di Bonzo et al., 2009).

In the liver scenario of CLDs, both chronic inflammation as well as the specific pattern of fibrosis may further affect the extent of angiogenesis and favor CLD progression. Chronic inflammation is known to have a major role in CLD progression (Friedman, 2008; Parola et al., 2008; Dranoff and Wells, 2010; Povero et al., 2010; Lee and Friedman, 2011) and various inflammatory-related mediators generated during CLDs are known to stimulate cells in the microenvironment to express VEGF and other pro-angiogenic factors (Carmeliet and Jain, 2011). Similarly, several mediators known to be effectively generated during CLDs such as platelet-derived growth factor (PDGF), nitric oxide (NO) and hepatocyte growth factor (HGF) can contribute to angiogenesis.

On the other hand, the specific pattern of fibrosis (post-necrotic or bridging fibrosis, peri-cellular or peri-sinusoidal fibrosis, biliary fibrosis or centrilobular fibrosis, see Parola et al., 2008; Povero et al., 2010) can affect the extent of angiogenesis and favor CLD progression, representing at the same time a key limiting factor for fibrosis reversibility. Along these lines, one should recall the impressive histo-pathological scenario in liver specimens obtained, for example, from chronic HCV or HBV patients (i.e., patients in which post-necrotic or bridging fibrosis predominates) where neo-angiogenesis, vascular remodeling, altered angio-architecture and formation of bridging septa between portal and central vein areas are particularly impressive.

Evidence strictly correlating hypoxia, angiogenesis and fibrogenesis in CLDs is now consistent and supported by several unequivocal observations. The first emerged evidence has been provided by pathologists and scientists observing that angiogenesis and fibrogenesis were developing in parallel in both clinical and experimental conditions. In fact, all major human conditions of CLD (chronic HCV and HBV infection, alcoholic or autoimmune liver disease, or with either primary biliary cirrhosis or primary sclerosing cholangitis) as well as the most relevant animal models of CLDs are characterized by the presence of a high number of endothelial cells and microvascular structures in chronically injured liver, the latter being particularly evident in portal tracts and fibrotic septa (Medina et al., 2004; Fernandez et al., 2009; Valfrè di Bonzo et al., 2009; Rosmorduc and Housset, 2010). Histo-pathological evidence also confirmed a simple but critical issue: VEGF-A overexpression co-localizes with hypoxic areas in experimental and human CLDs and fibrogenic progression is intrinsically associated with a progressive increase in hypoxic areas in liver parenchyma undergoing chronic injury, from early changes to established cirrhosis (Corpechot et al., 2002a; Medina et al., 2004; Fernandez et al., 2009; Valfrè di Bonzo et al., 2009; Rosmorduc and Housset, 2010; Novo et al., 2012). The same experimental and clinical studies also revealed that the response to hypoxia and VEGF expression are features involving, in addition to endothelial cells, mainly hepatocytes and, as recently shown, HSC/MFs (Novo et al., 2007, 2012). In this connection, positive nuclear staining for HIF-2α was clearly identified in α-smooth-muscle actin (α-SMA)-positive MFs of fibrotic septa that, in turn, are located in close contact with HIF-2α-positive hepatocytes and then are likely to be affected in their behaviour by pro-angiogenic cytokines released by hypoxic parenchymal cells (Novo et al., 2007, 2012). Along these lines, MFs have been reported to represent an additional source of pro-angiogenic cytokines in CLDs (VEGF, angiopoietin 1) and related receptors (VEGFR-2, Tie- 2) (Novo et al., 2007), and HIF-2α–positive staining is mainly evident in MFs of developing septa and at the border of more mature and larger fibrotic septa (Novo et al., 2007, 2012). A role for HIF-1α in the progression of fibrogenesis has been unequivocally provided by experiments performed in HIF-1α liver-conditional knock-out mice using the bile duct ligation (BDL) model of biliary fibrosis (Moon et al., 2009) indicating that hypoxia and HIF-1α-recruitment preceded fibrosis and

that hepatocyte-specific silencing of HIF-1α resulted in a significant reduction of ECM deposition. Interestingly, hypoxia may indirectly favor the predominance of fibrogenic responses, also indirectly, by negatively affecting the hyperplastic response of hepatocytes: it has been shown that hypoxia is able to rapidly inhibit c-met expression in hepatocytes and also to down-regulate synthesis and release by HSCs of hepatocyte growth factor (HGF), the most potent mitogen for parenchymal cells (Corpechot et al., 2002b).

