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Hypoxia and Hypoxia Inducible Factors: Diverse Roles in Liver Diseases

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Abstract

Hypoxia has been shown to have a role in the pathogenesis of several forms of liver disease. The Hypoxia Inducible Factors (HIFs) are a family of evolutionarily conserved transcriptional regulators that affect a homeostatic response to low oxygen tension and have been identified as key mediators of angiogenesis, inflammation, and metabolism. In this review, we summarize the evidence for a role of HIFs across a range of hepatic pathophysiology. We describe regulation of the hypoxia inducible factors and review investigations that demonstrate a role for HIFs in the development of liver fibrosis, activation of innate immune pathways, hepatocellular carcinoma, as well as other liver diseases in both human disease as well as murine models.

Keywords

Hypoxia-Inducible Factor; Steatosis; Liver injury; Fibrosis; Inflammation

Introduction

The liver has a unique anatomical and functional niche within the body that profoundly affects its physiology and pathophysiology including its oxygen homeostasis. Afferent blood flow to the liver derives from both highly oxygenated blood in the hepatic artery as well as oxygen-depleted blood in the hepatic portal vein. Furthermore, the directional flow of mixed oxygenated and deoxygenated blood towards the central vein of the hepatic lobule creates a physiological oxygen gradient.(1) This gradient has been reported to result in oxygen tensions from about 60–65mmHg in periportal blood, falling to about 30–35 mmHg in perivenous portions of the liver parenchyma; by comparison, physiological arterial oxygen concentration in most other body tissues is about 74–104 mmHg, and venous oxygen concentration is 34–46 mmHg.(1) Hypoxia has profound consequences for tissues of an aerobic organism. In recent decades, our knowledge of the homeostatic response to hypoxia has increased to molecular genetic mechanisms.

The Hypoxia Inducible Factors are a family of heterodimeric transcription factors that act as master regulators of a homeostatic transcriptional response to hypoxia in virtually all cells and tissues. Active Hypoxia-Inducible Factor (HIF) consists of an alpha subunit and a beta subunit. Three alpha subunits, named Hypoxia Inducible Factor 1-α [HIF1α], Hypoxia Inducible Factor 2-α [HIF2α], or Hypoxia Inducible Factor 3α [HIF3α], have been described in humans, mice, and rats; all bind to a common β subunit named, alternatively, HIF1β, or the Aryl-Hyrdocarbon-Nuclear Receptor Translocator [ARNT].(2) Active HIF is named by its alp(1)ha subunit, hence, HIF1 is the active transcription factor consisting of

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HIF1α and ARNT, HIF2 is the dimer of HIF2α and ARNT, etc. HIF1 and HIF2 are the major hypoxia-inducible factors in humans, mice, and rats. Far less is known about the function of HIF3.(2)

Post-translational degradation by the proteasome is a major pathway of HIF regulation. Under normoxia, and in the absence of other metabolic perturbations, the alpha subunits of HIF are rapidly hydroxylated by prolyl-hydroxylases (PHD1, PHD2, or PHD3) and scaffolded on a multimeric protein complex that includes the product of the Von Hippel Lindau tumor suppressor gene. Prolyl hydroxylation and presentation of HIF on the VHL scaffold leads to rapid ubiquitination and proteasomal degradation.(3) Under conditions of hypoxia, or perturbations in cellular redox state, HIFs escape hydroxylation and are free to form dimers with ARNT. Active HIF then translocates to the nucleus, where it binds to hypoxia-responsive elements (HREs) in the promoter region of target genes. HIF1 and HIF2 activate transcription of a broad range of target genes, (e.g., vascular endothelial growth factor, VEGF) with some overlap between the two factors.(4) Numerous other pathways have been implicated in posttranslational HIF regulation and have been reviewed elsewhere.(5) A simplified version of post-translational regulation of HIF is illustrated in Figure 1.

Hypoxic gene targets and their regulation by HIF

Dozens, even hundreds, of genes have been reported to be regulated by hypoxia and the hypoxia inducible factors.(4, 6) Notably, pivotal recent work in the biology of HIF has identified that a large number of hypoxia response genes many of which have been identified as HIF targets, lack a HRE in their promoter sequences, but that genes that contain an HRE in their promoter region are more likely to respond to hypoxic stimuli across a range of cell types.(6) Conversely, genes that are reported to be HIF targets may only be responsive to HIF within a narrow range of tissue types, depending upon cooperative interactions with other molecules including, for example, CBP-P300, sirtuin, beta catenin, and many others.(7, 8)

While a comprehensive list of HIF targets would be well beyond the scope of this article, several HIF targets that have been described in liver disease as summarized in Table 1. Notably, the gene families represented include proinflammatory and profibrotic mediators, as well as genes involved in tumor progression.(9–17)

Oxygen and the Liver

The physiological gradient of oxygen tension across the hepatic lobule has profound effects on the function of hepatic parenchymal and nonparenchymal cells. Periportal hepatocytes and perivenous hepatocytes differ in their expression of many enzymes involved in glucose transport or metabolism, including insulin receptor, glucagon receptor, phosphoglycerate kinase (PGK1), L-type pyruvate kinase, and numerous others.(18) Consequently, periportal hepatocytes tend to sub-specialize in oxidative energy metabolism, glucose production, and synthesis of urea and bile, whereas perivenous hepatocytes are major sites of glucose uptake, glutamine formation and xenobiotic metabolism.(1)

