Neuropilin and Liver Fibrosis: Hitting Three Birds with One Stone?

Cao S, Yaqoob U, Das A, Shergill U, Jagavelu K, Huebert RC, et al. Neuropilin-1 promotes cirrhosis of the rodent and human liver by enhancing PDGF/TGF-beta signaling in hepatic stellate cells. J Clin Invest 2010;120:2379-2394. (Reprinted with permission.)

Abstract

PDGF-dependent hepatic stellate cell (HSC) recruitment is an essential step in liver fibrosis and the sinusoidal vascular changes that accompany this process. However, the mechanisms that regulate PDGF signaling remain incompletely defined. Here, we found that in two rat models of liver fibrosis, the axonal guidance molecule neuropilin-1 (NRP-1) was upregulated in activated HSCs, which exhibit the highly motile myofibroblast phenotype. Additionally, NRP-1 colocalized with PDGF-receptor beta (PDGFRbeta) in HSCs both in the injury models and in human and rat HSC cell lines. In human HSCs, siRNA-mediated knockdown of NRP-1 attenuated PDGF-induced chemotaxis, while NRP-1 overexpression increased cell motility and TGF-beta-dependent collagen production. Similarly, mouse HSCs genetically modified to lack NRP-1 displayed reduced motility in response to PDGF treatment. Immunoprecipitation and biochemical binding studies revealed that NRP-1 increased PDGF binding affinity for PDGFRbeta-expressing cells and promoted downstream signaling. An NRP-1 neutralizing Ab ameliorated recruitment of HSCs, blocked liver fibrosis in a rat model of liver injury, and also attenuated VEGF responses in cultured liver endothelial cells. In addition, NRP-1 overexpression was observed in human specimens of liver cirrhosis caused by both hepatitis C and steatohepatitis. These studies reveal a role for NRP-1 as a modulator of multiple growth factor targets that regulate liver fibrosis and the vascular changes that accompany it and may have broad implications for liver cirrhosis and myofibroblast biology in a variety of other organ systems and disease conditions.

Comment

Chronic liver disease afflicts millions of patients and is among the 10 leading causes of death in the United States.[1] The great majority of chronic liver disease is caused by hepatitis B, hepatitis C, nonalcoholic fatty liver disease, and alcoholic liver disease. In most cases, these diseases progress slowly over several decades in characteristic stages, with hepatic fibrosis setting the stage for the development of cirrhosis and, in some cases, hepatocellular carcinoma (HCC). Hepatic stellate cells (HSCs) have emerged as the main profibrogenic cell type in the liver, and the transformation from quiescent, vitamin A storing to activated HSCs with a myofibroblastic phenotype is believed to be a key event in the progression to fibrosis and cirrhosis. Numerous studies have characterized signaling pathways that contribute to HSC activation such as transforming growth factor β (TGFβ), platelet-derived growth factor (PDGF), angiotensin II, lipopolysaccharide/Toll-like receptor 4, hedgehog signaling, cannabinoids, leptin, and adiponectin, among many others.[2] Evidence from transgenic and knockout mice as well as pharmacological studies have revealed PDGF and TGFβ as probably the two most important contributors to HSC activation and liver fibrosis.[2] In HSCs, the binding of PDGF to the PDGF cell surface receptor stimulates several profibrogenic signaling cascades, including the phosphoinositide 3-kinase (PI3K)–AKT-p70S6 kinase, the mitogen-activated protein kinase (MAPK)/c-Jun N-terminal kinase (JNK) pathway, and the Ras/MEK/extracellular signal-regulated kinase (ERK) pathway to stimulate HSC proliferation and motility.[3,4] TGFβ binds the TGFβ receptor complex to promote HSC activation both through Smad transcription factors as well as Smad-independent pathways such as Ras-MEK-ERK and TGFβ-activated kinase 1/ MAPK kinase–p38/JNK.[5]

