Short Review

Metabolic zonation of the liver: The oxygen gradient revisited

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ABSTRACT

The liver has a multitude of functions which are necessary to maintain whole body homeostasis. This requires that various metabolic pathways can run in parallel in the most efficient manner and that futile cycles are kept to a minimum. To a large extent this is achieved due to a functional specialization of the liver parenchyma known as metabolic zonation which is often lost in liver diseases. Although this phenomenon is known for about 40 years, the underlying regulatory pathways are not yet fully elucidated. The physiologically occurring oxygen gradient was considered to be crucial for the appearance of zonation; however, a number of reports during the last decade indicating that β-catenin signaling, and the hedgehog (Hh) pathway contribute to metabolic zonation may have shifted this view. In the current review we connect these new observations with the concept that the oxygen gradient within the liver acinus is a regulator of zonation. This is underlined by a number of facts showing that the β-catenin and the Hh pathway can be modulated by the hypoxia signaling system and the hypoxia-inducible transcription factors (HIFs). Altogether, we provide a view by which the dynamic interplay between all these pathways can drive liver zonation and thus contribute to its physiological function.

1. Introduction

The liver is the central metabolic organ responsible for maintaining blood glucose levels, ammonia metabolism, for biotransformation of xenobiotics and endogenous metabolic byproducts of metabolism, as well as for bile synthesis. All these processes require that a number of pathways and enzyme reactions are running in parallel in the most efficient manner. To achieve this, the liver parenchyma displays a functional organization known as metabolic zonation. Although known as a specific phenomenon for years, it is still largely unknown which regulatory mechanism(s) establish this pattern. Research during the last decade has shown that the Wnt/β-catenin pathway plays a major role for determining the zonal pattern in addition to the dynamic factors coming from the blood flow. From the latter, oxygen and reactive oxygen species (ROS) have been shown to be able to contribute to some features of metabolic zonation. The current review aims to connect the new observations and the improved understanding between the regulatory processes that can drive liver zonation and their potential impact on liver diseases.

2. Anatomy, functional units and zonation

At a first glance the macro and micro anatomy of the liver gives a quite uniform impression of tissue composition. However, the liver is heterogeneous in terms of cell types and functional organization (for review see [1]). On the histological level the lobule represents the smallest unit [2]. Classically it is of almost hexagonal shape with a central vein in the middle. The corners of the hexagon are formed by so called portal triads consisting of a branch from the portal vein also called terminal portal vein, and a branch from the hepatic artery also called terminal hepatic arteriole as well as a bile duct (Fig. 1A). In the lobule the parenchymal cells of the liver, the hepatocytes, are connected with each other and are visible as cords. The cords radiate out from the central vein towards the portal triads. In the cords the hepatocyte membranes are interconnected and face blood channels called sinusoids at either side. The sinusoids can almost be considered as tubes wrapped with lines of fenestrated endothelial cells; sinusoids are also populated with resident macrophages, the Kupffer cells. Importantly, there is a small space between the endothelial cell lining and the apical membrane of the hepatocytes called the Space of Disse; it is involved in lymph draining and provides a residence niche for Stellate cells which store fat and vitamin A [2] (Fig. 1B).

While the lobule represents a more structural unit, the hepatic acinus is considered to be the functional unit in terms of blood flow [2]. The acinus can be visualized by connecting two portal triads with a line from which it extends into the direction of the two adjacent central veins. Initially two zones, one around the portal triads, i.e. (the periportal zone), and a second one around the central vein (the perivenous, pericentral, or centrilobular zone) were distinguished. In the meantime, an additional intermediary zone (zone 2) is also considered (for review see [1]) (Fig. 1A). Despite the almost homogenous appearance on the histological level, the liver lobules/acini
display an enormous heterogeneity with respect to subcellular [3,4], biochemical and physiological functions [5]. Accordingly, the key enzymes of various pathways and thus the metabolic capacities are found to be preferentially in one or the other zone (Fig. 1C), a pattern which became commonly known as metabolic zonation [6]. The zonation provides several advantages to main organ and whole body homeostasis. For example, it allows that opposing pathways are spatially separated which prevents competition for a common substrate and futile cycles. Further, complementing pathways can be linked, and substrate demanding activities can be carried out at sites with the best substrate provision. Although not all metabolic activities need to be zonated, metabolic zonation has been shown for carbohydrate, amino acid, lipid, ammonia, and xenobiotic metabolisms (covered in many excellent reviews [7–13] (Fig. 1C)). Moreover, the localization and functional activities of nonparenchymal cells also are zonated [1,14].