Physiological exposure of hepatocytes to varying levels of oxygen tension also has consequences for the ability of hepatocytes to respond to hypoxic stress. Primary rat hepatocytes cultured in conditions approximating periportal oxygen tensions were able to survive transient anoxia with less cell death and cytokine release than hepatocytes cultured in conditions approximating perivenous oxygen tension. This suggests that in conditions of oxygen deprivation, such as increased hepatic metabolic demand, tissue ischemia, or other conditions, perivenous hepatocytes may be primed to increased injury when oxygen tension drops beneath a threshold level.(19)

Ischemia-Reperfusion Injury

Understanding and controlling ischemia reperfusion (IR) injury is a major goal of liver biology, particularly as IR injury often occurs in the context of reperfusion of the transplanted liver and in emergencies with low arterial pressure. Through a variety of mechanisms, including the production of reactive oxygen species and inflammatory mediators, IR injury can cause major morbidity including predisposing to graft failure. HIF1α induction has been described as an early event, preceding apoptosis, in ischemia-reperfusion injury.(20) Hepatic ischemiareperfusion has been described to upregulate the HIF target Vascular Endothelial Growth Factor (VEGF).(21) Unsurprisingly, HIF1α tends to accumulate during ischemia, but HIF1α DNA binding has been shown to decrease during reperfusion.(22) Some data suggests that HIF1α-dependent upregulation of the transferrin gene contributes to reactive species formation and liver injury in reperfusion, likely through iron-dependent reactive species accumulation.(23) A protective effect of HIF1α induction on ischemia-reperfusion injury has also been decribed in *in vitro* models.(24) Consistent with those results, knockout or silencing of the HIF-degrading PHD1 gene recently has been shown to attenuate IR injury.(25) Blocking the HIF1α target VEGF via a soluble VEGF antagonist reduced IR injury by inhibiting leukocyte migration and cytokine production.(26) More recent work describes a protective effect of HIF1α stabilization on hepatocyte apoptosis in ischemia reperfusion injury via an interaction between the Wnt signalling pathway and HIF, presenting data that suggests that a stabilizing interaction between beta-catenin and HIF1α promotes hepatocyte survival in ischemia/reperfusion injury.(8) Much of the work on HIFs in ischemia reperfusion injury relies upon HIF1α, and further work may clarify the role of other isoforms, such as HIF2α.

Hypoxia in Obstructive Sleep Apnea and Liver Disease

An association described between obstructive sleep apnea (OSA) and non-alcoholic fatty liver disease and/or NASH remains controversial.(27) Several studies have linked OSA, and in particular, the incidence of apneic-hypopneic episodes, to elevation of liver enzymes and the histologic appearance of NASH.(28, 29) A major confounding factor is the frequent comorbidity of obesity and/or the metabolic syndrome; however, one recent study suggested that even among obese patients, nocturnal oxygen desaturation contributed to insulin resistance and liver injury, including fibrosis, inflammation and ballooning necrosis, but not the appearance of steatosis.(30) A study of 83 patients with OSA and matched controls suggested that there was a relationship between OSA and progression of steatosis to steatohepatitis, based on serum levels of type III procollagen.(31) In a larger study of 218 patients with OSA, severe OSA (defined as greater than 50 Apneic/Hypopneic episodes/ hour, AHI) was associated with an increased liver enzymes (OR 5.9, $p<0.02$). Patients with AHI greater than 50/hour were also much more likely to have steatosis, lobular necrosis, and fibrosis by liver biopsy.(32)

Several studies in mouse models have offered some data to corroborate these observations. In one study, chow-fed mice were exposed to either room air or 12 hours of room air and 12 hours of chronic, intermittent hypoxia (CIH, approximately 5% oxygen for periods of 30 seconds followed by 21% oxygen for periods of 30 seconds). After 12 weeks on the CIH regimen, mice developed increased serum ALT, serum triglycerides, and serum cholesterol, as well as increased NFκB DNA- binding activity in liver nuclear extracts.(33) In mice genetically predisposed to obesity, CIH increased liver triglycerides and phospholipids, as well genes of lipid biosynthesis, including SREBP1c, Acetyl-coenzyme A Carboxylase, and Steroyl-CoA Desaturases 1 and 2.(34) In a third study, WT mice were maintained on a high-fat diet and exposed to either room air (21% oxygen) or room air with period of intermittent hypoxia (as described above) for six months. At the conclusion of the study, CIH mice had a

marked elevation in serum AST and ALT, some increase in inflammatory cytokines, and increased serum and liver malondialdehyde, myeloperoxidase, and alpha-1 collagen; however, liver triglycerides were unchanged, and only a mild enhancement of oil-red O staining was observed in CIH-treated mice.(35) The lack of an increase in steatosis compared to controls in perhaps not unexpected given that control group mice were also treated with 6 months of high-fat-diet feeding.