The study of Cao et al.[6] introduces neuropilin-1 (NRP-1) as a new element in profibrogenic signaling pathways in HSCs and suggests that NRP-1 serves as an important amplifier of the two major profibrogenic signaling pathways, PDGF and TGFβ. Neuropilins were first discovered as receptors for class 3 semaphorins, polypeptides with key roles in the nervous system such as axonal guidance.[7] Subsequently, it was found that neuropilins are also involved in other signaling pathways such as vascular endothelial growth factor (VEGF) signaling. Recent evidence, including the results presented by Cao et al., also imply a role for NRP-1 in the cellular response to PDGF and TGFβ.[8,9] NRP-1 has a very short intracellular domain that lacks a defined signaling role. It is therefore widely believed that NRP-1 mediates functional responses as a result of complex formation with other receptors (e.g., plexins and VEGF receptors).[7] NRP-1 functions are best studied in the nervous system and the vasculature, and knockout mice demonstrate

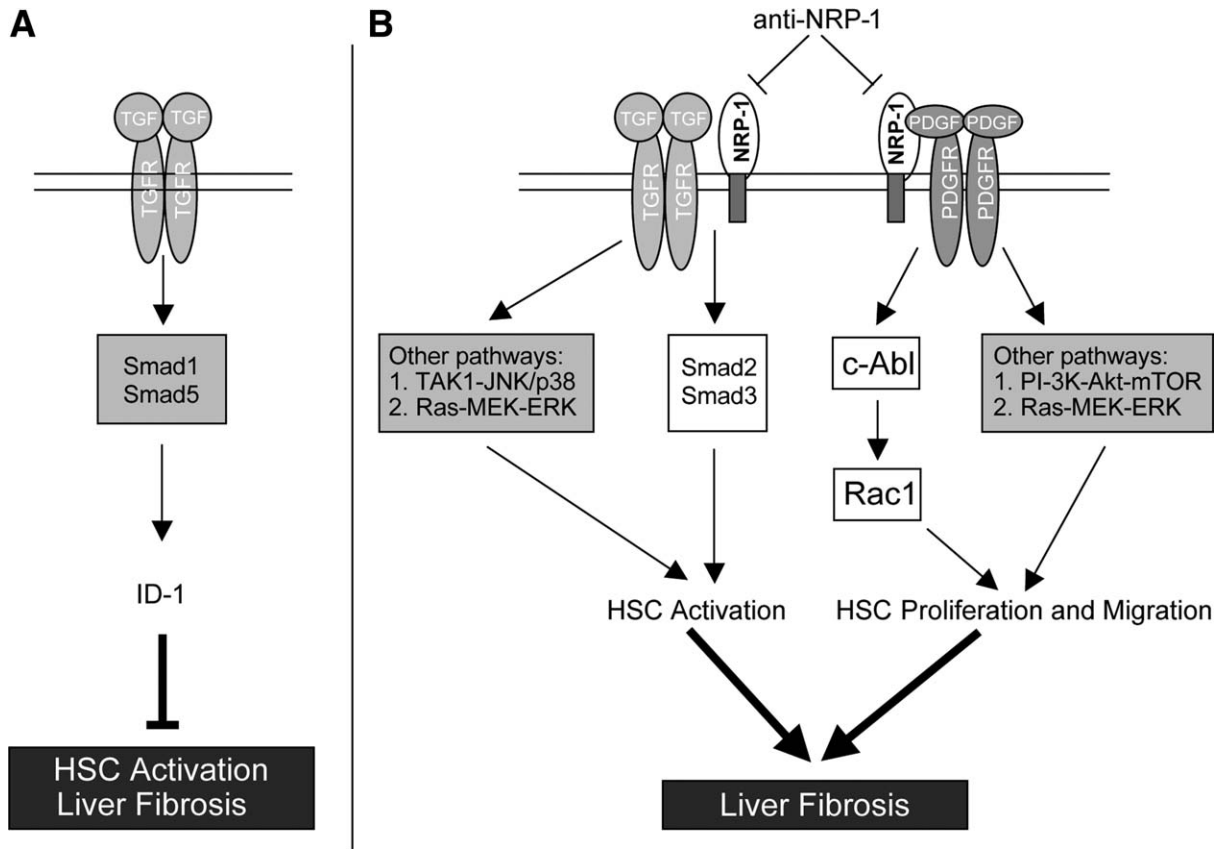

Fig. 1. Role of NRP-1 in TGFβ and PDGF signaling. (A) HSCs with low NRP-1. In the absence of NRP-1, TGFβ is bound to the TGF receptor, leading to an activation of Smad1 and Smad5 signaling, up-regulation of inhibitor of differentiation (ID-1), and subsequent inhibition of HSC activation and liver fibrosis. (B) HSCs with high NRP-1. In the presence of NRP-1, PDGF binding to its receptor is increased, and NRP-1 promotes activity of the c-Ab1/Rac1 pathway, leading to migration of HSCs without affecting the PI3K/Akt/mammalian target of rapamycin (mTOR) and Ras/MEK/ERK pathways. At the same time, NRP-1 also promotes TGFβ-induced activation of Smad2 and Smad3. Together, these pathways promote HSC activation and liver fibrosis, both of which are blocked by NRP-1 antagonism. NRP-1–regulated pathways are represented by white boxes.

decreased neural vascularization and hypoplasia of segments of the arch arteries and dorsal aorta and die during embryogenesis.[10]