The strict relationships between angiogenesis and fibrogenesis in CLD progression have received direct confirmation by several studies indicating that experimental anti-angiogenic therapy is highly effective in significantly reducing fibrogenic progression. Whatever the specific drug or tool employed, anti-angiogenic therapy resulted not only in the reduction of angiogenesis but also in a significant reduction of the inflammatory infiltrate, the number of α-smooth muscle actin (α-SMA)-positive MFs, the extent of fibrosis and, in some models, even of portal pressure (Fernandez et al., 2009; Valfrè di Bonzo et al., 2009; Paternostro et al. 2010). To this purpose, several anti-angiogenic drugs and tools have been used in experimental animal models of fibrosis including: i) receptor kinase inhibitors like sunitinib (Tugues et al., 2007) or sorafenib (Mejias e al., 2009), with sunitinib recently shown to act directly on hepatic MFs by reducing collagen synthesis, migration and pro-angiogenic potential (Majumder et al., 2013); ii) antibodies versus either VEGF receptor type-2 (VEGFR-2) or PlGF (Yoshiji et al., 2003; Van Steenkiste et al., 2011); iii) adenovirus expressing soluble Tie-2 (AdsTie-2), the receptor for Ang-1, resulting in the block of Ang-1 signalling and in a significant prevention of both angiogenesis and fibrosis (Taura et al., 2008); iv) the angiostatic chemokine CXCL9, which displayed strong anti-proliferative and anti-migratory effects on VEGF-stimulated endothelial cells and HSC/MFs by down-regulating VEGFR-2, phospholipase CÁ and extracellular signal-regulated kinase (ERK) phosphorylation (Sahin et al., 2012); v) the JWH-015 agonist of cannabinoid receptor type 2 or CB2 (Huang et al., 2012); vi) a drug able to block apelin receptor APJ (Reichenbach et al., 2012); vii) the histone deacetylase inhibitor largazole, found to be effective by inhibition of both TGF, and VEGF-A signaling (Liu et al., 2013); viii) rifaximin-mediated de-contamination of mouse intestinal microbiota, reported to down-regulate the lipopolysaccharide (LPS)-Toll-like Receptor-4 (TLR-4) pathway (Zhu et al., 2012).

Although these experimental studies are unequivocal in their results, it is correct to say that the question of whether anti-angiogenic therapy has an adequate rationale to be considered for therapy in patients with cirrhosis and portal hypertension still remains unanswered and several concerns have been reviewed (Shah and Bruix, 2009) with caution advised, particularly on optimal dosage to be used and the knowledge of severe side effects that some of the employed drugs (for example sorafenib or bevacizumab plus sunitinib) have displayed in HCC patients. The need for caution seems even more obvious on the basis of recent studies which offer both positive as well as negative findings. For example, a study performed on a series of cirrhotic patients with advanced HCC and treated with sorafenib showed a significant decrease in portal venous flow of at least 36% (Coriat et al., 2011). On the other hand, a very recent systematic review and meta-analysis of anti-angiogenic studies in HCC from 1995 to 2011 has revealed that the use of sorafenib was associated with an increased risk of bleeding in HCC patients (Duffy et al., 2013).

Hepatic myofibroblasts in liver angiogenesis and fibrogenesis: hypoxia-dependent mechanisms

As already mentioned, VEGF over-expression is strictly associated with hypoxic areas and is mostly limited to hepatocytes as well as to activated HSC/MFs (Novo et al., 2007; Fernandez et al., 2009; Valfrè di Bonzo et al., 2009; Rosmorduc and Housset, 2010). Hepatic MFs, in particular HSC/MFs, in CLDs are likely to represent a hypoxia-sensitive and cyto- and chemokine-modulated cellular crossroad between critical features like necro-inflammation, pathological angiogenesis and fibrogenesis. This view is sustained by several studies that have outlined a number of major concepts. HSC and HSC/MFs are primary actors in angiogenesis since they respond to the exposure of low oxygen tension by up-regulating, in a HIF-1α-related way, expression of VEGF, Ang-1 as well as of their related receptor VEGFR-2 and Tie2 (Ankoma Sey et al., 2000; Wang et al., 2004; Aleffi et al., 2005; Novo et al., 2007). However, the same cells are also sensitive targets for the action of VEGF and Ang-1; VEGF has been reported to stimulate HSC/MFs proliferation and synthesis of ECM components (Corpechot et al., 2002b) as well as to stimulate oriented migration (i.e., chemotaxis) of these cells (Novo et al., 2012). Moreover, hypoxia itself is able to induce oriented migration of either HSC/MFs or MF-like mesenchymal stem cells from bone marrow by eliciting a biphasic mechanism: i) an early phase of migration switched on by ROS released by mitochondria and requiring redox-dependent activation of Ras/ERK and c-Jun-NH2-terminal kinase isoforms (JNKs); ii) a delayed and sustained phase of migration depending on HIF-1α-mediated, ROS-stabilized, up-regulation of VEGF expression, resulting in the subsequent chemotactic action of extracellularly released VEGF (Busletta et al., 2011; Novo et al., 2012). Relationships between hypoxia and ROS generation outlined in HSC/MFs have a counterpart in vivo in liver specimens from HCV cirrhotic patients, as documented by positive nuclear staining for HIF-2α and cytoplasmic staining for heme-oxygenase 1 (HO-1, a redox-dependent target gene) in hepatocytes of regenerative nodules and in cells of fibrotic septa having morphology of α-SMA-positive MFs (Novo et al., 2012). What is

interesting in this in vivo scenario is a critical correspondence: positive staining for both HIF-2α and HO-1 is mainly evident in MF-like cells in developing septa and at the border of more mature and larger fibrotic septa (Novo et al., 2012) and this remarkably corresponds to what was previously described for the expression of VEGF, Ang-1 and related receptors VEGFR-2 and Tie-2 (Novo et al., 2007). In both human and rat fibrotic/cirrhotic livers hepatic MFs able to express concomitantly VEGF, Ang-1 or the related receptors VEGFR-2 and Tie-2, were found at the leading edge of tiny and incomplete developing septa, but not in larger bridging septa. This specific distribution has been proposed to reflect two distinct phases of the angiogenic process during CLDs: i) an early phase, occurring in developing septa, in which fibrogenesis and angiogenesis may be driven/modulated by HSC/MFs, and then likely by hypoxia itself; ii) a later phase, occurring in larger and more mature fibrotic septa, where chronic wound healing is likely to be less active and fibrogenic progression more established. In such a late setting pro-angiogenic factors, particularly receptors like VEGFR-2 or Tie-2, are expressed only by endothelial cells, a scenario that is likely to favor stabilization of the newly formed vessels. Even more relevant, all these data are fully compatible with those reported in a murine model of hepatocyte-specific knock out of HIF-1α (Moon et al., 2009).