Acetaminophen Poisoning

The model that is emerging from these studies suggests that milder periods of hypoxia, such as moderate OSA, are insufficient by themselves to cause progression to hepatitis/steatohepatitis. However, when CIH is added to a primary insult, such as diet-induced steatosis, there is a predilection towards progression of liver injury. Corroborating evidence from other disease models includes the observation that sublethal acetaminophen poisoning resulted in fulminant liver failure when given in combination with CIH. (36) A recent study combined CIH with daily injections of low-dose acetaminophen (APAP, 200mg/kg) in mice for 4 weeks. At the end of the study period, CIH/APAP mice had markedly elevated liver enzymes, including serum AST, ALT, GGT, and total bilirubin whereas no elevation was observed in mice with APAP alone, and only AST increased in mice with CIH alone.(36)

Some evidence relates to the interaction between the HIF pathway and acetaminophen toxicity. HIF1α nuclear protein was observed to accumulate within 1 hour after a toxic dose (300mg/kg) of APAP; this increase in HIF1α was prevented by N-Acetylcysteine.(37) Pretreatment with Salidroside, an extract of an herbal compound used in Chinese medicine to ameliorate mountain sickness, was able to prevent ALT, AST, and serum TNFα in a mouse model of sublethal APAP toxicity as well as a decrease in HIF1α immunostaining compared to untreated controls.(38) It is unknown whether the role of HIF1α in APAP injury is protective or deleterious; for example, treatment of APAP-challenged mice with hyperbaric oxygen was able to improve survival, even though it increased HIF1 protein levels and the downstream target Glut1.(39) Recent work showed that deletion of HIF1α was protective against APAP toxicity at 6 but not 24 hours, suggesting that HIF1α may play more of a role in early, rather than late APAP toxicity. (40) In that study, a conditional knockout of HIF1α was generated through an inducible ubiquitin promoter, resulting in whole body deficiency of HIF1α. Mice were then exposed to acetaminophen, and mice with HIF deficiency appeared to have decreased liver damage at 6 hours, but not at 24 hours. The emerging picture suggests that HIF1α is a component of the cellular response to acetaminophen injury, but that the complexity of this metabolic insult involves other factors as it develops over time. It is possible that further research will identify therapies that can modify hypoxia inducible factor activity and thereby alter the progression of acetaminophen toxicity at particular points in the evolution of liver injury._

Alcohol Mediated Liver Injury

For several decades, it has been appreciated that alcohol creates alterations in hepatic portal blood flow and oxygen utilization within the liver.(41, 42) A seminal study in the field identified centrilobular injury as a consequence of hepatic oxygen demand in alcohol fed rats that were briefly exposed to low atmospheric oxygen tension, and were able to demonstrate protection against this effect with the co-administration of the thioamide anti hyperthyroid drug propylthiouracil.(41) The diverse effects of chronic ethanol on cellular signaling, cellular metabolism and organ physiology has been reviewed elsewhere. (43) The development of hypoxia in alcohol-exposed liver has also been reviewed elsewhere.(44) Exposure of thin liver slices to acute ethanol was reported to increase oxygen consumption over three decades ago.(45) Acute ethanol causes a rapid increase in liver metabolism,

including rapid induction of alcohol detoxifying enzymes and hepatic oxygen consumption within 2–3 hours.(46) More recently, acute ethanol was found to result in increased areas of staining using the hypoxia-specific marker pimonidazole; this effect appeared to be at least partially dependent on functional hepatic Kupffer cells, and was also apparent in a model of chronic ethanol treatment.(47, 48)

Recent gene array data from ethanol-fed and pair-fed mice demonstrated upregulation of multiple genes in the glycolytic pathway, as well as genes in lipid metabolic pathways in the livers of chronically alcohol fed mice.(49) Although not explored in that publication, most, if not all of these genes may be regulated by HIF1α. An earlier report suggested an upregulation of HIF1α mRNA in the livers of chronic alcoholics.(50) One group offered some data to indicate that HIF1α mRNA is cyclically regulated with the urinary alcohol cycle in a model on continuous, intragastric ethanol feeding.(51) More recently, in the hypercholesterolemic ApoE(−/−) mouse, ethanol significantly increased HIF1α protein in liver, and a synergistic upregulation with tobacco smoke was observed.(52) Our own recent work has demonstrated that chronic alcohol administration increased HIF activation in the liver and that increased hepatiic steatosis in the setting of alcohol developed in a HIF-dependent manner.(53)

It has been well established that alcoholic liver disease proceeds in part through a combination of prolonged metabolic insult coupled with activation of signaling through innate immune mechanisms. Given the role of HIFs in innate immunity as described further below, it is quite possible that the contribution of HIFs to alcoholic liver disease proceeds both through pathways of innate immunity as well as through pathways in the hepatocyte, including hepatic lipid accumulation. A schematic illustrating the convergence of innate immune pathways with HIF1α in an experimental model of steatohepatitis is given in Figure 2.