Zonation is rather dynamic and not static since most gene expression patterns and consequently enzyme distributions change in response to nutrition, drugs, hormones, and other blood borne factors. Although glutamine synthetase was long considered to be an example of static zonation, recent findings showing that its expression area can extend in response to thyroid hormones [15,16] and rspondins (RSPO) [17] changed this view. Accordingly, it can now be also considered to be dynamic, though the signals changing its expression may be scarce.

3. Factors involved in the regulation of zonation

Over the years a number of findings gave rise to different concepts with which the pattern of zonation could be explained. All of them, such as the streaming liver [18], developmental [19], the cell–matrix [20], and the post-differentiation patterning concept have their pros and cons. Up to now, it appears that the post-differentiation pattern concept provides a quite comprehensive view. In this concept, gradients of morphogens such as Wnt, hedgehog, hormones or growth factors such as HGF, and other factors such as oxygen act in concert, in order to restrict gene expression to differentiated hepatocytes located in specific zones of the liver acinus. Thereby, they appear to act in a hierarchical fashion with the gradients of morphogens being basic and the gradients of nutrients, hormones, intracellularly formed prostanoids, cytokines, and oxygen being modulatory with quite significant impact.

The blood flow and liver metabolism within the acinus, rather than differences in the autonomic nervous system, and biomatrix, appear to be crucial for the generation of the modifier gradients [21,22]. The blood coming from the branches of the portal vein and the hepatic artery in the portal tracts flows as a mixture through the sinusoids to the central vein. Due to metabolism and elimination the composition of blood changes and gradients of substrates, products, hormones, and oxygen are formed. The latter is of particular importance and ranges from about 60–65 mm Hg (84–91 µmol/L) in the periportal blood to about 30–35 mm Hg (42–49 µmol/L) in the perivenous blood [24]. Accordingly, the intracellular pO2 is about 15 mm Hg lower, i.e. 45–50 mm Hg in periportal cells and 15–20 mm Hg in perivenous cells (for review see [22,23]) (Fig. 1B). This goes in line with differences in the number and structure of mitochondria [3,4,25] as well oxidative capacities in periportal and perivenous zones [26,27].

Fig. 1. Liver micro architecture, the oxygen gradient and zonation of metabolism. (A) Classic hexagonal shaped liver lobule with a central vein (CV) in the middle and portal triad (PT) corners with branch from the portal vein also called terminal portal vein (TPV, blue dot), and a branch from the hepatic artery also called terminal hepatic arteriole (THA, red dot) as well as a bile duct (BD, green dot). The acinus extends from a PT into

the direction of two adjacent central veins. Three zones can be distinguished. 1, the periportal zone; 2, the intermediary zone; 3, the perivenous, pericentral, or centrilocular zone. (B) Liver sinusoid and oxygen gradient. Hepatocytes (HC), are connected with each other, bile canaliculi (BC) transport the bile formed in HC into the bile duct (BD). Sinusoids are wrapped with fenestrated endothelial cells (EC). HC and EC are separated by the Space of Disse which is the residence niche for hepatic stellate cells (HC). Resident macrophages, the Kupffer cells (KC) are also to be found in the sinusoid. (C) Distribution of major metabolic pathways. pp, periportal; pv, perivenous; AA, amino acids; Cho, cholesterol synthesis; CYP, cytochrome P450 enzymes; G6a, glycogen; Lac, lactate; GPX, glutathione peroxidase; GS, glutamin synthesis; GST, glutathione transferase. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
Since oxygen constitutes the basis for formation of reactive oxygen species (ROS), it is plausible that also an intra-acinar redox gradient exists. Indeed, redox images obtained from perfused livers showed a gradient curve that decreased sigmiodally from the perportal to the pericentral region [28,29].

The importance of oxygen as a regulator of carbohydrate metabolism has long been known from studies in perfused rat livers [30,31] and cultured primary hepatocytes [32–34]. While the role of oxygen for amino acid, ammonia, and lipid metabolism has been less studied, a recent mathematical model points to a role of oxygen in lipid metabolism and stresses the role of insufficient oxygen supply for the development of steatosis [13,35].

In vivo evidence for the role of oxygen as a modulator of zonation came from the observation that in livers of mice transgenic for the human erythropoietin (EPO) gene, human EPO mRNA was detected only in the less aerobic perivenous hepatocytes [36].