A more recent and very elegant study has proposed an additional concept, suggesting that HIF-2α may have a predominant role (as compared to HIF-1α) in sustaining fibrogenesis in experimental non-alcoholic steatohepatitis or NASH (Qu et al., 2011). These Authors have performed their experiments using simultaneous, hepatocyte-specific VHL and HIF-1α or HIF-2α mouse mutants. In these mice hepatocyte specific disruption of VHL (and then with an increased expression of both HIF-1α and HIF-2α) resulted in steatosis as well as in increased inflammation and fibrosis. All these events were prevented when simultaneous deletion of HIF-2α, but not HIF-1α, was carried out. Whether these data are really relevant in the emerging scenario of diverse role of HIFs in liver diseases (Nath and Szabo, 2012), has to be confirmed in other experimental models and in human liver specimens.

Hepatic myofibroblasts in liver angiogenesis and fibrogenesis: hypoxia-independent mechanisms

It is relevant to recall that HIF-dependent events, including angiogenesis, can also be regulated/modulated by a long list of hypoxia-independent factors and mediators which currently can include oncogenes, growth factors, cytokines, chemokines, adipokines, metabolic or mitochondrial stress and ROS (Parola et al., 2008; Friedman, 2008). All these factors and mediators are known to be generated in conditions of CLDs and have been reported to lead to either increased HIF-1 mRNA transcription or, as for ROS following direct interaction, to HIF-1 increased stabilization (Dewhirst et al., 2008). This hypoxia-independent scenario is likely to play a critical role either under conditions of CLDs or in the development of HCC.

A first relevant example is the well characterized pro-angiogenic role of PDGF, which has been elucidated for the liver by an elegant study showing that this growth factor can directly affect HSC. PDGF can indeed promote an angiogenic phenotype of HSC that is reported to be able to regulate HSC-driven vascular tube formation in vitro and enhance coverage of sinusoids in vivo (Semela et al., 2008). PDGF-dependent regulatory events can significantly affect relevant vascular functions that are mediated through pericytes, including vascular permeability and pressure regulation. PDGF-related angiogenic signaling was found in this study to require the involvement of the multifunctional ephrin-B2 receptor tyrosine kinase, once again stressing the concept that HSCs represent a key cell type in the modulation of microvascular structure and function in liver parenchyma (Semela et al., 2008).

Another well documented example of a pro-angiogenic mediator is that of leptin, an adipocytokine that has been suggested to exert a pro-fibrogenic effect in promoting the progression from non-alcoholic fatty liver disease (NAFLD) to non-alcoholic steatohepatitis (NASH) (Friedman, 2008; Parola et al., 2008; Dranoff and Wells, 2010). The original study describing the direct pro-angiogenic role of leptin showed its ability to up-regulate in human HSC/MFs the expression of VEGF and Ang-1 expression as well as of the pro-inflammatory chemokine monocyte-chemoattractant protein 1 (MCP-1 or CCL2) (Aleffi et al., 2005). HSC/MFs express the specific leptin receptor ObR and other receptor isoforms and the binding of leptin to these receptors is followed by the activation of several signaling pathways, including extracellular-signal - regulated kinase (ERK), Akt and Nuclear Factor κB, the latter being relevant for chemokine expression. Unexpectedly, leptin was found to operate as a pro-angiogenic mediator by recruitment/stabilization of HIF-1α and nuclear translocation of HIF-1 (Aleffi et al, 2005). The relevance of these findings was confirmed by in vivo experimental studies in which ObR expression was found to co-localize with VEGF and α-SMA after induction of fibrosis. The link between leptin, angiogenesis and NAFLD/NASH was confirmed by a study performed on Zucker rats, animals that carry leptin receptor mutations, receiving the steatogenic choline-deficient and aminoacid defined (CDAA) diet (Kitade et al., 2006). More recently, a study has shown that the pro-angiogenic effect of both leptin and PDGF-BB in human HSC/MFs occurs through a common mechanism leading to up-regulation of VEGF and its release in the extracellular medium. This was documented by experiments in which conditioned media from HSCs treated with either leptin or PDGF-BB were able to induced tube formation in cultured human umbilical vein

endothelial cells (Aleffi et al., 2011). The common pro-angiogenic signaling of leptin and PDGF required: i) the activation of the mammalian target of rapamycin (mTOR) pathway and the increase of HIF-1α and VEGF expression in HSCs; the use of rapamycin, a specific mTOR inhibitor, or the silencing of Raptor (a component of the mTORC1 complex) abolished mTOR activation by leptin and PDGF, which resulted in reduction of VEGF expression and HSC migration, without affecting HIF-1α modulation (Aleffi et al., 2011); ii) a significant increase in the intracellular generation of ROS in HSCs, which was reduced by NADPH-oxidase inhibitors or antioxidants. The overall message is that both leptin and PDGF-BB can up-regulate VEGF expression in human HSC/MFs by activation of mTOR signaling and generation of ROS via NADPH-oxidase, the latter event being apparently relevant for HIF-1α stabilization but not for mTOR activation (Aleffi et al., 2011).