Hypoxia inducible factors and immunity

In alcoholic liver disease (ALD) and non-alcoholic steatohepatitis (NASH), activation of Toll-like receptor 4 (TLR4) by gut-derived endotoxin has been demonstrated to contribute to disease pathogenesis. (54) Several reports indicate that activation of HIF1α plays a pivotal role downstream of LPS signaling through TLR4. LPS upregulated hepatic HIF1α in rats, as well as HIF1α target gene aldolase.(55) In macrophages, LPS stimulation upregulated HIF1α target genes, including VEGF, PAI-1, and iNOS, as well as HIF DNA binding and HIF1α mRNA and protein.(56) Using a cre-lox system of targeted HIF1α mutation to a transcriptionally inactive form, one group recently reported that knockdown of HIF1α transcriptional activity in cells of the myeloid lineage (LysMCre/HIFflox/flox mice) resulted in protection from LPS-induced sepsis. LysMCre/HIFflox/flox mice had lower levels of pro-inflammatory cytokines, including IL-6, IL-12, and TNFα, and maintained blood pressure and body temperature in the face of LPS challenge at levels that induced septic shock in wild-type mice.(57) Subsequent work indicated that LPS-induced HIF1α activity is dependent upon transcriptional regulation through the inflammatory master regulator group of proteins NFκB.(58) NFκB transcriptional activity is predominantly regulated through the inhibitory action of Inhibitor of κB proteins (IκB), which themselves are targeted for degradation by phosphorylation via the action of IκB Kinases (IKKα, IKKβ, the latter being the major isoform.) IKKβ deletion, then, renders cells unable to phosphorylate IκB and thereby inhibits NFκB signaling. Stimulation of bone-marrow-derived macrophages from mice in which IKKβ had been deleted by cre-lox mediated recombination (IKKβ-null mice) resulted in diminished expression of HIF1α target gene mRNAs. Additionally, HIF1α mRNA was suppressed in IKKβ-null mice prior to any stimulation, indicating that NFκB may regulate HIF1α at the transcriptional level.(59) Although a role for HIF1α activation in

NASH has not been thoroughly investigated, pharmacological inhibition of IKK proteins, analogous to IKKβ-null strategies, was able to prevent steatosis and the development of NASH.(60)

These data suggest that the activation of the pro-inflammatory cascade downstream of LPS-TLR4 signaling may be at least partially dependent upon functional HIF1α signaling. In contrast, in other cell types, some data suggests that HIF1α may suppress T-cell mediated inflammation. HIF1α knockout in T-lymphocytes prevented sepsis and mortality after cecal ligation and puncture (CLP), and T-Cell specific HIF1α(−/−) mice had significantly lower levels of serum ALT 72 hours after CLP challenge than WT mice.(61) Knockout of HIF1α in T-and exvivo stimulation of T-cells from T-cell specific HIF1α (−/−) mice resulted in higher levels of IL2 and IFNg, suggesting that the survival benefit of T-Cell specific HIF1α knockout may be at least partially due to a derepression of HIF1α inhibition of pro-inflammatory cytokine release.(62)

Hypoxia Inducible Factors: A Common Mechanism of Lipid Accumulation?

Multiple lines of evidence suggest that hypoxia and/or HIF may play a role in hepatic lipid accumulation. Numerous studies have implicated a role for hypoxia in altering lipid storage in various cell types. Rats exposed to chronic hypoxia accumulated foam cells in pulmonary alveoli.(63) Hypoxia was described to cause lipid-loading of macrophages, and this effect was prevented by HIF1α siRNA treatment.(63, 64) The differentiation of 3T3-L1 preadipocytes to an adipocytic phenotype was found to be partially dependent on HIF2α, which is transcriptionally regulated in adipocytic differentiation.(65) Forced expression of HIF1α in cardiomyocytes resulted in increased lipid accumulation, and was correlated to a suppression of peroxisomeproliferator-alpha DNA binding.(66) A recent study in breast cancer cell lines demonstrated an increase in HIF1 expression downstream of Akt signaling resulting in an increase in fatty acid synthase (FAS) which is over-expressed in several types of solid tumors.(67)

In hepatocytes, germline deletion of HIF2α resulted in neonatal death and a phenotype of severe steatosis.(68) Although this study suggests that the absence of HIF2α predisposes to steatosis, numerous other studies in vitro and in vivo have suggested that this observation does not apply to the role of HIFs in the adult liver. Hepatocyte specific deletion of the VHL gene is accompanied by a phenotype of hypervascularity and steatosis.(69) Simultaneous introduction of degradation-resistant transgenic constructs of HIF1α and HIF2α resulted in a similar phenotype of hepatic lipid accumulation; in that study, introduction of degradation-resistant HIF1α alone caused a mild phenotype of lipid accumulation, and introduction of degradation-resistant HIF2α alone caused a phenotype of hypervascularity, including the formation of cavernous hemangioma, without lipid accumulation.(4) More recently, a different group described lipid accumulation in a murine model of liver-specific HIF2 activation.(70) In that study, mouse models with cre-lox mediated deletion of VHLH, HIF1α, and/or HIF2α resulted in mice in which both HIF1 and HIF2 or only one or the other isoform was active. HIF2 appeared to play a major role in regulating hepatic lipid by various mechanisms, including the upregulation of lipid biosynthetic pathways, the suppression of fatty acid β-oxidation, or upregulation of the lipid droplet surface protein ADFP.(70) Newer studies have further extended and verified the dominant role of hepatic HIF2α on regulating hepatic lipid accumulation.(71) Our own group has shown that while hepatocyte-specific disruption of HIF1α is able to decrease the upregulation of hepatic lipid that occurs with chronic ethanol administration, constitutively active HIF1, using the HIF1dPA model of hepatocyte-specific HIF1α, results in steatosis that is further exacerbated by chronic ethanol exposure. Using in vitro approaches, we also described an upregulation of HIF1α in cultured hepatocytes that were exposed to the chemokine MCP-1,

suggesting a pathway between cytokine stimulation of hepatocytes and hepatic lipid accumulation.(53) Further work is needed to clarify the relative contributions of HIF1α and HIF21α to hepatocyte lipid accumulation.