4. Hypoxia-inducible transcription factors (HIFs)

The zonal distribution of key enzymes is mainly controlled by a zonated gene expression pattern which is to a large extent the result of differential transcription factor action. Important transcription factors mediating the regulation of gene expression in response to changes in the oxygen availability are hypoxia-inducible factors (HIFs). To date three HIF transcription factors (HIF-1α, HIF-2α, and HIF-3α) are known (for review see [37]). They act in a heterodimeric complex where the beta subunit is represented by an ARNT protein. The heterodimer binds to DNA areas known as hypoxia responsive elements (HREs) in target genes thereby increasing or decreasing their transcription [38]. HIF-1α and HIF-2α have overlapping but also distinct target genes (for review see [37]) and it has been suggested that HIF-1α is responsible for an acute response to low pO2, whereas HIF-2α responds to states with more chronic hypoxia [39]. Much less is known about HIF-3; several splice variants have been detected which are even supposed to have an inhibitory function [40–42].

In line with the oxygen gradient in liver, all HIFαs were found with higher levels in the less aerobic perivenous zone [43]. The importance of HIF transcription factors for structural maintenance of the liver is exemplified in studies from mice with hepatocyte-specific HIF-1α deficiency. Those mice displayed an extension of hepatic lobules, an impaired fatty acid beta-oxidation, decreased lipogenic gene expression of the HIF-2 target gene erythropoietin and stimulates red blood cell synthesis [59]. With respect to metabolism it was found that PHD2 inhibition improves glucose and lipid metabolism as well as protects against obesity and metabolic dysfunction [60].

Apart from oxygen, a number of signaling substances, stress inducers and signaling pathways have been shown to be able to regulate HIFs under normoxia, i.e., in the presence of oxygen and ROS; the interested reader is referred to a number of reviews covering that topic in more detail [61–66]. In brief, heavy metals, hormones such as insulin, growth and coagulation factors such as HGF, IGF, thrombin, cytokines such as TNFα, lipopolysaccharides (LPS), and mechanical stress have been shown to contribute to that regulation by involving one or more intracellular signaling pathways such as the phosphatidylinositol-3-kinase (PI3K) protein kinase B/Akt, the mTOR, p70S6K1, and RAS/RAF/ERK1/2 as well as mitochondrial metabolites such as the PHD/FIH substrate α-ketoglutarate [65].

5. HIF, PHDs, redox and zonation

ROS have been shown to be important signaling molecules in a number of the above mentioned pathways and they also play an important role in HIF signaling (for review see [66]). Both HIF-1α and HIF-2α can be modified by ROS in a direct and indirect manner. Direct regulation requires presence of redox factor-1 (Ref-[1]) and affects transactivation of HIF-1α at Cys800 and of HIF-2α at Cys848 [67] as well as recruitment of coactivators such as steroid receptor coactivator-1 and transcription intermediary factor 2 [68]. Another direct redox effect is oxidation of the Cys present in the DNA-binding domain of HIF-2α, but not HIF-1α [69]. The indirect effects of ROS are mediated via regulation of PHDs, FIH, redox-sensitive kinases, and phosphatases (for review see [66,70]). As mentioned the PHDs hydroxylate HIF-1α and HIF-2α at critical proline residues thereby inducing HIF degradation under normoxia. The PHDs belong to a family of oxygen, Fe2+, 2-oxoglutarate, and ascorbate dependent dioxygenases which need a radical cycling system to regenerate the iron after each catalytic cycle [71]. Even though ascorbate is a key agent in the regeneration of iron, glutathione could substitute it in mice deficient in vitamin C synthesis, pointing to the importance of thiol oxidation/reduction cycles [72]. In line, a pair of cysteine residues in one of the PHDs was described to modulate its redox sensitivity, again highlighting the potential of thiol oxidation in regulating PHD activity.
though in endothelial cells subjected to hypoxia, no variation in PHD 
cysteine oxidation was observed [72].