Because HSCs express many neural markers such as neural cell adhesion molecule and glial fibrillary acidic protein,[2] the expression of NRP-1 in activated HSCs as demonstrated by Cao et al. is not entirely surprising. Cao et al. not only show that NRP-1 increases in HSCs isolated from CCl_4-treated and bile duct–ligated livers but also demonstrate an up-regulation of NRP-1 in cirrhotic livers from patients with hepatitis C virus and nonalcoholic steatohepatitis. The clinically most relevant result of the study by Cao et al. is the reduction of liver fibrosis as assessed by hydoxyproline levels and multiple fibrogenesis markers such as Col1a1, α smooth muscle actin, and Tgfβ messenger RNA by an inhibitory NRP-1 antibody. Further mechanistic studies revealed that small interfering RNA knockdown of NRP-1 inhibited PDGF-induced chemotaxis independently of VEGF receptor and Sema3a, whereas overexpression of NRP-1 increased HSC motility. Moreover, *in vitro* binding studies demonstrated that NRP-1 increases PDGF binding affinity for PDGFR-expressing cells. HSCs from NRP-1–deleted mice exhibited decreased migration in response to PDGF, whereas overexpression of NRP-1 promoted selective activation of Rac1 in the presence of PDGF without affecting Akt and ERK activity. Interestingly, Rac activity was diminished in c-Abl–deficient mouse embryonic fibroblasts overexpressing NRP-1, suggesting that NRP-1 directs the PDGFR signals to Rac1 through its ability to bind and activate c-Abl (Fig. 1). Furthermore, Cao et al. investigate the role of NRP-1 in the regulation of collagen deposition induced by the PDGF and TGF-β pathways. Surprisingly, both cytokines induce collagen deposition after overexpression of NRP-1. Collagen deposition is inhibited in NRP-1– and c-Abl–deficient mouse embryonic fibroblasts after treatment with PDGF or TGF-β, suggesting the presence of an NRP-1/c-Abl pathway in enhancing

collagen deposition. In a recently published parallel study, the same group demonstrated that NRP-1 mediates R-SMAD signaling via TGFβ[11] and suggested that NRP-1 amplifies TGFβ-induced myofibroblast activation by increasing the profibrogenic Smad2/3 pathway and suppressing the antifibrogenic Smad 1/5 pathway. In summary, these studies convincingly establish NRP-1 as an amplifier for profibrogenic signaling pathways such as PDGF and TGF-β, leading to increased HSC activation and fibrosis in the liver.