HIFs and Metal Accumulation in Liver Disease

Iron accumulation has a role in the pathogenesis of several hepatic diseases, including alcoholic liver disease, and hereditary hemochromatosis. Macrophage iron increased the severity of alcoholic liver disease in a rodent model.(72) In conditions of chronic iron deficiency, iron export is limited by production of hepcidin, which in turn degrades the iron efflux protein ferroportin. Using a model of hepatocyte specific HIF1α deletion, Peysonnaux and colleagues demonstrated that functional HIF1α is partially responsible for the downregulation of hepcidin in chronic iron deficiency.(73) In support of this, endothelial-cell ARNT-knockout mice, which are completely defective in HIF signaling, accumulated high levels of iron.(74) Hypoxia Inducible factors have been implicated in gut iron absorption, where some recent data showed that deletion of HIF2α, but not HIF1α, in intestinal cells resulted in downregulation of serum iron and intestinal expression of the divalent metal ion transporter-1 (DMT1). (75) A similar effect of HIF1α expression on DMT1 was observed *in vitro* in HEPG2 cells.(76)

Liver Fibrosis

Recent evidence indicates a profound effect of HIF1α on cholestatic liver injury. Moon et al. recently described the effect of HIF1α deletion in bile-duct ligated mice, a model of cholestatic liver injury.(77) Mice with a floxed HIF1α exon were mated to Mx-Cre mice enabling near total excision of floxed genetic elements in cells of the immune system and the liver and partial deletion in other body tissues following serial injections of poly-I:C. Deletion of HIF1α was followed with bile duct ligation (BDL) or sham ligation. In WT mice, an increase of pimonidazole stained areas and accumulation of HIF1α was observed as early as 3 days following BDL, indicating hypoxia. Both HIF1αflox/MxCre and WT mice displayed similar increases in ALT, AST, and serum bile acids, but HIF1αflox/MxCre mice were protected from increases in collagen synthesis and alpha-smooth muscle actin staining, both markers for tissue fibrosis, as well as profibrotic mediators including PAI-1 and Platelet-Derived Growth Factor (PDGF) A and PDGF-B.(77) In a series of in vitro experiments, the same group reported that production of profibrotic mediators was induced by culturing mouse hepatocytes in 1% oxygen. Using an siRNA approach, the authors demonstrated that the production of profibrotic mediators was completely prevented in ARNT-null cells, but only partially prevented in HIF1α-null cells, suggesting that other HIF isoforms (particularly, HIF2) play a role.(78)

These data in support of a role for HIF in liver fibrosis are rendered more compelling by evidence in other models of liver fibrosis. After five weeks of once-weekly diethylnitrosamine (DEN) injections (100mg/kg), pronounced collagen septa may be observed, and progression to cirrhosis is observed by 8 weeks. In DEN-treated mice, Vascular Endothelial Growth Factor (VEGF) isoforms were increased with increasing time of treatment, becoming strongly positive by northern blot and immunohistochemical staining by 8 weeks of DEN treatment, and were correlated with increase in tissue hypoxia as observed by pimonidazole staining.(11)

In vitro models have been in concordance with the previous findings. Stellate cell activation has been described as an initiating factor in liver fibrosis, and stellate cells cultured under hypoxia had increased collagen mRNA transcripts. (11) Exposure of the hepatic stellate cell line LX-2 to hypoxia stimulated HIF1α and VEGF mRNA accumulation by 8 hours, and was associated with evidence of increased signaling through the TGFb-SMAD dependent

pathway. Comparison of a gene array using LX-2 cells in normoxia and hypoxia revealed several targets, including fibroblast growth factor-4, that have been implicated in fibrogenesis or inflammation.(79, 80) Another study also reported activation of HSC by hypoxia, and demonstrated that this activation was accompanied by secretion of proangiogenic cytokines, such as VEGF and ANG-1, which were able to stimulate HSC chemotaxis in an autocrine or paracrine fashion. Isolated stellate cells from HIF1α (−/−) mice also demonstrated that genes involved in fibrosis, including angiogenic and collagen-deposition factors, were at least partially dependent upon functional HIF1α.(81)

More recent work has argued for a dominant role for HIF2α in regulating hepatic fibrogenesis in the setting of steatohepatitis. Simultaneous, hepatocyte-specific VHL and HIF1α or HIF2α mouse mutants were generated and assessed for a number of fibrotic markers. The authors described that when mice with disruption of VHL (e.g., with increased expression of HIF1α and HIF2α) were treated for two weeks with an ethanol containing diet, they developed increased fibrosis and increased expression of the fibrosis marker smooth muscle actin. However, when simultaneous deletion of HIF2α, but not HIF1α, was carried out, this increase was prevented.(71)