A further confirmation of PDGF as a pro-angiogenic mediator having a role in vascular remodeling in cirrhosis, has been provided by an elegant experimental study in which the Authors have shown that cholangiocytes and HSC/MFs can produce and then release, mainly in response to PDGF, Hedgehog (Hh) ligands in microparticles. This event, potentially relevant since Hh ligands are known to activate Hh signaling in endothelial cells (for example in embryogenesis), was found to be critical in experimental biliary cirrhosis (BDL model). This study suggests that in normal liver the low amount of Hh ligands released by rare immature ductular-type progenitors is efficiently counteracted by the expression of HIP (Hh interacting protein) expressed by either quiescent HSCs or fenestrated SEC. However, this scenario changes in conditions of chronic injury where HIP expression is repressed and the activation of ductular-type progenitor cells can result in PDGF-BB up-regulation and release. PDGF-BB, in turn, can lead HSC/MFs and ductular cells to produce Hh ligands that can promote proliferation and survival of cholangiocytes and HSC/MFs, as well as induce changes in SEC gene expression, resulting in capillarization of sinusoids and in the release of vasoactive factors such as NO, then contributing to vascular remodeling in cirrhosis (Witek et al, 2009).

Still in relation to the role of PDGF-BB, macrophages are known to play an integral role in the development of liver fibrosis by releasing mediators, such as platelet-derived growth factor-B (PDGF-B) and transforming growth factor-ß1 (TGF-ß1), which stimulate hepatic stellate cell proliferation, chemotaxis, and collagen production. Along these lines, a study has tested the hypothesis that chronic liver injury may activate HIFs in macrophages by then affecting their production of pro-fibrogenic mediators (Copple et al. 2012). This hypothesis has been tested in myeloid cell-specific HIF-1α or HIF-1ß knockout mice subjected to bile duct ligation (BDL) and the study reports that levels of α-SMA and type I collagen in the liver were reduced compared with those of mice with normal levels of HIFs. The deficiency of HIFs in macrophages did not affect liver injury or inflammation after BDL but reduced PDGF-B mRNA and protein, suggesting that HIF activation in macrophages may promote fibrosis by regulating the production of PDGF-B. Consistent with a role for HIFs in liver fibrosis in cholestatic liver disease, nuclear HIF-1α protein was detected in macrophages, hepatocytes, and fibroblasts in the livers from patients with primary biliary cirrhosis and primary sclerosing cholangitis (Copple et al., 2012).

The cross-talk by inflammatory response and the pro-angiogenic/pro-fibrogenic role of hepatic MFs is also suggested by a study reporting a related involvement and activation of TLR-4-dependent signaling by LPS as generated from intestinal microbes that can enter the liver through the portal vein. In this study, LPS has been reported to stimulate HSC/MFs expression of fibronectin (FN) following interaction with TLR-4 receptor and that FN released from these MFs can effectively promote liver endothelial cell angiogenesis (Zhu et al., 2012). Accordingly, intestinal decontamination obtained by in vivo rifaximin administration was found to inhibit the LPS-TLR-4 pathway and to suppress fibrosis and angiogenesis. This microbiota - and LPS - related scenario is even more complex because of two additional concepts to recall: i) LPS can also bind to TLR-4 expressed on sinusoidal endothelial cells, then promoting more directly angiogenesis (Jagavelu et al, 2010); ii) LPS, through its binding to TLR4 expressed by hepatic MFs, is known to be able to promote also TGF-,-stimulated fibrogenesis (Seki et al., 2007).

A final mention is deserved by the recently proposed involvement of the apelin system in fibrogenesis and angiogenesis. Apelin is the endogenous ligand of the angiotensin-like-receptor 1 (AGTRL1) and its serum levels are significantly increased in either cirrhotic patients or cirrhotic animals (Principe et al., 2008). Interestingly, apelin and its related receptor APJ are expressed by MFs in cirrhotic animals and are also detected in the splanchnic vasculature of portal hypertensive rats (Principe et al., 2008; Tiani et al., 2009; Yokomori et al., 2011). An elegant study has shown that apelin synthesis by HSC/MFs is up-regulated by angiotensin II and by endothelin-1 (ET-1) but not by hypoxia, with recombinant apelin being able to directly stimulate collagen-I and PDGF receptor ß (PDGF-Rß) expression in MF-like cells (Melgar-Lesmes et al., 2010). To complete this scenario, experimental therapy employing apelin receptor antagonists significantly reduced liver angiogenesis and hepatic fibrosis as well as splanchnic angiogenesis and porto-systemic collateral vessel formation (Principe et al., 2008; Tiani et al., 2009; Melgar-Lesmes et al., 2010; Yokomori et al., 2011 Reichenbach et al., 2012).

Concluding remarks

Experimental and clinical studies have provided

unequivocal evidence that liver angiogenesis is intimately linked to fibrogenesis and to progression of CLDs towards the end-point of cirrhosis and related complications. Available data support a major pathogenic role for hypoxia and related hypoxia inducible factors, with a major role actually proposed for HIF-1α and HIF-2α as well as for the involvement of ROS and redox mechanisms. Increasing evidence also suggests that several CLD-related but hypoxia-independent mediators and mechanisms are also involved in the fibrogenic progression of CLDs and in the modulation of associated angiogenesis and vascular remodeling. The link between angiogenesis and fibrogenesis is so intimate that hepatic neo-angiogenesis is believed to favor fibrogenesis and then progression of CLDs. This is also suggested by experimental evidence that anti-angiogenic therapy can block fibrogenesis and significantly affect the perpetuation of inflammatory response and critical features like hyper-dynamic splanchnic and systemic vascularization, formation of porto-systemic collaterals and the increase in portal pressure. Accordingly, anti-angiogenic drugs, particularly those that have been already approved in the treatment of HCC (such as sorafenib), are currently considered as a putative alternative therapeutic tool to prevent or significantly slow down fibrosis progression towards cirrhosis.