A few questions have yet to be answered, however. Culture-activated HSCs and HSC cell lines employed for mechanistic experiments in this study may differ significantly from *in vivo*–activated HSCs.[12,13] In this regard, additional *in vivo* studies may be helpful to further delineate whether NRP-1 promotes HSC activation and liver fibrosis acting through its role as a VEGF and semaphorin coreceptor. Notably, the two antibodies employed in the present studies differ in their epitope binding, with NRP-1a blocking semaphoring binding and NRP-1b blocking VEGF binding. Because NRP-1b antibody reduced CCl_4-induced liver fibrosis, one needs to consider whether the VEGF blocking abilities of this antibody played a role in the improved fibrosis observed *in vivo*. Importantly, angiogenesis has been suggested to contribute to hepatic fibrosis.[14] Although Cao et al. investigated the role of NRP-1 in regulating the ability of HSCs to promote the formation of vascular tubes, they did not investigate angiogenesis *in vivo*. Moreover, the current study did not employ genetic methods to inhibit NRP-1 expression *in vivo*. The floxed NRP-1 mice employed for *in vitro* experiments in this study should ideally be used to delete NRP-1 in HSCs during liver fibrosis. Finally, it is intriguing that NRP-1 was strongly up-regulated in HSCs from CCl_4-treated livers but only very moderately in HSCs isolated form bile duct–ligated livers. Thus, it would be important to study the transcriptional regulation of NRP-1 in HSCs as well as the functional contribution of NRP-1 in additional models such as bile duct ligation or genetic models of liver fibrosis. With this additional information, future studies can possibly attempt to target NRP-1 in patients and to "hit three birds with one stone": namely PDGF, TGFβ, and most likely also VEGF signaling. Antibodies to human NRP-1 are currently studied in phase l trials and might be available for antifibrotic therapies in the near future. In view of several studies showing antitumor effects of NRP-1 inhibition,[15,16] it would also be interesting to investigate whether NRP-1 is expressed in HCCs or the hepatic tumor microenvironment, and whether it promotes growth or angiogenesis of HCC.

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Potential conflict of interest: Nothing to report.

Primary Prophylaxis Against Gastric Variceal Bleeding: Is There a Sticky Solution at Last?

Mishra SR, Sharma BC, Kumar A, Sarin SK. Primary prophylaxis of gastric variceal bleeding comparing cyanoacrylate injection and beta-blockers: a randomized controlled trial. J Hepatol 2011;54:1161-1167.

Abstract

In this randomized single center trial, 89 cirrhotic patients with GOV2 (eradicated esophageal varices) or IGV1 (both at least 10 mm size) not previously bled were selected for randomization over a 3 year period. Patients were randomized to: (1) Cyanoacrylate (n=30); (2) Propranolol (n=29); or (3) No treatment. There was complete obturation of GV in all patients after a mean of 1.6 ± 0.4 sessions. Propranolol was commenced at 20mg BD and titrated to aim for a heart rate of 55/min (mean dose 140 mg). There was no discontinuation of propranolol due to side effects. Hepatic venous pressure gradient (HVPG) measurements were performed at baseline and after 1 year in all groups and within 24h of bleeding. Most patients had alcoholic or cryptogenic cirrhosis and GOV2 (85%) of 20mm median size. The median follow up time was 26 (3-34) months. There was significantly lower gastric variceal bleeding with cyanoacrylate in (10% versus 38% and 53% for propranolol and no treatment respectively). There was no difference in bleeding between propranolol and no treatment. There was a significant reduction in HVPG in the propranolol group (35% had HVPG response) and an increase in the other groups. HVPG at baseline and HVPG response did not predict bleeding. There was a significant difference in overall and bleeding related mortality in favor of the cyanoacrylate group compared with no treatment (7 versus 26%). No difference in mortality was seen between propranolol and the other groups.