Viral Hepatitis

Several studies have illuminated the role of HIFs in the pathogenesis of viral hepatitis, including hepatitis B, hepatitis C, and hepatitis E. In a series of HCC cases secondary to primary HBV infection, Hepatitis B Virus X protein [HBx] was found to correlate with HIF1α expression, and transfection of HBx in HepG2 cells was found to increase HIF1α protein accumulation.(82) An earlier study similarly reported the stabilization of HIF1α protein in the presence of the HBx protein, and that this stability correlated with promoter activity of HIF1 at the Multi Drug Resistance-1 protein, an efflux drug transporter thought to be primarily responsible for chemoresistance in HCC. HIF1α siRNA treatment was able to abolish the activation of an MDR-1-luciferase construct induced by HBx transfection.(83) These findings were confirmed and extended in another study that reported that HBx protein increased levels of Metastasis Associated Protein 1 (MTA1) and Histone Deacetylase 1 (HDAC1). These two proteins in turn physically associated with HIF1α, and contributed to HIF1α stability.(84)

The hepatitis E virus (HEV) open reading frame protein 3 (ORF3) is a viral protein thought to be required for infection. In an in vitro system of hepatocyte cell lines expressing HEV ORF3, upregulation of several glycolytic pathway enzymes was reported, and correlated with increased expression and DNA-binding activity of HIF1α. This expression was correlated with increased Akt phosphorylation as well as increased phosphorylation of the CBP/p300 transcriptional co-activator via an ERK-dependent mechanism.(85)

Hepatitis C infection may interact with the HIF1α pathway via multiple mechanisms. Huh7 cells expressing the HCV core protein were reported to have increased VEGF expression and increased HIF1α DNA binding by EMSA; this binding was partially abrogated in the presence of PD98059, an ERK inhibitor.(86) Transient HCV infection in Huh7 cells was associated with HIF1α stabilization by 3 days; furthermore, in Huh7 cells expressing subgenomic HCV replicons, HIF1α was also stabilized. This stabilization again appeared to be dependent on multiple kinase and transcriptional pathways, as functional ERK and PI3K inhibition was able to prevent HIF1α protein accumulation, as was Stat3 inhibition and NFκB inhibition. HIF1α stability was accompanied by production of functional VEGF. (86) HIF stabilization by HCV was demonstrated to be insensitive to antioxidant treatment, and dependent upon derangement of mitochondrial respiration in HCV-infected cells.(87)

HIF in Liver Regeneration

HIF1α is rapidly induced in liver after partial hepatectomy, and remains upregulated for up to 24 hours.(12) Prolactin treatment was able to increase the proliferative response after partial hepatectomy, and was also able to upregulate HIF1α protein and VEGF.(88) However, in another study, hyperbaric oxygen pretreatment, which upregulates HIF1α protein, was unable to accelerate liver regeneration after partial hepatectomy; however, BRDU uptake, and indicator of cellular proliferation, was upregulated in hepatic sinusoidal endothelial cells.(89) More recent work has demonstrated that HIF1α deletion resulted in delayed recovery after partial hepatectomy, an effect that was attributed to decreased hepatic gluconeogenesis.(90)

Oncostatin M (OSM) is an IL-6-type cytokine secreted by leukocytes that has been described to have a role in liver regeneration, liver development, and angiogenesis.(91) A recent report offered data to demonstrate that OSM is able to up regulate HIF1α protein levels and HIF1α target genes, including PAI-1 and VEGF, in a Stat3 dependent mechanism. Further, the upregulation of HIF1 appeared to be at the transcriptional level.(91)

Hepatocellular Carcinoma

Significant evidence indicates that the HIFs play an important role in the pathogenesis and pathophysiology of hepatocellular carcinoma (79–90). HIF1α and VEGF were found to be expressed at higher levels in dysplastic nodules and implicated in malignant transformation. (92) This finding was confirmed in humans and extended by the description of HIF1 expression in chemically-induced preneoplastic lesions in mice.(93) Notably, this expression was independent of tissue hypoxia, as HIF1 positive areas did not differ from other regions of the liver in terms of needle-electrode measured oxygen tension and pimonidazole staining; however, HIF1 levels were effectively reduced by treatment with the PI3K inhibitor LY294002, raising the possibility of a PI3K-Akt dependent mechanism.(94) Recent data also suggest that inhibition of HIF may have a role in cancer therapy. Nonresectable hepatocellular carcinomas may be treated by transarterial catheter embolization (TAE) in which tumor vessels are occluded via catheter-guided placement of a coil or other occluding agent. Drawbacks of this approach include an uncertain survival benefit, as well as a possible induction of tumor neovascularization following TAE. Following the observation that neovascularization of embolized tumors proceeds with upregulation of VEGF, delivery of antisense oligonucleotides against HIF1α in combination with TAE was able to improve efficacy of TAE in promoting tumor necrosis and preventing neovascularization.(94) Furthermore, in that study, the ability of tumor cells to survive on glycolytic metabolism alone (the so-called Warburg effect) was inhibited through suppression of HIF1α glycolytic target genes, including the glucose transporter GLUT1 and Lactate Dehydrogenase A.(94)

The data from clinical studies paint a similar picture. In one series of cases, up to 50% of HBV-associated hepatocellular carcinomas expressed high levels of HIF1α, and HIF1α expression correlated with metastases and decreased survival.(82) Poor prognosis was also associated with expression of Metastasis Associated Protein-1 (MTA-1), which is a stabilizer of HIF1α.(95) Patients with MTA-1 positive cancer had larger tumors with increased incidence of microvessel invasion and nodal extension. The incidence of extrahepatic metastases was almost twofold higher (23% versus 12%, $p<0.001$) in patients with MTA positive lesions than in patients with MTA negative lesions. The prevalence of MTA-1 positive staining was higher in patients with HCC secondary to primary HBV infection than from other causes, including HCV infection or nonviral etiologies.(95)

