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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Hypoxia and Hypoxia-Inducible Factor-1α 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. Later studies showed that damage to the liver with compounds such as monocrotaline or acetaminophen also produce regions of hypoxia. 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. To identify regions of hypoxia, many of these studies have used a chemical called pimonidazole, marketed as hypoxyprobe. In addition to this method, activation of HIF-1α has been used as a surrogate marker of hypoxia. HIF-1α has been detected in the livers of mice treated with ethanol, acetaminophen, or carbon tetrachloride (CCL4). Treatment of rats with diethylnitrosamine or after bile duct ligation (BDL), both of which produce severe damage to the liver with compounds such as monocrotaline or acetaminophen also produce regions of hypoxia. As discussed earlier, HIF-1α is activated in hepatocytes in the liver after chronic treatment of rats with diethylnitrosamine or after bile duct ligation (BDL), both of which produce severe fibrosis. We have confirmed these findings in mice and demonstrated that HIF-1α is activated in several cell types in the liver after BDL. In particular, HIF-1α 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-1α protein in hepatocytes and scar-associated macrophages near regions of bridging fibrosis in livers from patients with primary biliary cirrhosis or primary sclerosing cholangitis. We also detected HIF-1α in α-smooth muscle actin (α-SMA) expressing myofibroblasts within regions of bridging fibrosis. Because HIF-1α regulates a number of genes that have been implicated in fibrosis development, including platelet-derived growth factor (PDGF), fibroblast growth factor-2 (FGF-2), vascular endothelial growth factor (VEGF), plasminogen activator inhibitor-1 (PAI-1), and many others, our laboratory and others have conducted studies to evaluate the role of HIF-1α in the development of fibrosis.

Role of Hypoxia-Inducible Factor-1α in the Development of Liver Fibrosis

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

Profibrotic Function of Hypoxia-Inducible Factors in Hepatocytes

As discussed earlier, HIF-1α is activated in hepatocytes in mice subjected to BDL and in patients with primary biliary cirrhosis and primary sclerosing cholangitis. Early studies by Kietzmann et al. demonstrated that HIF-1α is activated in hypoxic hepatocytes and that HIF-1α directly regulates PAI-1 in these cells. Our laboratory confirmed these findings and also showed that HIF-2α was activated in hypoxic hepatocytes and was needed for full induction of PAI-1 in these cells. In addition to PAI-1, our studies demonstrated that hypoxia up-regulates VEGF, adrenomedullin-1 (ADM-1), and ADM-2 in a HIF-1α and HIF-2α-dependent manner. Interestingly, although HIF-1α 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 β (TGF-β) signaling pathways. In this study, our laboratory demonstrated that hypoxic hepatocytes activate latent TGF-β1 in a HIF-dependent manner. 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-β1.

Collectively, these in vitro studies demonstrated that HIF-1α and HIF-2α are activated in hypoxic hepatocytes and regulate expression of PAI-1 and VEGF as well as regulate activation of latent TGF-β1, which could promote the development of liver fibrosis. Since these studies, other laboratories have investigated the role of hypoxia HIF in the development of liver fibrosis in vivo. In a study by Scott et al., HIF-1β (aryl hydrocarbon nuclear transporter) floxed mice were crossed with mice...
that express Cre recombinase under control of the albumin promoter to generate hepatocyte-specific HIF-1β knockout mice. As discussed earlier, HIF-1β heterodimerizes with the basic helix-loop-helix-PER-ARNT-SIM (bHLH/PAS) transcription factor family, which includes HIFs, and is thus required for both HIF-1α and HIF-2α transcriptional activity. Mice with and without deletion of HIF-1β in hepatocytes were treated with the hepatotoxicant thioacetamide to induce liver fibrosis. Although the pattern of fibrosis was similar in both, there was a decrease in macrophage infiltration as well as a decrease in the mRNA expression of profibrotic genes, including TGF-β1 and TGF-β2, collagen type 1 and 5, and tissue inhibitor of metalloproteinases 1 and 5 (Timp1 and 5) in hepatocyte-specific HIF-1β knockout mice. These results provided support for the importance of HIFs in regulating profibrotic gene expression in hepatocytes in vivo after liver injury.