Comment

Gastric variceal bleeding (GVB) remains an important clinical problem. The management of gastric varices is controversial, with a lack of consensus regarding therapies for the primary prevention of gastric variceal hemorrhage. Risk factors for GVB are similar to those of esophageal varices and include size of fundal varices, child's class, and red spots.[1] The risk of bleeding is lower than with esophageal varices, yet the transfusion requirements and mortality associated with a bleeding episode are both higher.[1] Gastric varices are supplied by the short gastric, left gastric and polar veins, and unlike esophageal varices, they lie deep within the submucosa.[3] The widely used classification described by Sarin et al.[2] defines four types of gastric varices according to site and risk of bleeding. The most common types are gastro-esophageal varices types 1 and 2 (GOV1 and GOV2), which are continuations of esophageal varices along the lesser and greater curve, respectively. Isolated gastric varices (IGV) type 1 occur in isolation in the fundus, are less common, and bleed less frequently (albeit more severely).[2] GOV1 are treated like esophageal varices, and GOV2 and IGV1 require specific therapy. The 2-year bleeding risk for larger gastric varices can be as much as 65%.[2] Therefore, it would seem appropriate to concentrate on therapies to prevent bleeding in patients with GOV2 and IGV1 (Fig. 1).

Clinical trials investigating primary prophylaxis of GVB are lacking, perhaps because gastric varices are less common than esophageal varices. The recruitment of patients sufficient for studies of primary prophylaxis of moderate to large esophageal varices has proved difficult.[4] Uncontrolled studies have demonstrated the efficacy of endoscopic therapies in eradicating gastric varices.[5,6] There has been some interest in balloon-occluded retrograde obliteration (B-RTO) of gastric varices, wherein large gastric varices are obliterated by injection of a sclerosant through gastro-renal shunts under fluoroscopic guidance. A small prospective study comparing B-RTO with no treatment revealed reduced bleeding and mortality with B-RTO. These findings must be interpreted with caution, because the study was not randomized, and other investigators have found that B-RTO can increase the long-term risk of bleeding in patients with coexisting esophageal varices.[7] Both the American Association for the Study of

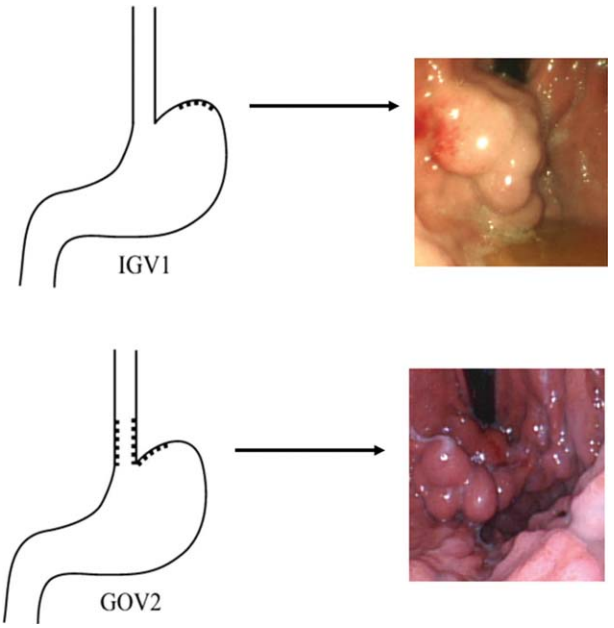

Fig. 1. High-risk gastric varices based on the classification of Sarin et al.[3] GOV2, gastro-esophageal varices type 2; IGV1, isolated gastric varices type 1.