Acknowledgements. Financial support was from the Italian Ministero dell'Università e della Ricerca (MIUR, Rome - PRIN 2009 Project 2009ARYX4T, to M.P.), the Regione Piemonte (Torino, to M.P. and E.N), the Fondazione CRT (Torino, to M.P.), the Fondazione CariPLO (Milan, to MP).

References

Aleffi S., Petrai I., Bertolani C., Parola M., Colombatto S., Novo E., Vizzutti F., Anania F.A., Milani S., Rombouts K., Laffi G., Pinzani M. and Marra F. (2005). Upregulation of proinflammatory and proangiogenic cytokines by leptin in human hepatic stellate cells. Hepatology 42, 1339-1348.

Aleffi S., Navari N., Delogu W., Galastri S., Novo E., Rombouts K., Pinzani M., Parola M. and Marra F. (2011). Mammalian target of rapamycin mediates the angiogenic effects of leptin in human hepatic stellate cells. Am. J. Physiol. Gastrointest. Liver. Physiol. 301, G210-219.

Ankoma-Sey V., Wang Y. and Dai Z. (2000). Hypoxic stimulation of vascular endothelial growth factor expression in activated rat hepatic stellate cells. Hepatology 31, 141-148.

Archer S.L., Gomberg-Maitland M., Maitland M.L., Rich S., Garcia J.G. and Weir E.K. (2008). Mitochondrial metabolism, redox signaling, and fusion: a mitochondria-ROS-HIF-1a-Kv1.5 O2-sensing pathway at the intersection of pulmonary hypertension and cancer. Am. J. Physiol. Heart Circ. Physiol. 294, H570-H578.

Bozova S. and Elpek G.O. (2007). Hypoxia-inducible factor-1alpha expression in experimental cirrhosis: correlation with vascular endothelial growth factor expression and angiogenesis. APMIS 115, 795-801.

Busletta C., Novo E., Valfrè Di Bonzo L., Povero D., Paternostro C., Ievolella M., Mareschi K., Ferrero I., Cannito S., Compagnone A., Bandino A., Colombatto S., Fagioli F. and Parola M. (2011). Dissection of the biphasic nature of hypoxia-induced motogenic action in bone marrow-derived human mesenchymal stem cells. Stem Cells 29, 952-963.

Carmeliet P. (2003). Angiogenesis in health and disease. Nature Med. 9, 653-660.

Carmeliet P. and Jain R.K. (2011) Molecular mechanisms and clinical applications of angiogenesis. Nature 473, 298-307.

Copple B.L., Kaska S. and Wentling C. (2012). Hypoxia-inducible factor activation in myeloid cells contributes to the development of liver fibrosis in cholestatic mice. J. Pharmacol. Exp. Ther. 341, 307-316.

Coriat R., Gouya H., Mir O., Ropert S., Vignaux O., Chaussade S., Sogni P., Pol S., Blanchet B., Legmann P. and Goldwasser F. (2011). Reversible decrease of portal venous flow in cirrhotic patients: a positive side effect of sorafenib. PLoS One 6:e16978.

Corpechot C., Barbu V., Wendum D., Kinnman N., Rey C., Poupon R., Housset C. and Rosmorduc O. (2002a). Hypoxia-induced VEGF and collagen I expressions are associated with angiogenesis and fibrogenesis in experimental cirrhosis. Hepatology 35, 1010-1021.

Corpechot C., Barbu V., Wendum D., Chignard N., Housset C., Poupon R. and Rosmorduc O. (2002b). Hepatocyte growth factor and c-Met inhibition by hepatic cell hypoxia: a potential mechanism for liver regeneration failure in experimental cirrhosis. Am. J. Pathol. 160, 613-620.

Dewhirst M.W., Cao Y. and Moeller B. (2008). Cycling hypoxia and free radicals regulate angiogenesis and radiotherapy response. Nature Rev. Cancer 8, 425-437.

Diez H., Fischer A., Winkler A., Hu C.J., Hatzopoulos A.K., Breier G. and Gessler M. (2007). Hypoxia-mediated activation of Dll4-Notch-Hey2 signaling in endothelial progenitor cells and adoption of arterial cell fate. Exp. Cell Res. 313, 1-9.

Dranoff J.A. and Wells R.G. (2010). Portal fibroblasts: Underappreciated mediators of biliary fibrosis. Hepatology 51, 1438-1444.

Du R., Lu K.V., Petritsch C., Liu P., Ganss R., Passegue E., Song H., Vandenberg S., Johnson R.S., Werb Z. and Bergers G. (2008). HIF1a induces the recruitment of bone marrow-derived vascular modulatory cells to regulate tumor angiogenesis and invasion. Cancer Cell 13, 206-220.

Dufour J.-F. and Johnson P. (2010). Liver cancer: from molecular pathogenesis to new therapies: summary of the EASL single topic conference. J. Hepatol. 52, 296-304.

Duffy A., Wilkerson J. and Greten T.F. (2013). Hemorrhagic events in hepatocellular carcinoma patients treated with antiangiogenic therapies. Hepatology 57, 1068-1077.

El-Serag H.B. and Rudolph K.L. (2007). Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 132, 2557-2576.

Fernández M., Semela D., Bruix J., Colle I., Pinzani M. and Bosch J. (2009). Angiogenesis in liver disease. J. Hepatol. 50, 604-620.

Ferrara N., Gerber H. and LeCouter J. (2003). The biology of VEGF and its receptors. Nature Med. 9, 669-676.