Both HIF1α and HIF2α isoforms may be overexpressed in hepatocellular cancer. In one series, HIF2α expression was found to be present in 52% of HCC, and correlated with tumor size, capsule infiltration, portal vein invasion, and necrosis.(96) A subsequent larger study found HIF2α expression in 69.5% of HCC cases, as well as 55% of adjacent tissue, but no expression in noncancerous tissue, suggesting that HIF2α expression may be a preneoplastic feature of tumors or a feature of tumor-associated stroma. In that study, high HIF2α expression was also correlated with vascular endothelial growth factor expression, microvessel density, and decreased survival.(97)

Multiple studies have suggested that HIF1α is a prognostic factor for tumor recurrence in human and murine HCC.(98, 99) Some studies have shown that high expression of HIF1α in non-malignant liver tissue adjacent to resected HCC is a negative predictor of disease-free survival, and may correlate to upregulation of HIF1α dependent genes involved in cell migration and invasion.(100) Lastly, patients with HCC whose tumors had high levels of expression of p28(GANK) had a high risk of recurrence, metastasis, and high mortality; interestingly, high p28(GANK) expression correlated with higher levels of HIF1α.(101)

These observations imply that HIF1α inhibition may play a role in anticancer therapeutics. RNA-mediated inhibition of HIF1α was able to slow tumor growth.(102) The antitumor efficiency of doxorubicin was increased when combined with a HIF1α antisense oligonucleotide.(103) Rapamycin inhibits signaling by the Mammalian target of Rapamycin (mTOR) complex pathway, and has shown some efficacy against hepatocellular carcinoma. The prevention of HCC tumor growth by rapamycin in a rodent model was correlated to suppression of HIF1α by rapamycin.(104) Another compound, silibinin, was demonstrated to have some antitumor efficacy through phosphorylation of mTOR, and was also associated with suppression of HIF1α signaling.(105)

Summary

Hypoxia has been implicated in the pathogenesis of a broad range of hepatic disease. In most of these models, some data exists to implicate a role for hypoxia inducible factors. Consideration of the role of HIFs in liver diseases should include multiple cell types, as HIF1α activity has been implicated in hepatocytes as well as myeloid (Kupffer cells) and lymphoid (T-cells) lineage immune cells. Taken collectively, these findings strongly suggest that anti-HIF therapies promise useful interventions in the management of hepatic diseases of various etiologies.

List of abbreviations

AHI	apneic/hypopneic episodes/hour
ALD	alcoholic liver disease
ALT	alanine aminotransferase
APAP	acetaminophen
ARNT	aryl-hyrdocarbon-nuclear receptor translocator
BDL	bile duct ligation
CIH	chronic, intermittent hypoxia
CLP	cecal ligation and puncture
DEN	diethylnitrosamine
DMT1	divalent metal ion transporter-1

FAS	fatty acid synthase
GGT	gamma-glutamyl transferase
HBx	hepatitis B virus X protein
HCC	hepatocellular carcinoma
HDAC1	histone deacetylase 1
HEV	hepatitis E virus
HIF1	hypoxia inducible factor 1
HIF1α	hypoxia inducible factor 1-α
HIF2α	hypoxia inducible factor 2-α
HIF3α	hypoxia inducible factor 3α
HIFs	hypoxia inducible factors
HREs	hypoxia-responsive elements
HSC	hepatic stellate cells
IKK	IκB Kinases
iNOS	inducible nitric oxide synthase
IR	ischemia reperfusion
IκB	inhibitor of κB proteins
LPS	lipopolysaccharide
MCP-1	monocyte chemoattractant protein-1
MDR-1	multi-drug resistance 1
MTA1	metastasis associated protein 1
NASH	non-alcoholic steatohepatitis
NFκB	nuclear factor kB
ORF3	open reading frame protein 3
OSA	obstructive sleep apnea
OSM	oncostatin M
PAI-1	plasminogen-activator-inhibitor-1
PDGF	platelet-derived growth factor
PGK1	phosphoglycerate kinase
siRNA	small interference RNA
SREBP-1c	sterol regulatory element binding protein 1-c
TAE	transarterial catheter embolization
TGFb-SMAD	transforming growth factor-beta
TLR	toll-like receptor
TNFα	tumor necrosis factor alpha
VEGF	vascular endothelial growth factor

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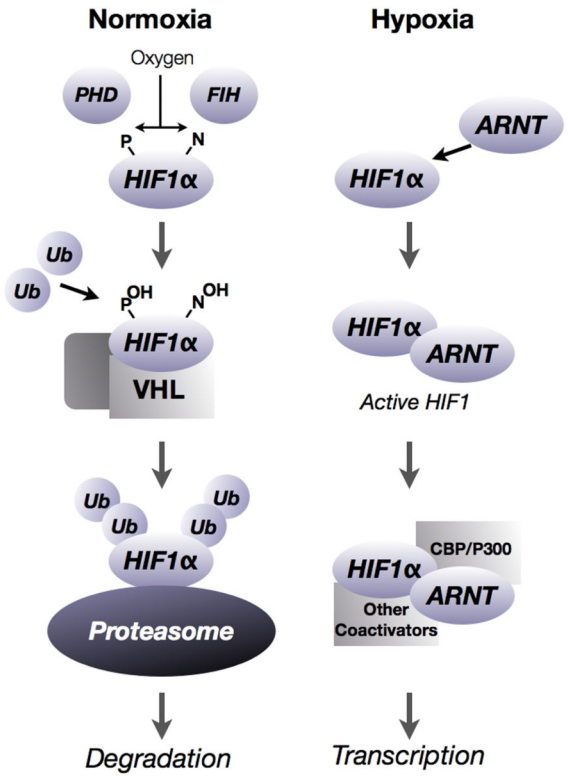

Figure 1.