Liver Diseases guidelines[8] and the latest Baveno V[9] consensus do not provide definitive guidance, although nonselective beta-blockers (NSBBs) are suggested by Baveno V.[9]

The work by Mishra et al.[10] is the first randomized controlled trial comparing therapies in the primary prevention of GVB, and as such makes an important contribution to the literature and merits closer review. More than 90% of screened patients (n = 1,050) were excluded because they failed to meet the strict inclusion criteria. Therefore, the investigators carefully selected patients who had the highest risk of bleeding. Perhaps this explains the relatively small sample size required to show differences between cyanoacrylate, NSBBs, and no treatment. There were significant differences in favor of cyanoacrylate for bleeding and survival when compared with no treatment ($P = 0.046$), and only for prevention of bleeding when compared with propranolol. The latter observation is interesting, because there was a significant reduction in the hepatic venous pressure gradient (HVPG) with propranolol and a rise in HVPG in the other groups. The lack of HVPG response in predicting bleeding is in contrast to that for esophageal variceal bleeding, where HVPG response to NSBBs has been shown to predict both bleeding and the formation of varices.[11] This finding perhaps reinforces our understanding of the risk factors for GVB. It has been shown that gastric varices can bleed at lower pressures compared with esophageal varices, suggesting that reduction in portal pressure will have less influence in bleeding risk or that a greater magnitude in pressure reduction is necessary to protect against bleeding.[12] Other risk factors (in particular the size of gastric varices) that in turn influence wall tension may also be important. The median size of gastric varices in the study was 20 mm and obturation of varices was achieved in all patients. Patients treated with cyanoacrylate all had a reduction in the size of gastric varices, in contrast to over a third of patients in the other arms having an increase in size of gastric varices. There was no difference in the appearance of esophageal varices or appearance/worsening of portal hypertensive gastropathy during follow-up in the two groups.

Certain aspects of the findings by Mishra et al.[10] findings must be considered carefully. It is not clear from the three-arm study whether a Bonferroni multiple comparison correction was used. Therefore, the findings may not withstand close statistical scrutiny. In practice, particularly outside of large specialized units, many patients may be ineligible for treatment given the strict inclusion criteria. Although no complications from cyanoacrylate were observed, in less expert hands this may not always be the case. It may be difficult to convince patients or clinicians to accept prophylactic cyanoacrylate if it has not been shown to be more effective than propranolol in improving survival. This brings into question the choice of NSBBs. The recent demonstration that carvedilol was more effective than band ligation in preventing bleeding from esophageal varices makes one wonder how this drug would compare with cyanoacrylate.[13] Only one-third of patients in the Mishra et al. study responded to propranolol, and because carvedilol has been shown to be more effective at lowering portal pressure in a greater proportion of patients,[14] the results could have been different. NSBBs would also treat esophageal varices and portal hypertensive gastropathy. The caveat is that NSBBs should be used with caution in patients with advanced cirrhosis, in particular those with refractory ascites.[15]

In conclusion, it is clear that carefully selected patients with large gastric varices should receive prophylactic treatment to prevent bleeding. Despite the promise shown by cyanoacrylate, further controlled trials comparing cyanoacrylate with beta-blockers such as carvedilol or even thrombin injection[16] are necessary. The latter therapy shows promise and, due to ease of use and lack of complications compared with cyanoacrylate, may be a more attractive option; however, it has yet to be studied in a controlled clinical trial.

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Does It Matter Not Only How Much but Also When We Eat to Induce Fatty Liver?

Feng D, Liu T, Sun Z, Bugge A, Mullican SE, Alenghat T, Liu XS, Lazar MA. A circadian rhythm orchestrated by histone deacetylase 3 controls hepatic lipid metabolism. Science 2011;331:1315-1319. (Reprinted with permission.)