Forbes S.J. and Parola M. (2011). Liver fibrogenic cells. Best. Pract. Res. Clin. Gastroenterol. 25, 207-218.

Fraisl P., Mazzone M., Schmidt T. and Carmeliet P. (2009). Regulation of angiogenesis by oxygen and metabolism. Dev. Cell. 16, 167-179.

Friedman S.L. (2008). Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. Physiol. Rev. 88, 125-172.

Huang H.C., Wang S.S., Hsin I.F., Chang C.C., Lee F.Y., Lin H.C., Chuang C.L., Lee J.Y., Hsieh H.G. and Lee S.D. (2012). Cannabinoid receptor 2 agonist ameliorates mesenteric angiogenesis and portosystemic collaterals in cirrhotic rats. Hepatology 56, 248-258.

Jagavelu K., Routray C., Shergill U., O'Hara S.P., Faubion W. and Shah V.H. (2010). Endothelial cell toll-like receptor 4 regulates fibrosis-associated angiogenesis in the liver. Hepatology 52, 590-601.

Jain R.K. (2003). Molecular regulation of vessel maturation. Nature Med. 9, 685-693.

Jiang B.H., Rue E., Wang G.L., Roe R. and Semenza G.L. (1996). Dimerization, DNA binding, and transactivation properties of hypoxia-inducible factor 1. J. Biol. Chem. 271, 17771-17778.

Jungermann K. and Kietzmann T. (2000). Oxygen: modulator of metabolic zonation and disease of the liver. Hepatology 31, 255-260.

Kaelin W.G. Jr. and Ratcliffe P.J. (2008). Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. Mol. Cell 30, 393-402.

Kaur S., Tripathi D., Dongre K., Garg V., Rooge S., Mukopadhyay A., Sakhuja P. and Sarin S.K. (2013). Increased number and function of endothelial progenitor cells stimulate angiogenesis by resident liver sinusoidal endothelial cells (SECs) in cirrhosis through paracrine factors. J. Hepatol. 57, 1193-1198.

Keith B., Johnson R.S. and Simon M.C. (2012). HIF1α and HIF2α: sibling rivalry in hypoxic tumour growth and progression. Nature Rev. Cancer 12, 9-22.

Kitade M., Yoshiji H., Kojima H., Ikenaka Y., Noguchi R., Kaji K., Yoshii J., Yanase K., Namisaki T., Asada K., Yamazaki M., Tsujimoto T., Akahane T., Uemura M. and Fukui H. (2006). Leptin-mediated neovascularization is a prerequisite for progression of nonalcoholic steatohepatitis in rats. Hepatology 44, 983-991.

Krones A., Jungermann K. and Kietzmann T. (2001). Cross-talk between the signals hypoxia and glucose at the glucose response element of the Ltype pyruvate kinase gene. Endocrinology 142, 2707-2718.

Lando D., Peet D.J., Gorman J.J., Whelan D.A., Whitelaw M.L. and Bruick R.K. (2002). FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. Genes Dev. 16, 1466-1471.

Lee U.E. and Friedman S.L. (2011). Mechanisms of hepatic fibrogenesis. Best. Pract. Res. Clin. Gastroenterol. 25, 195-206.

Lee J.S., Semela D., Iredale J. and Shah V.H. (2007). Sinusoidal remodeling and angiogenesis: a new function for the liver-specific pericyte? Hepatology 45, 817-825.

Liu Y., Wang Z., Wang J., Lam W., Kwong S., Li F., Friedman S.L., Zhou S., Ren Q., Xu Z., Wang X., Ji L., Tang S., Zhang H., Lui E.L. and Ye T. (2013). A histone deacetylase inhibitor, largazole, decreases liver fibrosis and angiogenesis by inhibiting transforming growth factor-, and vascular endothelial growth factor signalling. Liver Int. 33, 504-515.

Llovet J.M., Burroughs A. and Bruix J. (2003). Hepatocellular carcinoma. Lancet 362, 1907-1917.

Loenarz C., Coleman M.L., Boleininger A., Schierwater B., Holland P.W., Ratcliffe P.J. and Schofield C.J. (2011). The hypoxia-inducible transcription factor pathway regulates oxygen sensing in the simplest animal, Trichoplax adhaerens. EMBO Rep. 12, 63-70.

Majumder S., Piguet A.C., Dufour J.F. and Chatterjee S. (2013). Study of the cellular mechanism of Sunitinib mediated inactivation of activated hepatic stellate cells and its implications in angiogenesis. Eur. J. Pharmacol. 705, 86-95.

Medina J., Arroyo A.G., Sanchez-Madrid F. and Moreno-Otero R. (2004). Angiogenesis in chronic inflammatory liver diseases. Hepatology 39, 1185-1195.

Mejias M., Garcia-Pras E., Tiani C., Miquel R., Bosch J. and Fernandez M. (2009). Beneficial effects of sorafenib on splanchnic, intrahepatic, and portocollateral circulations in portal hypertensive and cirrhotic rats. Hepatology 49, 1245-1256.

Melgar-Lesmes P., Casals G., Pauta M., Ros J., Reichenbach V., Bataller R., Morales-Ruiz M. and Jimenez W. (2010). Apelin mediates the induction of profibrogenic genes in human hepatic stellate cells. Endocrinology 151, 5306-5314.

Moon J.-K., Welch T.P., Gonzalez F.J. and Copple B.L. (2009). Reduced Liver Fibrosis in Hypoxia-inducible Factor-1α-Deficient Mice. Am. J. Physiol. - Gastrointest. Liver Physiol. 296, G582-592.

