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REVIEW

# Role of Hypoxia-Inducible Factors in the Development of Liver Fibrosis

Katherine J. Roth and Bryan L. Copple

*Department of Pharmacology and Toxicology, the Institute for Environmental Toxicology, and the Program in Cellular and Molecular Biology, Michigan State University, East Lansing, Michigan*

**SUMMARY**

Hypoxia-inducible factors (HIFs) play a critical role in the development of liver fibrosis. We summarize the important functions of HIFs in liver fibrosis and focus on the cell-specific role of these transcription factors in disease development.

**Liver fibrosis remains a significant clinical problem in the United States and throughout the world. Although important advances in the understanding of this disease have been made, no effective pharmacologic agents have been developed that directly prevent or reverse the fibrotic process. Many of the successes in liver fibrosis treatment have been targeted toward treating the cause of fibrosis, such as the development of new antivirals that eradicate hepatitis virus. For many patients, however, this is not feasible, so a liver transplant remains the only viable option. Thus, there is a critical need to identify new therapeutic targets that will slow or reverse the progression of fibrosis in such patients. Research over the last 16 years has identified hypoxia-inducible factors (HIFs) as key transcription factors that drive many aspects of liver fibrosis, making them potential targets of therapy. In this review, we discuss the latest work on HIFs and liver fibrosis, including the cell-specific functions of these transcription factors in the development of liver fibrosis.** ***(Cell Mol Gastroenterol Hepatol 2015;1:589–597; http://dx.doi.org/10.1016/j.jcmgh.2015.09.005)***

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Liver fibrosis has many causes, including alcohol, drugs, viruses, and genetic disorders, among others. All these agents produce hepatocellular injury to some extent, which initiates a reparative process. Part of this process involves differentiation of hepatic stellate cells (HSCs) into myofibroblasts, a process called "activation."[1,2] In instances of biliary injury, in addition to HSCs, portal fibroblasts become activated.[3,4] Once this occurs, these cells begin to proliferate, migrate, and synthesize collagen, which provides the initial matrix for repair. After an acute hepatic insult, the excess collagen is removed, and the myofibroblasts revert back to a quiescent phenotype. If liver injury persists, however, collagen continues to become deposited, resulting in fibrosis and ultimately cirrhosis that may lead to liver failure or cancer. Many of the mediators that regulate HSC activation have been identified (for a comprehensive review, see Friedman[2]), but the mechanisms that regulate production of these mediators during acute and chronic injury are not fully understood. Recent studies, however, have demonstrated that a group of transcription factors called hypoxia-inducible factors (HIFs) may be critical for this process.[5]

To adapt to varying levels of oxygen in the environment, organisms have developed oxygen-sensing systems that trigger adaptive transcriptional responses to maintain homeostatic conditions. These systems use HIF transcription factors, which are heterodimeric transcription factor complexes composed of $\alpha$ and $\beta$ subunits.[6,7] During hypoxia, HIF transcriptional activity leads to enhanced expression of various genes involved in cellular functions aimed at maintaining homeostasis, such as metabolism, proliferation, and migration. There are three known HIF transcription factors: HIF-1$\alpha$, HIF-2$\alpha$, and HIF-3$\alpha$.[8–10] Of these three, HIF-1$\alpha$ and HIF-2$\alpha$ are the best characterized. These $\alpha$ subunits heterodimerize with HIF-1$\beta$, also called the aryl hydrocarbon nuclear transporter, before they are able to regulate gene expression.[6,7] HIF-1$\beta$ is constitutively expressed and present in excess, whereas the $\alpha$ subunit is regulated in an oxygen-dependent manner.

Under normoxic conditions, the HIF $\alpha$ subunit is immediately targeted for degradation by the 26S proteasome.[11] In hypoxic conditions, however, the mechanisms that target the HIF $\alpha$ subunit for degradation are inhibited, allowing the HIF $\alpha$ unit to become stabilized and translocate to the nucleus. Once it enters the nucleus, it dimerizes with HIF-1$\beta$, forming a HIF complex that binds to specific hypoxia-responsive elements in target genes.[7,8]

***Abbreviations used in this paper:*** **BDL, bile duct ligation; $CCl_4$, carbon tetrachloride; Ccr, C-C chemokine receptor; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; HIFs, hypoxia-inducible factors; HSC, hepatic stellate cell; Jmjd, Jumonji domain-containing; PAI-1, plasminogen activator inhibitor-1; PDGF, platelet-derived growth factor; Rgs, regulator of G-protein signaling; $\alpha$-SMA, $\alpha$-smooth muscle actin; TGF-$\beta$, transforming growth factor $\beta$; VEGF, vascular endothelial growth factor.**

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## Hypoxia and Hypoxia-Inducible Factor-1$\alpha$ Activation in the Liver During Injury

Several studies, including our own, have demonstrated that regions of hypoxia develop in the liver after acute liver injury. Early studies showed that hypoxia develops in the liver after alcohol treatment.[12,13] Later studies showed that damage to the liver with compounds such as monocrotaline or acetaminophen also produce regions of hypoxia.[14,15] The mechanism by which this occurs is not known, but it most likely results from disruption of the hepatic architecture, which impedes blood flow through damaged regions; activation of the coagulation system, which leads to fibrin clot formation in the vasculature; and possibly production of vasoactive mediators that modulate hepatic blood flow to regions of injury.[16] To identify regions of hypoxia, many of these studies have used a chemical called pimonidazole, marketed as hypoxyprobe.[17] In addition to this method, activation of HIF-1$\alpha$ has been used as a surrogate marker of hypoxia. HIF-1$\alpha$ has been detected in the livers of mice treated with ethanol, acetaminophen, or carbon tetrachloride ($CCl_4$).[13,18–20]