Abstract

Disruption of the circadian clock exacerbates metabolic diseases, including obesity and diabetes. We show that histone deacetylase 3 (HDAC3) recruitment to the genome displays a circadian rhythm in mouse liver. Histone acetylation is inversely related to HDAC3 binding, and this rhythm is lost when HDAC3 is absent. Although amounts of HDAC3 are constant, its genomic recruitment in liver corresponds to the expression pattern of the circadian nuclear receptor Rev-erbα. Rev-erbα colocalizes with HDAC3 near genes regulating lipid metabolism, and deletion of HDAC3 or Rev-erbα in mouse liver causes hepatic steatosis. Thus, genomic recruitment of HDAC3 by Rev-erbα directs a circadian rhythm of histone acetylation and gene expression required for normal hepatic lipid homeostasis.

Comment

Circadian rhythms are responsible for daily variations in organ-specific functions and are essential in coordinating the timing of various physiological processes. Also, the gastrointestinal tract including the liver is subject to circadian rhythms, and a large number of genes involved in the maintenances of metabolic homeostasis is rhythmically expressed in the liver,[1] suggesting that circadian and metabolic regulatory networks are tightly connected. Circadian misalignment causes metabolic dysfunction, and mice with genetic disruption of circadian clock components develop hyperlipidemia, hyperglycemia, hypoinsulinemia, as well as hepatic steatosis.[2,3]

The nuclear receptor Rev-erbα is a key regulator of the circadian rhythm and is expressed in a circadian manner.[4] Rev-erbα is a transcriptional repressor of critical regulators of the circadian rhythms, and it is supposed that the circadian clock regulates metabolism mostly by regulating the expression of liver enzymes at the transcriptional level. Epigenetic alterations, such as hyperacetylation of the chromatin-associated histones, which is responsible for gene silencing, are critical regulators of gene transcription, involving multiple histone acetyltransferases and deacetylases (HDACs).

A recent report in *Science* demonstrates the existence of circadian changes in histone acetylation in mice, the dysregulation of which potentially causes major perturbations in normal metabolic functions and may also significantly affect the development and progression of nonalcoholic fatty liver disease (NAFLD) in men.[5]

Feng et al. discovered diurnal recruitment of HDAC3 to the liver genome of mice. In the light period, when the mice are inactive, HDAC bound to over 14,000 sites, whereas in the dark period when mice are active and feeding, the binding markedly reduced to only 120 sites. This HDAC3 recruitment pattern oscillated in a 24-hour cycle. Deletion of hepatic HDAC3 expression led to similar acetylation levels of histone H3 lysine 9 (H3K9) during the inactive time as observed in control mice during their activity period, indicating that the circadian clock is the pacemaker for the genomic HDAC3 recruitment. Associated with the observed decrease in H3K9 acetylation in mice with hepatic HDAC3 deletion, the authors found a decrease in polymerase II at the transcription start site of genes with HDAC3 binding sites and a reduced expression of these genes, respectively. Thus, diurnal recruitment of HDAC3 orchestrates a rhythm of epigenomic modification, polymerase II recruitment, and gene expression. Although the