Nath B. and Szabo G. (2012). Hypoxia and hypoxia inducible factors: diverse roles in liver diseases. Hepatology 55, 622-633.

Novo E., Cannito S., Zamara E., Valfrè di Bonzo L., Caligiuri A., Cravanzola C., Compagnone A., Colombatto S., Marra F., Pinzani M. and Parola M. (2007). Proangiogenic cytokines as hypoxia dependent factors stimulating migration of human hepatic stellate cells. Am. J. Pathol. 170, 1942-1953.

Novo E., Povero D., Busletta C., Paternostro C., Valfrè di Bonzo L., Cannito S., Compagnone A., Bandino A., Marra F., Colombatto S., David E., Pinzani M. and Parola M. (2012). The biphasic nature of hypoxia-induced directional migration of activated human hepatic stellate cells. J. Pathol. 54, 964-974.

Parola M., Marra F. and Pinzani M. (2008). Myofibroblast - like cells and liver fibrogenesis: emerging concepts in a rapidly moving scenario. Mol. Asp. Med. 29, 59-67.

Patel S.A. and Simon M.C. (2008). Biology of hypoxia-inducible factor-2alpha in development and disease. Cell Death Differ. 15, 628-634.

Paternostro C., David E., Novo E. and Parola M. (2010). Hypoxia, angiogenesis and liver fibrogenesis in the progression of chronic liver diseases. World J. Gastroenterol. 16, 281-288.

Pouysségur J. and Mechta-Grigoriou F. (2006) Redox regulation of the hypoxia-inducible factor. Biol. Chem. 387, 1337-1346.

Pouyssegur J., Dayan F. and Mazure N.M. (2006). Hypoxia signalling in cancer and approaches to enforce tumour regression. Nature 441, 437-443.

Povero D., Busletta C., Novo E., Valfrè di Bonzo L., Cannito S., Paternostro C. and Parola M. (2010). Liver fibrosis: a dynamic and potentially reversible process. Histol. Histopathol. 25, 1075-1091.

Principe A., Melgar-Lesmes P., Fernández-Varo G., del Arbol L.R., Ros J., Morales-Ruiz M., Bernardi M., Arroyo V. and Jiménez W. (2008). The hepatic apelin system: a new therapeutic target for liver disease. Hepatology 48, 1193-1201.

Pugh C.W. and Ratcliffe P.(2003). Regulation of angiogenesis by hypoxia: role of the HIF system. Nature Med. 9, 677-684.

Qu A., Taylor M., Xue X., Matsubara T., Metzger D., Chambon P., Gonzalez F.J. and Shah Y.M. (2011). Hypoxia-inducible transcription factor 2· promotes steatohepatitis through augmenting lipid accumulation, inflammation, and fibrosis. Hepatology 54, 472-483.

Ramirez-Bergeron D.L., Runge A., Dahl K.D., Fehling H.J., Keller G. and Simon M.C. (2004). Hypoxia affects mesoderm and enhances hemangioblast specification during early development. Development 131, 4623-4634.

Reichenbach V., Ros J., Fernández-Varo G., Casals G., Melgar-Lesmes P., Campos T., Makriyannis A., Morales-Ruiz M. and Jiménez W. (2012). Prevention of fibrosis progression in CCl4-treated rats: role of the hepatic endocannabinoid and apelin systems. J. Pharmacol. Exp. Ther. 340, 629-637.

Rosmorduc O. and Housset C. (2010). Hypoxia: a link between fibrogenesis, angiogenesis, and carcinogenesis in liver disease. Semin. Liver Dis. 30, 258-270.

Sahin H., Borkham-Kamphorst E., Kuppe C., Zaldivar M.M., Grouls C., Al-samman M., Nellen A., Schmitz P., Heinrichs D., Berres M.L., Doleschel D., Scholten D., Weiskirchen R., Moeller M.J., Kiessling F., Trautwein C. and Wasmuth H.E. (2012). Chemokine Cxcl9 attenuates liver fibrosis-associated angiogenesis in mice. Hepatology 55, 1610-1619.

Seki E., De Minicis S., Osterreicher C.H., Kluwe J., Osawa Y., Brenner D.A. and Schwabe R.F. (2007). TLR4 enhances TGF-beta signaling and hepatic fibrosis. Nature Med. 13, 1324-1332.

Semela D., Das A., Langer D., Kang N., Leof E. and Shah V. (2008). Platelet-derived growth factor signaling through ephrin-b2 regulates hepatic vascular structure and function. Gastroenterology 135, 671-679.

Semenza G.L. (2001). Hypoxia - inducible factor 1: oxygen homeostasis and disease patho-physiology. Trends Mol. Med. 7, 345-350.

Semenza G.L. (2004). Hydroxylation of HIF-1: oxygen sensing at the molecular level. Physiology (Bethesda) 19, 176-182.

Semenza G.L. (2011). Oxygen sensing, homeostasis, and disease. N. Engl. J. Med. 365, 537-547.

Semenza G.L. (2012). Hypoxia-inducible factors in physiology and medicine. Cell 148, 399-408.

Semenza G.L., Jiang B.H., Leung S.W., Passantino R., Concordet J.P., Maire P. and Giallongo A. (1996). Hypoxia response elements in the aldolase A, enolase 1, and lactate dehydrogenase A gene promoters contain essential binding sites for hypoxia-inducible factor 1. J. Biol. Chem. 271, 32529-32537.

Shah V.H. and Bruix J. (2009). Antiangiogenic therapy: not just for cancer anymore? Hepatology 49, 1066-1068.