In addition to hypoxia, however, HIF-1$\alpha$ can be activated in cells by other mediators, including cytokines, growth factors, and oxidative stress. Accordingly, caution should be used before equating HIF-1$\alpha$ activation with hypoxia. In fact, HIF-1$\alpha$ is activated in the liver after acetaminophen and $CCl_4$ treatment before the development of hypoxia, suggesting an important role for other mechanisms of HIF-1$\alpha$ regulation in the liver after exposure to these toxicants.[18,20]

Similar to acute injury, studies have shown that hypoxia is present in the liver during chronic injury. Rosmorduc et al[21,22] originally showed hypoxia in the liver after chronic treatment of rats with diethynitrosamine or after bile duct ligation (BDL), both of which produce severe fibrosis. We later confirmed these findings and demonstrated that HIF-1$\alpha$ is activated in several cell types in the liver after BDL.[5] In particular, HIF-1$\alpha$ was activated in macrophages and hepatocytes within and at the periphery of regions of necrosis, both areas where hypoxia was present. In addition to animal models, we detected HIF-1$\alpha$ protein in hepatocytes and scar-associated macrophages near regions of bridging fibrosis in livers from patients with primary biliary cirrhosis or primary sclerosing cholangitis.[23] We also detected HIF-1$\alpha$ in $\alpha$-smooth muscle actin ($\alpha$-SMA) expressing myofibroblasts within regions of bridging fibrosis.[23] Because HIF-1$\alpha$ regulates a number of genes that have been implicated in fibrosis development, including, platelet-derived growth factor (PDGF),[24] fibroblast growth factor-2 (FGF-2),[25] vascular endothelial growth factor (VEGF),[26] plasminogen activator inhibitor-1 (PAI-1),[27] and many others, our laboratory and others have conducted studies to evaluate the role of HIF-1$\alpha$ in the development of fibrosis.

## Role of Hypoxia-Inducible Factor-1$\alpha$ in the Development of Liver Fibrosis

HIF-1$\alpha$ knockout mice die during embryonic development.[28] Accordingly, to test the hypothesis that HIF-1$\alpha$ contributes to the development of liver fibrosis, our laboratory used Cre-lox technology to knockout HIF-1$\alpha$ in adult mice. In this study, HIF-1$\alpha$ floxed mice were crossed with mice that express Cre recombinase under control of the Mx interferon-inducible promoter.[5] In these mice, treatment with polyinosinic-polycytidylic acid ubiquitously increases Cre recombinase in most cell types.[29] These mice and control mice that did not receive polyinosinic-polycytidylic acid were subjected to BDL. In HIF-1$\alpha$-deficient mice, liver fibrosis was substantially reduced, which demonstrated for the first time a key role for HIF-1$\alpha$ in the development of liver fibrosis in vivo.[5] Deletion of HIF-1$\alpha$ prevented up-regulation of several key profibrotic mediators including PDGF-A, PDGF-B, PAI-1, and FGF-2.[5] All these proteins have been implicated in the development of liver fibrosis, suggesting that HIF-1$\alpha$ may promote fibrosis by regulating expression of these genes.[30–33] In fact, studies have shown that these genes are directly regulated by HIF-1$\alpha$ in some cell types.[24,25,27] Although this study indicated a key role for HIF-1$\alpha$ in the development of liver fibrosis, the cell-specific role of HIF-1$\alpha$ in the development of this disease remained unknown.

## Profibrotic Function of Hypoxia-Inducible Factors in Hepatocytes

As discussed earlier, HIF-1$\alpha$ is activated in hepatocytes in mice subjected to BDL and in patients with primary biliary cirrhosis and primary sclerosing cholangitis.[5,23] Early studies by Kietzmann et al[27] demonstrated that HIF-1$\alpha$ is activated in hypoxic hepatocytes and that HIF-1$\alpha$ directly regulates PAI-1 in these cells. Our laboratory confirmed these findings and also showed that HIF-2$\alpha$ was activated in hypoxic hepatocytes and was needed for full induction of PAI-1 in these cells.[34] In addition to PAI-1, our studies demonstrated that hypoxia up-regulates VEGF, adrenomedullin-1 (ADM-1), and ADM-2 in a HIF-1$\alpha$ and HIF-2$\alpha$-dependent manner.[34] Interestingly, although HIF-1$\alpha$ regulates PDGF-A and PDGF-B in some cell types, these were not increased in hypoxic hepatocytes.

Our studies also showed for the first time an interaction between the HIF and transforming growth factor $\beta$ (TGF-$\beta$) signaling pathways. In this study, our laboratory demonstrated that hypoxic hepatocytes activate latent TGF-$\beta$1 in a HIF-dependent manner.[35] Although the mechanism by which this occurs is not fully understood, we have evidence that hypoxia increases expression of several matrix metalloproteinases and thrombospondin-1 in hepatocytes (Copple, unpublished observations), all of which can activate latent TGF-$\beta$1.[36–38]

Collectively, these in vitro studies demonstrated that HIF-1$\alpha$ and HIF-2$\alpha$ are activated in hypoxic hepatocytes and regulate expression of PAI-1 and VEGF as well as regulate activation of latent TGF-$\beta$1, which could promote the development of liver fibrosis (Figure 1). Since these studies, other laboratories have investigated the role of hepatocyte HIFs in the development of liver fibrosis in vivo.

In a study by Scott et al,[39] HIF-1$\beta$ (aryl hydrocarbon nuclear transporter) floxed mice were crossed with mice