Both, HIF-1α and HIF-2α could be prevented from degradation by 
ROS that were generated by NOX4 [74] or at the Qo site of 
mitochondrial complex III [75,76]. From the ROS formed, hydrogen 
peroxide seems to be of major importance for HIF regulation since
overexpression of glutathione peroxidase or catalase, but not super-
oxide dismutase 1 or 2, prevented the hypoxic stabilization of HIF-1α
[75–77]. Together, ROS appear to constitute an important link for HIF
regulation especially in connection with altered mitochondrial activity
and abundance [78]. In this respect it is important to note that HIF-1
reduces ROS production under hypoxia via a subunit switch in
cytochrome c oxidase from the COX4-1 to COX4-2 regulatory subunit
that at the same time increases efficiency of complex IV [79]. HIF-1
also induces pyruvate dehydrogenase kinase 1 and 4 with the effect of
blocking pyruvate entry into mitochondria [80]. Moreover, HIF-1
contributes to mitochondrial autophagy under hypoxia via expression
of BNIP3 [81] and miRNA-210 [82], which blocks assembly of Fe/S
clusters that are required for oxidative phosphorylation and therefore
prevent increased ROS formation and cell death. All these mentioned
findings with respect to HIF and regulation of mitochondrial metabo-
limism are very much in line with the zonal differences in the number
and structure of mitochondria as well oxidative capacities [3,25–27] in
peripheral and perivenous zones, respectively.

Moreover, the recent findings that autophagy in liver is regulated by
a PI3K-protein kinase B/Akt-FOXO3 glutamine synthetase expression
network are also in agreement with the above mentioned findings [83].
The FOXO3 glutamine-synthetase-dependent autophagy occurs pri-
marily in the perivenous zone [84] in which FOXO3 appeared to be
primarily expressed and active. This feature goes nicely along with
findings showing that FoxO3α transcription can be upregulated by
HIF-1α in MEFs and NIH3T3 fibroblasts [85] and by the PHD
inhibitor dimethyldialyl glycine in a mouse glomerular vascular
endothelial cell line [86]. Moreover, polyhydrosylation of FOXO3 by
PHD1 (egln2) destabilized it by inhibiting its interaction with one of
the ubiquitin-specific protease family members (USP9x) [87].

As mentioned above, HIFα signaling is known to undergo a
crossstalk with both PI3K/Akt and RAS/RAF/ERK1/2 cascades where
ROS act as activators [88–90]. Further, p38 MAPKs and the p38
upstream kinases MKK3 and MKK6 [91] were shown to be involved in
the induction of HIF-1α by thrombin [92] and chromium (VI) [91]. In
addition, these substances can also induce HIF-1α mRNA levels in
several cell types [93–96]. By comparing the expression patterns of
peripheral and perivenous hepatocytes of liver tumors with activating
Ha-RAS mutations it was proposed that growth factor signals such as
that of HGF acting via the RAS/RAF/ERK1/2 pathway are important
for liver zonation [97]. Although HGF acts primarily as mitogen for
hepatocytes during liver regeneration [98–100] with higher activity in
the perivenous zone than in the perivenous zone [101], it is known that
HGF is able to stimulate HIF activity [102] via the redox-sensitive
transcription factor NFκB [94].

In liver, HIF-1α is a direct target gene of NFκB [103–107], which was
also found to be inducible by hypoxia [108] and ROS generated in
response to various stimuli like e.g. ethanol [109]. Thus, hypoxia and
ROS regulate also NFκB-dependent HIF-1α transcription [104,107],
that could have an impact on zonation. Interestingly, NFκB expression
and nuclear distribution of NFκB displayed a zonal pattern. In rat liver
the overall NFκB p65 subunit expression was higher in hepatocytes of
the perivenous area, whereas, more importantly, NFκB p65 displayed
a predominant nuclear localization in hepatocytes of the
perivenous area [110], in line with the findings of a higher HIFα mRNA
expression in the same area [43].

Another redox regulated transcription factor which regulates the
HIF system is Nrf2 (Nfe2l2; nuclear factor (erythroid-derived 2)-like 2)
[111]. Nrf2 is known to contribute to intermediary and xenobiotic
metabolism, bile production, as well as liver regeneration, and carci-
nogenesis [112–118]. Livers deficient in Nrf2 are reduced in size and
two thirds of Nrf2-disrupted mice display an impaired vascularization
with the appearance of a congenital infraportal shunt that directly
connects the portal vein to the inferior vena cava. This congenital
infraportal shunt reduced centrilobular hypoxia and decreased peri-
venous Cyp2e1 expression while phosphonolpyruvate carboxikinase,
normally confined to the periportal zone, exhibited both a periportal
and perivenous expression pattern [119].