Further studies conducted by Roychowdhury et al also suggested an important role for hepatocyte HIF-1α in the development of fibrosis. In their study, hepatocyte-specific HIF-1α-deficient mice were fed ethanol concomitantly with CCl4 treatment, resulting in the amplification of CCl4-induced hepatic fibrosis. Hepatocyte-specific HIF-1α deficiency prevented the ethanol-induced increase in CCl4-induced fibrosis compared with control mice, as measured by mRNA and protein levels of both type I collagen and α-SMA, indicating an important role for hepatocyte HIF-1α in the development of liver fibrosis in this model. However, when mice were treated with CCl4 alone without ethanol, HIF-1α deficiency in hepatocytes did not reduce liver fibrosis, which may indicate that hypoxia resulting from ethanol metabolism may enhance HIF-1α activation in hepatocytes after CCl4 treatment, allowing HIF-1α to play a greater role in the development of liver fibrosis.

Studies by Qu et al found that HIF-2α in hepatocytes may also be important for progression of fibrosis. In these studies, hepatocyte-specific Vhl knockout mice were produced, which results in constitutively activate HIF-1α and HIF-2α in hepatocytes. Gene expression profiles were then assessed in the livers of control mice and hepatocyte-specific Vhl knockout mice. Numerous genes critical to the development of liver fibrosis were found to be up-regulated. These included genes important for collagen formation, such as procollagen-lysine 2,oxoglutarate 5-dioxygenase 2 (PLOD2) and transglutaminase 2 (TGM2) as well as α-SMA. Furthermore, Vhl-disrupted mice fed an ethanol diet produced an increase in fibrosis. This increase was completely prevented in hepatocyte-specific Vhl-HIF-2α double knockout mice, but not in hepatocyte-specific Vhl-HIF-1α double knockout mice. Together, these data indicated that HIF-2α activation in hepatocytes is important for the development of liver fibrosis in this model.

One caveat of this study, however, was that although deletion of HIF-2α did prevent an increase in fibrosis, it also resulted in reduced liver injury overall. Because liver injury is the driving factor leading to fibrosis, it is possible that HIF-2α did not play a direct role in the progression of fibrosis, but rather played a role in producing liver injury. Further studies are needed to more definitively elucidate the role of HIF-2α in hepatocytes in the development of fibrosis.

**Kupffer Cells**

Kupffer cells are resident macrophages of the liver. Although these cells perform key beneficial functions, they...
also contribute to the pathogenesis of a number of liver disorders, including ischemia-reperfusion injury, toxin-induced liver injury, fatty liver disease, and liver fibrosis.\textsuperscript{43,44} Rivera et al.\textsuperscript{13} were the first to show a key role for macrophages in the development of liver fibrosis in rats after chronic CCl\textsubscript{4} treatment. Subsequent studies have confirmed these results, reaffirming an essential role for these cells in the development of liver fibrosis.\textsuperscript{45} Work from Karlmark et al.\textsuperscript{46} indicated that the macrophage population that promoted fibrosis was recruited from bone marrow in a manner dependent on C-C chemokine receptor type 2 (Ccr2) and type 6 (Ccr6). Immunophenotyping of these cells indicated that they were CD11b\textsuperscript{+}F4/80\textsuperscript{+}Gr1\textsuperscript{+} monocyte-derived macrophages.

Although these studies have clearly indicated that macrophages are important for the development of fibrosis, the mechanism by which they contribute to this process was not known. Early studies by Friedman and Arthur\textsuperscript{31} showed that conditioned medium from cultured Kupffer cells stimulated HSC proliferation in culture in a PDGF-dependent manner. In addition, studies provided evidence that intrahepatic CD11b\textsuperscript{+}F4/80\textsuperscript{+}Gr1\textsuperscript{+} macrophages could stimulate HSCs to produce collagen in a TGF-\beta-dependent manner.\textsuperscript{47,48} Collectively, these studies demonstrated that macrophages are important for the development of fibrosis and that they may contribute to fibrosis by producing growth factors that stimulate HSC proliferation and collagen production. However, the mechanism by which chronic liver injury stimulated these cells to produce profibrotic mediators remained unknown.

As discussed earlier, our studies demonstrated that HIF-1\textsubscript{a} was activated in scar-associated macrophages in both mice and humans with liver fibrosis.\textsuperscript{5,23} In subsequent studies, we showed that exposure of Kupffer cells to hypoxia in vitro activated HIF-1\textsubscript{a} but not HIF-2\textsubscript{a}, suggesting that HIF-1\textsubscript{a} signaling may predominate in Kupffer cells from nondiseased livers.\textsuperscript{49} This, however, could potentially change in diseased livers. In the presence of certain cytokines, macrophages polarize to different phenotypes, including classically activated (M1) and alternatively activated (M2) macrophages. Takeda et al.\textsuperscript{50} showed that HIF-1\textsubscript{a} is up-regulated and HIF-2\textsubscript{a} is down-regulated in classically activated macrophages, whereas HIF-2\textsubscript{a} is up-regulated and HIF-1\textsubscript{a} down-regulated in alternatively activated macrophages. It is possible that Kupffer cells in nondiseased livers are skewed toward a classically activated phenotype, which may explain the lack of HIF-2\textsubscript{a} activation in these cells. In diseased livers, however, we have observed hepatic macrophages with activated HIF-1\textsubscript{a} or HIF-2\textsubscript{a}, indicating that both transcription factors can be activated in hepatic macrophages in vivo after injury (Copple, Mochizuki, and Roth, unpublished observation).