Sharp F.R. and Bernaudin M. (2004). HIF1 and oxygen sensing in the brain. Nature Rev. Neurosci. 5, 437-448.

Talks K.L., Turley H., Gatter K.C., Maxwell P.H., Pugh C.W., Ratcliffe P.J. and Harris A.L. (2000). The expression and distribution of the hypoxia-inducible factors HIF-1alpha and HIF-2alpha in normal human tissues, cancers, and tumor-associated macrophages. Am. J. Pathol. 157, 411-421.

Taura K., De Minicis S., Seki E., Hatano E., Iwaisako K., Osterreicher C.H., Kodama Y., Miura K., Ikai I., Uemoto S. and Brenner D.A. (2008). Hepatic stellate cells secrete angiopoietin 1 that induces angiogenesis in liver fibrosis. Gastroenterology 135, 1729-1738.

Tiani C., Garcia-Pras E., Mejias M., de Gottardi A., Berzigotti A., Bosch J. and Fernandez M. (2009). Apelin signaling modulates splanchnic angiogenesis and portosystemic collateral vessel formation in rats with portal hypertension. J. Hepatol. 50, 296-305.

Tillmanns J., Rota M., Hosoda T., Misao Y., Esposito G., Gonzalez A., Vitale S., Parolin C., Yasuzawa-Amano S., Muraski J., De Angelis A., Lecapitaine N., Siggins R.W., Loredo M., Bearzi C., Bolli R., Urbanek K., Leri A., Kajstura J. and Anversa P. (2008). Formation of large coronary arteries by cardiac progenitor cells. Proc. Natl. Acad. Sci. USA 105, 1668-1673.

Tugues S., Fernandez-Varo G., Muñoz-Luque J., Ros J., Arroyo V., Rodés J., Friedman S.L., Carmeliet P., Jiménez W. and Morales-Ruiz M. (2007). Antiangiogenic treatment with sunitinib ameliorates inflammatory infiltrate, fibrosis, and portal pressure in cirrhotic rats. Hepatology 46, 1919-1926.

Valfrè di Bonzo L., Novo E., Cannito S., Busletta C., Paternostro C., Povero D. and Parola M. (2009). Angiogenesis and liver fibrogenesis. Histol. Histopathol. 23, 1324-1341.

Van Steenkiste C., Ribera J., Geerts A., Pauta M., Tugues S., Casteleyn C., Libbrecht L., Olievier K., Schroyen B., Reynaert H., van Grunsven L.A., Blomme B., Coulon S., Heindryckx F., De Vos M., Stassen J.M., Vinckier S., Altamirano J., Bataller R., Carmeliet P., Van Vlierberghe H., Colle I., and Morales-Ruiz M. (2011). Inhibition of placental growth factor activity reduces the severity of fibrosis, inflammation, and portal hypertension in cirrhotic mice. Hepatology 53, 1629-1640.

Wang G.L., Jiang B.-H., Rue E.A. and Semenza, G.L. (1995). Hypoxia inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O_2 tension. Proc. Natl. Acad. Sci. USA 92, 5510-5514.

Wang Y.Q., Luk J.M., Ikeda K., Man K., Chu A.C., Kaneda K. and Fan S.T. (2004). Regulatory role of vHL/HIF-1alpha in hypoxia-induced VEGF production in hepatic stellate cells. Biochem. Biophys. Res. Commun. 317, 358-362.

Ward J.P. (2008). Oxygen sensors in context. Biochim. Biophys. Acta 1777, 1-14.

Weir E.K., Lopez-Barneo. J., Buckler K.J. and Archer S.L. (2005). Acute oxygen-sensing mechanisms. N. Engl. J. Med. 353, 2042-2055.

Weis S.M. and Cheresh D.A. (2011). Tumor angiogenesis: molecular pathways and therapeutic targets. Nature Med. 17, 1359-1370.

Witek R.P., Yang L., Liu R., Jung Y., Omenetti A., Syn W.K., Choi S.S., Cheong Y., Fearing C.M., Agboola K.M., Chen W., Diehl A.M. (2009). Liver cell-derived microparticles activate hedgehog signaling and alter gene expression in hepatic endothelial cells. Gastroenterology 136, 320-330.

Yokomori H., Oda M., Yoshimura K., Machida S., Kaneko F. and Hibi T. (2011). Overexpression of apelin receptor (APJ/AGTRL1) on hepatic stellate cells and sinusoidal angiogenesis in human cirrhotic liver. J. Gastroenterol. 46, 222-231.

Yoshiji H., Kuriyama S., Yoshii J., Ikenaka Y., Noguchi R., Hicklin D.J., Wu Y., Yanase K., Namisaki T., Yamazaki M., Tsujinoue H., Imazu H., Masaki T. and Fukui H. (2003). Vascular endothelial growth factor and receptor interaction is a prerequisite for murine hepatic fibrogenesis. Gut 52, 1347-1354.

Zhu P., Huang L., Ge X., Yan F., Wu R. and Ao Q. (2006). Transdifferentiation of pulmonary arteriolar endothelial cells into smooth muscle-like cells regulated by myocardin involved in hypoxia-induced pulmonary vascular remodelling. Int. J. Exp. Pathol. 87, 463-474.

Zhu Q., Zou L., Jagavelu K., Simonetto D.A., Huebert R.C., Jiang Z.D., DuPont H.L. and Shah V.H. (2012). Intestinal decontamination inhibits TLR4 dependent fibronectin-mediated cross-talk between stellate cells and endothelial cells in liver fibrosis in mice. J. Hepatol. 56, 893-899.

Accepted August 29, 2013