Further, proper vascular development and morphogenesis of hepatic
sinusoids was shown to be dependent on vascular endothelial
growth factor (VEGF) [120], another hypoxia-inducible gene [121].
Thereby, an intercellular crosstalk is also important and lack of VEGF
from liver epithelial lineages during midgestational development
disturbed zonal endothelial and hepatocyte cell differentiation as well
as formation of a three-dimensional vascular and zonal architecture
[120].

In vivo, these signaling pathways do not act per se alone and thus it
is easy to envision that HIFs are regulated by interconnected signaling
which may, with time, and under certain physiological or pathological
conditions, induce or prolong HIFα abundance. For example, ischemia
due to an acute reduction in blood flow stabilizes HIF-1α [122].
Further, ROS generated during reperfusion can inactivate the PHDs,
leading also to HIFα accumulation. Consequently, the following
remodeling processes again alter oxygen and nutrient supply which
also have an impact on HIF levels and signaling. Thus, it is likely that
small changes in tissue pO2 or activation of non-hypoxic pathways in
the less aerobic perivenous zone of the liver (i.e. ∼4–8% O2), becomes
highly significant for liver function.

6. Beta-catenin and zonation

Interesting seminal discoveries have shown that the Wnt/β-catenin
pathway is an important driver of hepatic zonation [123,124]. Similar
to HIFs, β-catenin abundance is regulated post-translationally and in
the absence of Wnt signals, β-catenin resides in a multiprotein complex
which is the prerequisite for recruitment of the ubiquitin ligase β-
TRCP that ubiquitylates β-catenin; thus, marking it for degradation by
the proteasome [125,126]. In the canonical Wnt pathway, binding of
Wnt ligands to its receptor, Frizzled, and co-receptors, such as the low-
density lipoprotein receptor-related protein 5 (LRP5) and LRP6
[127,128], destroys the destruction complex. As a consequence, β-
catenin becomes stabilized and is transported into the nucleus where it
acts as a coactivator for the transcription of Wnt target genes by
binding to transcription factors from the T-cell factor (TCF) and
lymphoid enhancer factor (Lef) family [129].

Stabilized and active β-catenin is found in perivenous hepatocytes,
whereas the negative regulator of β-catenin, APC, is predominantly
localized in periportal hepatocytes [123]. Importantly, genetic ablation
of APC activates the β-catenin pathway also in the perivenous zone.
Reciprocally, inhibition of β-catenin signaling in the liver acinus leads
to a periportal phenotype in the perivenous hepatocytes [123].

Experiments with fetal rodent hepatocyte cultures supported the
role of the Wnt/β-catenin system for metabolic zonation. When these
hepatocytes were differentiated in culture to mature hepatocytes only
peripheral gene expression markers could be detected. However, when
the cells were differentiated in the presence of a β-catenin activator this
induced a reversible expression of perivenous marker genes [130,131].

Recent findings have indicated that proteins from the Rspondin
(Rspo) family are important Wnt pathway activators [132].
Accordingly, conditional deletion of Rspo3 in mice abrogates proper
perivenous zonation. Further, overexpression of another Rspo member,
Rspo1, induced expression of perivenous marker genes peripherally
indicating that Rspo members may be important angiocrine signals
modulating the β-catenin-dependent liver zonation [133].

Responds possess their own receptors, the LGRs, which together with Rsps prevent clearance of frizzled receptors from the membrane. As a consequence, Wnt signaling is promoted [134]. The LGR system has been shown to be of importance for stem cell renewal, a process also evidently necessary for liver regeneration. In particular LGR5, but not LGR4, is expressed at high levels in damage-activated liver stem cells [135] and with a zonated expression pattern; LGR5 mRNA was exclusively found in perivenous hepatocytes [136], the area showing the lowest oxygen content but an active Wnt/β-catenin signaling in adult livers. In line with these observations are current findings indicating that deletion of LGR5/5 in mouse liver deregulated the expression of peripoortal and perivenous marker genes such as glutamine synthetase-1 within the entire acinus [148]. Although this pattern was similar to that seen upon loss of β-catenin, the authors did not find down-regulation of Dicer or any microRNAs in β-catenin-deficient liver and suggested involvement of an indirect mechanism. Interestingly, Dicer1 was found to be inducible by hypoxia and to be necessary for the function of HIFs and full expression of HIF target genes [149] from which some are eventually β-catenin repressors.