Under conditions of normoxia, HIF alpha subunits are rapidly hydroxylated at proline and asparagine residues by hydroxylase enzymes. Hydroxylation of HIF1α and assembly on a protein scaffold consisting of the VHL tumor suppressor, along with other co-factors, results in the rapid ubiquitination of the alpha subunit and subsequent degradation by the proteasome. Conversely, in conditions of hypoxia, HIF alpha subunits escape degradation and are free to dimerize with the binding partner, the Aryl Hydrocarbon Nuclear Translocator (ARNT). The HIF heterodimer is named by the alpha subunit (hence, HIF1α-ARNT heterodimer is the active HIF1 transcription factor) which translocates to the nucleus and affects transcription of target genes, typically by binding to a hypoxia response element in the upstream promoter region of the target gene. CBP/P300 and other cofactors may be implicated in HIF transcription. Other pathways of HIF regulation have been described in detail elsewhere.

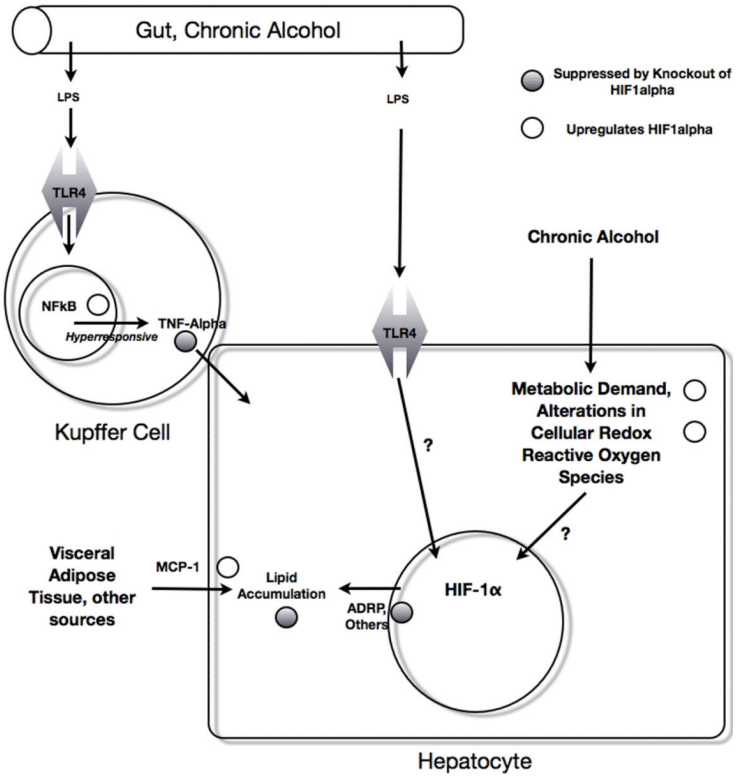

Figure 2.

Pathways of HIF involvement in the Pathogenesis of Alcoholic Liver Disease Chronic alcohol causes increased gut permeability, which results in increased LPS entering the portal circulation. This stimulates TLR4 receptors in the hepatic macrophage, which in turn cause TNFα secretion via an NFκB, HIF1α dependent mechanism. LPS may also stimulate hepatic TLR4 to result in HIF1α activation (Nath, in submission.) Chronic alcohol increases results in an increase in HIF1α in whole liver, possibly mediated through the known effects of ethanol on increased metabolic demand, alteration in cellular redox state, and the creation of reactive oxygen species, all of which can increase HIF1α. HIF1α activation increases lipid accumulation. MCP-1 also increases lipid accumulation, and was shown to increase HIF1α in a hepatoma cell line in vitro.

Table 1

Several gene targets of hypoxia inducible factors described in liver are listed, along with the broad category or categories of physiologic or pathologic activity to which major functions of the gene are attributed. Further information appears in the accompanying text.

Gene	Function	Reference
Plasminogen activator Inihibitor-1 (PAI-1)	Inflammation, thrombosis, metastasis	Kietzmann (1999), Anh (2010)
Prolyl-4 hydroxylase alpha-2	Fibrosis, collagen stabilization	Copple (2011)
Heme Oxygenase 1 (HO1)	Oxidative stress, inflammation	Yeligar (2011)
Vascular endothelial growth factor	Angiogenesis, tumor vascularization, ischemia-reperfusion	Corpechot (2002), Maeno (2005), Nakamura (2005), Redaelli (2005)
Erythropoietin	Erythrocyte proliferation, liver regeneration	Rankin (2007)
Ceruloplasmin	Copper metabolism	Martin (2005)
Glukokinase	Metabolism	Roth (2004)
Bone Morphogenetic Protein	Tumorigenesis	Maegdefrau (2009)