Because HIF-1\textsubscript{a} and HIF-2\textsubscript{a} regulate overlapping and distinct sets of genes, it would be interesting to evaluate the pattern of gene expression in hypoxic Kupffer cells either skewed toward a classic phenotype with activated HIF-1\textsubscript{a} or an alternative phenotype with activated HIF-2\textsubscript{a}. It is possible that these two macrophage populations may play very different roles in liver disease in part due to differential expression of HIF-1\textsubscript{a} and HIF-2\textsubscript{a}.

Studies have shown that hypoxic Kupffer cells produce profibrotic mediators in vitro. Our laboratory showed that in vitro exposure of Kupffer cells to hypoxia increased expression of several genes involved in angiogenesis, including VEGF and angiopoietin-1, in a HIF-dependent manner.\textsuperscript{49} In addition, PDGF-B was also up-regulated in hypoxic Kupffer cells in a HIF-dependent manner.\textsuperscript{51} In mice subjected to BDL, knocking out HIF-1\textsubscript{b} selectively in macrophages using Cre/lox did not affect liver injury, markers of cholestasis, or hepatic inflammation, including up-regulation of cytokines and hepatic neutrophil and macrophage accumulation.\textsuperscript{52} Deletion of HIF-1\textsubscript{b} did, however, reduce liver fibrosis as measured by type I collagen and \(\alpha\)-SMA mRNA and protein levels.\textsuperscript{23} In addition, knocking out HIF-1\textsubscript{b} in macrophages prevented an increase in PDGF-B mRNA and protein.\textsuperscript{53} It also prevented up-regulation of FGF-2, another profibrotic growth factor, but did not affect levels of active TGF-\beta protein.\textsuperscript{54} Similar results were observed in mice in which HIF-1\textsubscript{a} was knocked out in macrophages, suggesting that HIF-1\textsubscript{a} in macrophages is profibrotic (Figure 1).\textsuperscript{55} This is consistent with reports suggesting that classically activated, inflammatory macrophages primarily promote fibrosis.\textsuperscript{46} As mentioned earlier, HIF-1\textsubscript{a} is up-regulated in classically activated macrophages. It would be interesting, however, to investigate the role of HIF-2\textsubscript{a} in macrophages and determine whether it may play an opposing role and promotes fibrosis reversal.

### Hepatic Stellate Cells

Despite being originally described in the 19th century, it was not until 1985 that Friedman et al.\textsuperscript{1} discovered that HSCs are the main collagen-producing cell type in the liver. Recent studies using sophisticated cell-tracing techniques have further confirmed the importance of these cells in collagen deposition during fibrosis.\textsuperscript{51} During quiescence, these cells store and release retinoids. After liver injury, these cells become activated and differentiate into \(\alpha\)-SMA–expressing myofibroblasts that produce collagen, proliferate, and migrate throughout the liver.\textsuperscript{2} Because liver injury produces regions of hypoxia, Corpechot et al.\textsuperscript{52} tested the hypothesis that hypoxia stimulates HSCs to produce collagen. In this study, exposure of rat HSCs to hypoxia in vitro up-regulated type I collagen, indicating that hypoxia is a stimulus for collagen production by these cell types. In addition, we recently confirmed these findings in culture-activated, primary human HSCs (Copple, unpublished findings).

Although this process is not fully understood, it most likely evolved to provide an initial structural matrix to facilitate repair processes, such as angiogenesis. During chronic injury, however, persistent hypoxia provides a continuous stimulus for collagen production by HSCs, leading to fibrosis. A later study by Shi et al.\textsuperscript{52} confirmed that hypoxia up-regulates collagen in a human HSC line, LX-2.
cells. In this study, they also observed an increase in TGF-β, suggesting that hypoxia stimulates autocrine production of TGF-β, which could stimulate collagen production.52 This same group also observed an increase in matrix metalloproteinase 2, which could process TGF-β to its active form.52 Whether these processes require HIFs and occur in vivo during fibrosis development, however, remain to be determined.