While the above mentioned findings indicate the links between hypoxia and β-catenin regulation, it is also of utmost importance that the expression of the negative β-catenin regulator APC is suppressed by hypoxia and HIF [150]. Upon exposure of several cell lines to hypoxia (1% O2) APC levels were decreased. This decrease was found to be transcriptionally mediated via HIF-1α since depletion of HIF-1α with siRNA restored the APC levels. Further analyses identified a functional hypoxia-responsive element in the APC promoter. Reciprocally, APC was able to mediate a repression of HIF-1α; a process which, in addition to wildtype APC, required low levels of β-catenin and NFκB activity [151]. Importantly, in this context the action of APC appears to involve several cellular compartments such as the nucleus, and mitochondria [152–154]. The latter are being found at higher number in the perportal zone and constitute major sites of ROS production [3,4,25]. Both, β-catenin and HIF-1α signaling are modulated by ROS [155] and in particular superoxide and H2O2 are considered to serve as messengers in this regulation [65]. Hence, changes in the oxygen availability and consequently in superoxide production may have a major influence on β-catenin and HIF-1α signaling as well as on zonation. Indeed, hepatocyte-specific deletion of manganese superoxide dismutase (MnSOD; sod2) which generates H2O2, caused a disruption of the zonal gene expression [156]. Further, HIF-1α as well as β-catenin were absent in hepatocyte-specific MnSOD-deficient mice which were also more prone to chemically-induced carcinogenesis [157].

Together, these findings are in line with the view that the low oxygen content in perivenous hepatocytes leads to induced HIF function which mediates APC repression and consequently stabilization and nuclear localization of β-catenin. Vice versa, loss of HIF function in the more oxygenated and ROS enriched perportal zone allows full APC expression with the result that β-catenin signaling is suppressed.

8. Hedgehog signaling

Recently it was proposed that Hedgehog (Hh) signaling, that is in particular important during development and regeneration, has also a determining role for metabolic zonation [158]. In adult liver, Hh signaling is low in hepatocytes whereas hepatic stellate cells and cholangiocytes are Hh positive [159,160]. In particular Hh signaling becomes activated upon certain states of liver damage as seen in non-alcoholic fatty liver disease (NAFLD), liver cirrhosis, and hepatocellular carcinoma (HCC) as well as after partial hepatectomy [161,162]. The known Hedgehog ligands (Sonic-Hh, Indian-Hh, and Desert-Hh) exert their effects after binding to Patched (PTCH1, -2) receptors on the surface of Hh-responsive cells. In the canonical Hh pathway this relieves the inhibitory actions of PTCH’s on the signaling co-receptor
Smoothened (SMO) which then promotes the nuclear localization and activation of the glia-associated oncogene transcription factors GLI1, GLI2, and GLI3 [163].

Mouse liver displayed a perivenous zonation of HH [164] and conditional hepatocyte-specific deletion of SMO resulted in peripoortal lipid accumulation and up-regulation of key lipogenic transcription factors such as SREBP and PPARs and enzymes such as FASN [165]. The latter displayed a reversed zonation in the SMO deleted mice; instead of being perivenous [8,166] like in the control mice, FASN was detected in the perizonal zone of the SMO knockouts. Further, from the GLI transcription factors especially GLI3 appeared to be responsible for the observed effects, in particular in the SMO-deletion mediated development of steatosis [165]. Importantly, cholesterol biosynthesis, glycogen content, and glycolysis were not altered in hepatocyte-specific SMO deleted mice suggesting that the major role of the Hh pathway consist in, is not limited, to regulate lipid metabolism and its zonation.

Interestingly, the regulation of IGF1 and IGFBP1 which were found to be expressed in the periporal and perivenous area, respectively [167], was also affected in the same SMO knockout model; Deletion of SMO decreased IGF1 but increased IGFBP1 expression [168].

9. Hypoxia, HIFs, and hedgehog signaling: another liaison

Although the findings with respect to lipid metabolism as well as IGF1 and IGFBP1 expression in the hepatocyte specific SMO-deficient mice support a role of Hh signaling for metabolic zonation, they correlate also well with the role of oxygen in the regulation of zonation.

With respect to fatty acid synthesis it has been shown that FASN can be induced by hypoxia [169]. Thereby, hypoxic induction of FASN appears to be an indirect HIF effect involving first HIF-dependent up-regulation of SREBP1 and then an action of SREBP1 on the promoter of FASN [169]. By contrast and similar to APC, HIF-1 inhibited β-oxidation by suppressing expression of medium- and long-chain acyl-CoA dehydrogenases (MCAD and LCAD) [170] which is in line with the old findings that under starvation, oxidation of FAs is more pronounced in peripoortal hepatocytes.

The regulation of IGF1 expression by oxygen is supported from findings showing that serum IGF-1 levels were lower in cyanotic than in acyanotic congenital heart disease patients [171,172] which fits with the reduced perivenous IGF1 appearance. The role of oxygen, ROS, and the HIF system was much more explored with respect to IGFBP1 expression in primary rat hepatocytes where perivenous oxygen tensions enhanced IGFBP1 expression. Experiments with the iron chelator desferrioxamine and H2O2 supported the concept that ROS and ROS induction of SMO decreased IGF1 but increased IGFBP1 expression [168].

Remarkably and similar to the connection between HIF and β-catenin pathways, there appears to exist also a feed-back regulation between the HIF and the Hh system. In particular, the SHh-GLI1 pathway was able to upregulate HIF-2α levels under normoxic conditions [178]. Predominantly this crosstalk appears to be of importance during liver damage, where cholangiocytes and myofibroblasts secrete Hh ligands in order to promote survival and proliferation of both cell types [159,160]. Upon liver damage, hepatic stellate cells become activated myofibroblasts via an Epithelial-to-Mesenchymal-Transition (EMT) and the Hh pathway was shown to be a major regulator of the stellate cell to myofibroblast transition [160]. During this transition a metabolic switch occurs which is in favor of aerobic glycolysis and GLI transcription factors and HIF-1α appeared to be involved in this switch. In mice with different types of liver injury genetical or pharmacological inhibition of SMO, attenuated HIF-1α expression and suppressed glycolytic gene expression. Reciprocally, activating SMO up-regulated HIF-1α mRNA expression, and chromatin immunoprecipitation assays (ChIP) identified that GLI proteins interact with the hif-1α promoter [179].

Moreover, the Hh pathway undergoes also a cross talk with the Wnt/β-catenin signaling pathway. This crosstalk appears to be mutual and even complex with the involvement of common components but different functional aspects of the GLI transcription factors and tissue specificity. In adult tongue epithelium dominant activation of β-catenin lead to a significant up-regulation of Shh which then diminished β-catenin signaling [180]. Vice versa, IHH is a target of Wnt/β-catenin, and in liver it has been shown that the perivenous area expressing HH is extended upon activation of β-catenin signaling [164]. However, these regulations may further divide at the level of GLI or TCF transcription factors. In quail embryos GLI2 and GLI3 were shown to be regulated by Wnt/β-catenin signaling whereas GLI1 activation in somites was shown to be controlled by Shh signaling [181]. In mouse chondrocytes activation of hedgehog signaling selectively inhibited β-catenin-induced FGFP18 expression. The selectivity was shown to be due to the Hh mediated induction of a dominant negative isoform of TCF7L2 (dnTCF7L2) which complexes with GLI transcription factors and tissue specificity.

The multitude of functions including being the major metabolic organ puts the liver into an important strategic position for maintaining whole body homeostasis. Metabolic zonation of the liver is an important feature that helps to achieve this. Research during the past decade provided considerable information into the complex underlying networks involved in liver zonation. In particular, these studies have established a major role for β-catenin signaling, and the involvement of the Hh pathway in metabolic zonation. These findings are still well in accordance with the “old” concept that the oxygen gradient within the liver acinus is a regulator of zonation. This is underlined by a number of facts showing that β-catenin signaling and the Hh pathway can be modulated by the HIF system. Given the zonation of non-parenchymal cells such as bile duct cells and hepatic stellate cells which are both predominantly found in the portal tract and peripoortal area, respectively, they may well be secretors of Hh signals. The Hh signals in turn can, by leaving a gradient, spread into the perivenous direction. In the oxygen rich peripoortal area Hh signaling could, at least in a large part, inhibit β-catenin signaling. The low oxygen content in the perivenous zone would, via the HIF system, activate β-catenin, and...
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References