Because of the results described here, our laboratory next determined whether HIFs are activated in hypoxic HSCs. In these studies, exposure of culture-activated, primary mouse HSCs to hypoxia activated both HIF-1α and HIF-2α.53 Hypoxia also increased the expression of several genes that could contribute to fibrosis development. For instance, hypoxia increased expression of two genes involved in collagen metabolism, prolyl-4-hydroxylase α1 and prolyl-4-hydroxylase α2, which are key enzymes that contribute to the formation of stable collagen triple helices.53 As expected, hypoxia also increased expression of several genes involved in angiogenesis, including VEGF, angiopoietin-like-4, placental growth factor, and macrophage-migration inhibitory factor.53

It has been proposed that angiogenesis is a critical factor in the development of liver fibrosis;21 although this remains a rather controversial area. For instance VEGF has been shown to stimulate HSCs to produce collagen, to proliferate, and to migrate in vitro.54 Furthermore, neutralization of VEGF was shown to reduce fibrosis in CCl4-treated mice.55 More recent studies, however, have shown that VEGF is critical for reversal of fibrosis,56,57 The antifibrotic effects of VEGF are most likely due to effects of VEGF on sinusoidal endothelial cells rather than HSCs. Accordingly, the importance of VEGF and angiogenesis to liver fibrosis is complicated and remains an evolving field.

In addition to proangiogenic mediators, hypoxia increased the expression of receptors involved in HSC migration and activation. For instance, hypoxia increased the expression of the chemokine receptors Ccr1 and Ccr5.58 In the presence of chemokines, activation of these receptors stimulates HSC migration. In support of a role for these receptors in the development of fibrosis, it was shown that Ccr1 and Ccr5 knockout mice have reduced liver fibrosis.59 Two other receptors of interest were increased by hypoxia in HSCs. These were interleukin-13 receptor α1 and adrenergic receptor α2b, which are activated by interleukin-13 and catecholamines, respectively.53 In vitro, activation of these receptors on HSCs stimulates collagen production, and in vivo both these pathways have been shown to play an important role in liver fibrosis development.59–61

Although up-regulation of some of the genes described here required HIF-1α, up-regulation of some did not require HIF-1α, suggesting an important role for HIF-2α or potentially other hypoxia-regulated transcription factors in regulation of these genes.53 Of particular interest, up-regulation of many of these genes by hypoxia only occurred in culture-activated HSCs and did not occur in quiescent HSCs, suggesting that HSC activation increases the sensitivity of HSCs to HIF-mediated gene expression changes.53 The mechanism by which this occurs is not known, but it may result from changes in histone modifications or DNA methylation changes that modify access of HIFs to the promoters of certain HIF-regulated genes in activated HSCs.

In addition to the genes already described, hypoxia also increased expression of genes that have the potential to globally affect HSC function in a profound manner. Two of these were the Jumonji domain-containing proteins Jmjd6 and Jmjd1a.54 Jmjd6 regulates mRNA splicing, and Jmjd1a is a histone demethylase.60,61 Up-regulation of Jmjd1a by HIFs could increase expression of a number of genes by modifying histone methylation, whereas up-regulation of Jmjd6 could promote the formation of alternatively spliced mRNAs that code for proteins with modified functions that promote fibrosis.

In addition to Jumonji proteins, hypoxia increased expression of regulator of G-protein signaling 2 (Rgs2) and Rgs4.55 These proteins act as GTPase-activating proteins for G protein α subunits, thereby decreasing signaling by Gα1, Gα2a, and Gαq subtypes.62 Up-regulation of Rgs proteins could substantially affect signaling by G protein-coupled receptors in HSCs. The importance of this in regulating HSC function during the development of fibrosis remains to be determined. The various processes regulated by HIFs in HSCs that may contribute to fibrosis are summarized in Figure 2.

Although numerous genes were increased in hypoxic HSCs, surprisingly few were decreased by hypoxia. Two genes of particular interest that were decreased by hypoxia were hepatocyte growth factor (HGF) and α1-integrin.53 HGF is important for liver regeneration, and studies have shown that HSCs are a source of HGF during regeneration.65–67 In addition to our study, Corpechot et al68 demonstrated in 2002 that hypoxia decreases HGF in hypoxic HSCs. In this study, they proposed that decreased HGF expression in hypoxic HSCs may contribute to liver regeneration failure in cirrhotic livers.68 Alpha-1-integrin is a receptor for collagen, and studies have shown that collagen production by fibroblasts from α1-integrin knockout mice is enhanced, suggesting a role for this receptor in feedback inhibition of collagen production.69 The importance of the decrease in α1-integrin in hypoxic HSCs, however, remains to be determined. Furthermore, whether HIF-1α contributes to the reduction in HGF and α1-integrin is not known.

In consideration of the impact of hypoxia and HIF activation on the function of HSCs in vitro, studies were conducted to evaluate the role of HIF-1α in HSCs in vivo on the development of liver fibrosis. In these studies, HIF-1α floxed mice were crossed with mice that express Cre recombinase under the glial fibrillary acidic protein promoter, which, in the liver, is only expressed by HSCs.70 This resulted in deletion of HIF-1α in HSCs but no other liver cell types. Treatment of control mice in this study with a single hepatotoxic dose of CCl4 increased the expression of types I, III, and IV collagens by 48 hours after treatment.70 Deletion of HIF-1α in HSCs completely prevented up-regulation of all three collagens in the liver at this time point.70 Surprisingly,
by 72 hours after CCl₄ administration, the levels of type I collagen mRNA and protein were much greater in the livers of mice in which HIF-1α was knocked out in HSCs. In addition, there were greater levels of α-SMA mRNA and protein, indicating enhanced HSC activation.

These results were quite contrary to in vitro studies and suggested a potential role for HIF-1α in limiting HSC activation and collagen production in vivo after liver injury. Further analysis, however, demonstrated that there was a failure of macrophages to become activated in the livers of these mice, which prevented removal of necrotic hepatocytes from the liver. We proposed that the persistence of necrotic cells most likely maintained HSC activation and enhanced production of collagen in mice deficient in HIF-1α in HSCs.

Interestingly, this phenotype is also observed in plasminogen and urokinase plasminogen activator knockout mice, where there is a failure to clear necrotic hepatocytes, leading to enhanced collagen production. According to the research, while HIF-1α appeared to be important for collagen up-regulation early after injury, it also stimulated HSCs to produce a HIF-1α-dependent factor(s) that activated macrophages, which is essential for phagocytic removal of necrotic hepatocytes. The mechanism by which this occurs is not known; however, considering the similar phenotype in mice deficient in plasmin, it is possible that HIF-1α activation in HSCs modulates plasmin formation after injury.

Figure 2. Genes regulated by hypoxia-inducible factor 1α (HIF-1α) in hepatic stellate cells that may promote fibrosis. (See the text for full details.)

Summary and Perspectives for Targeting Hypoxia-Inducible Factors as a Therapy for Liver Fibrosis

Research over the past 16 years has provided important information on the role of HIFs in the development of liver fibrosis. This work has provided evidence that HIF-1α regulates a number of important profibrotic mediators in hepatocytes, HSCs, and Kupffer cells. It remains to be determined whether HIF-1α activation in other cell types contributes to this disease. For instance, VEGF, a key HIF-target gene, is increased in cholangiocytes after BDL. Whether this requires HIF-1α is not known. HIF-1α also regulates the function of various immune cell types, including T cells, natural killer T cells, and B cells, which may be important for fibrosis. Furthermore, very little
is known about the role of HIF-2α in liver fibrosis and in which cells HIF-2α is activated. Finally, there is a clear link between HIF-1α and HIF-2α activation and the development of cancer. Because cirrhosis can often progress to hepatocellular carcinoma, it would be interesting to determine whether HIFs are an important driving force for the progression of cirrhosis to cancer. Studies have shown that constitutive activation of HIF-1α and HIF-2α in hepatocytes produces hemangiomas in the liver, lending support for a role of HIFs in cancer development in the liver. Over the next several years, research should begin to fill these knowledge gaps.

Also, it is unclear whether pharmacologic inhibition of HIFs would be an effective therapy for liver fibrosis. Only one study has addressed this topic thus far. In a rat model of transarterial chemoembolization in which rats are treated with CCl4 and subjected to hepatic artery ligation, a putative HIF-1α inhibitor called LW6 prevented progression of liver fibrosis when started 2 weeks after the initiation of liver disease. Although these results are exciting, additional studies with other models of liver fibrosis and other well-characterized HIF inhibitors need to be conducted before HIFs are validated targets for liver fibrosis therapy.

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Correspondence
Address correspondence to: Bryan L. Copple, PhD, Department of Pharmacology and Toxicology, Michigan State University, 1355 Bogue Street, B403 Life Sciences Building, East Lansing, Michigan 48824. e-mail: copple@msu.edu.

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The authors disclose no conflicts.

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