[1] K. Jungermann, T. Kietzmann, Annu. Rev. Nutr. 16 (1996) 179–203.
[2] D. Sasse, U.M. Spornitz, I.P. Maly, Enzyme 46 (1992) 8–32.
[3] R.H. Wenger, D.P. Stiehl, G. Camenisch, Sci. STKE 2005 (2005) re12.
[4] G.L. Semenza, Annu. Rev. Pathol. 9 (2014) 47–66.
[5] S.T. Koury, M.C. Bondurant, M.J. Koury, G.L. Semenza, Blood 77 (1991) 657–661
[6] T. Jungermann, Histochem. J. 31 (2000) 255–260.
[7] T. Kietzmann, E.Y. Dimaova, D. Flügel, J.G. Scharf, Z. Gastroenterol. 44 (2006) 67–76.
[8] R. Gregor, M. Biehle, Springer Verlag, Berlin-Heidelberg, 1996, pp. 2427–2447.
[9] D.L. Schmucker, J.S. Mooney, A.L. Jones, J. Cell Biol. 78 (1981) 319–337.
[10] K. Jungermann, Semin. Liver Dis. 8 (1988) 329–341.
[11] K. Jungermann, Naturwissenschaften 72 (1985) 76–84.
[12] K. Jungermann, T. Kietzmann, Kidney Int. 51 (1997) 402–412.
[13] B. Sato, A. Tanaka, S. Morii, N. Yanabu, T. Kita, A. Tokuka, et al., Biochim. Biophys. Acta Mol. Cell Res. 1266 (1995) 20–28.
[14] C. Balle, K. Jungermann, Eur. J. Biochem. 158 (1986) 13–18.
[15] H. Bartels, B. Vogt, K. Jungermann, FEBS Lett. 221 (1987) 277–283.
[16] M. Nauck, D. Wölfe, N. Katz, K. Jungermann, J. Eur. Biochem. 119 (1981) 657–661.
[17] A. V. Loud, J. Cell Biol. 37 (1968) 27–46.
[18] A. Vinicius, M. Pasanen, K.I. Kivirikko, J. Myllyharju, Cell Mol. Life Sci. 68 (2011) 4538–4558.
[19] M. Heikkila, A. Pasanen, K.I. Kivirikko, J. Myllyharju, Cell Mol. Life Sci. 68 (2011) 4538–4558.
[20] J.C.A. Mompox, W.J. Wilson, H. Tanaka, L. Poellinger, et al., J. Biol. Chem. 277 (2002) 32405–32408.
[21] M. Heikkinen, A. Pasanen, K.I. Kivirikko, M. Mylläryharju, Cell Mol. Life Sci. 68 (2011) 3985–3991.
[22] T. Kietzmann, Y. Cornesse, K. Brechtel, S. Modiressi, K. Jungermann, Biochem. J. 354 (2001) 531–537.
[23] G.L. Semenza, Annu. Rev. Respir. Physiol. 9 (2014) 47–71.
[24] J.C.A. Mompox, W.J. Wilson, H. Tanaka, L. Poellinger, et al., J. Biol. Chem. 277 (2002) 32405–32408.
[25] K. Tsukada, T. Tajima, S. Hori, T. Matsuura, R.S. Johnson, N. Goda, et al., Microcirculation 20 (2013) 385–393.
[26] A.L. Wang, R. Suzuki, K. Lee, T. Tran, J.E. Gunton, A.K. Saha, et al., Cell Metab. 9 (2009) 565.
[27] A. Tajima, N. Goda, N. Fujikawa, T. Matsuura, N. Nishiyama, N. Senoo-Matsuda, et al., Proc. Natl. Acad. Sci. USA 105 (2008) 4745–4750.
[28] Z. Khan, G.K. Michalopoulos, D.B. Stolz, Am. J. Pathol. 169 (2006) 1251–1269.
[29] P.C. Mahon, K. Hirota, G.L. Semenza, Genes Dev. 15 (2001) 2675–2686.

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Fig. 2. Impact of the oxygen gradient on β-catenin and hedgehog signaling and metabolic zonation. Non-parenchymal cells such as bile duct cells and hepatic stellate cells are more abundant in the oxygen rich periporal area and secrete Hh signals (nPC Hh) and inhibit β-catenin signaling. The low oxygen content in the perivenous zone activates the HIF system, induces LGR5 expression and activates β-catenin as well as suppresses expression of the negative β-catenin regulator APC. Rsponsins secreted from the central vein endothelial cells, activate β-catenin via LGR5 in perivenous hepatocytes. To maintain homeostasis, hypoxia activates expression of Hh components in hepatocytes (HC Hh) to feedback inhibit β-catenin. LGR5 expression as well as suppress APC expression. These, together with Rsponsins secreted from the central vein endothelial cells, may lead to an active β-catenin/TCF genetic program in perivenous hepatocytes. To keep balance and to maintain homeostasis, hypoxia activates expression of Hh components Hh, SMO and likely GLI1 at the same time (Fig. 2).