HDAC3 recruitment to the genome is diurnal, the abundance of HDAC3 was constant throughout the light/dark cycle. HDAC3 enzymatic activity requires interaction with nuclear receptor corepressors, and Feng et al. discovered that Rev-erbα protein oscillated in phase with HDAC3 recruitment (Fig. 1A), and remarkably, Rev-erbα bound to the majority of HDAC3 binding sites during the inactive period but not during the active period of the mice (Fig. 1B). The extent of HDAC3 with Rev-erbα binding was surprising because other nuclear receptors can also interact with corepressors and HDAC3. However, HDAC3 binding was reduced at many sites in Rev-erbα-deficient mice, consistent with a critical role of Rev-erbα. Still, residual HDAC3 binding sites in Rev-erbα-deficient mice reveal that other factors also contribute to HDAC3 recruitment. Of note, the set of genes bound by Rev-erbα and HDAC3 was enriched for genes encoding for proteins that function in lipid metabolic processes, and indeed, livers in which HDAC3 was deleted revealed a significant increase of neutral lipid content. In accord, chow fed Rev-erbα-deficient mice also developed liver steatosis, and the majority of genes up-regulated in livers depleted of Rev-erbα were bound by both Rev-erbα and HDAC3 during the sleeping period of the mice. At that time HDAC3 and Rev-erbα colocalized at more than 100 lipid biosynthetic genes and polymerase II recruitment to the transcription start site of many of these genes increased, when the mice were active and ate. These findings suggest that biosynthesis was actively suppressed, and indeed, Rev-erbα- and HDAC3-deficient mice revealed increased *de novo* biosynthesis of lipids (Fig. 1C). Thus, this fascinating report provides a molecular mechanism underlying the observation that hepatic lipogenesis in mice follows a diurnal rhythm that is antiphase to Rev-erbα and HDAC3 recruitment to the genome. HDAC3 was already known as a critical regulator of circadian rhythm and glucose metabolism,[6] and liver-specific deletion of HDAC3 has been described to cause fatty liver in mice.[7] The present report newly connects HDAC3 with the circadian rhythm and impressively demonstrates that not its abundance but its rhythmic recruitment to the genome in concert with Rev-erbα critically affects transcriptional regulation of hepatic lipid metabolisms. The significance of daily variations in hepatic gene expression is still not fully determined but may be related to different requirements of nutrient absorption, energy generation, and energy storage during the feeding and fasting state. In general, the suprachiasmatic nucleus harbors the central pacemaker of the circadian rhythm in mammals, but circadian oscillators exist in most peripheral tissues including the liver. Rats exposed to a light/dark cycle regimen mimicking shift-work during a period of 10 weeks revealed significantly changed hepatic lipid metabolism, including and noteworthy also, Rev-erbα expression.[8] In the study by Feng et al. the HDAC3 recruitment pattern to the liver genome was retained in constant darkness, whereas the rhythm of HDAC recruitment to the genome was quickly reversed when food was provided only during the inactive, sleeping period. Because the liver clock is entrained by food intake, these findings indicate that the "hepatic" circadian clock is the pacemaker for the genomic HDAC3 recruitment. It has been shown that temporal feeding restriction under light/dark or dark/dark conditions can change the phase of circadian gene expression in peripheral cell types by up to 12 hours, while leaving the phase of cyclic gene expression in the suprachiasmatic nucleus unaffected.[9] Hence, changes in metabolism can lead to an uncoupling of peripheral oscillators from the central pacemaker, and misalignment of fasting/feeding and sleep/wake cycles with endogenous circadian cycles of hepatic fuel utilization or energy storage cause hepatic steatosis. The liver seems to be prone to such a misalignment because food-induced phase resetting proceeds faster in liver than in other organs such as kidney, heart, or pancreas.[8] What may be the pathopyhsiological significance of such an imbalance? Feng et al. describe only modestly elevated hepatic transaminases in HDAC3-deficient mice, but this was probably due to the short observation time after induced HDAC3 depletion, because a previous study found progressive hepatocellular damage in HDAC-deficient mice with time.[7] Also, experimentally induced disruption of the circadian rhythm led to an abolished rhythm in the expression of both central clock as well as hepatic clock genes and caused an altered innate immune response with heightened release of proinflammatory cytokines in response to lipopolysaccharide (LPS) treatment.[10] Recent studies revealed the crucial role of innate immunity in the progression of (nonalcoholic) steatosis to (nonalcoholic) steatohepatitis (NASH).[11] Together, these studies suggest that disruption of the circadian rhythm affects not only hepatic (lipid) metabolisms but subsequently triggers the progression of NASH. In line with this, genetic variants of molecular clock genes have been identified as risk factors for the development of NAFLD,[12] rotating shift work increases the risk for developing the metabolic syndrome,[13] and interestingly, this appears to be particularly the case in individuals with elevated alanine aminotransferase serum levels.[14] Moreover, circadian disruption was found to accelerate liver carcinogenesis in mice, further

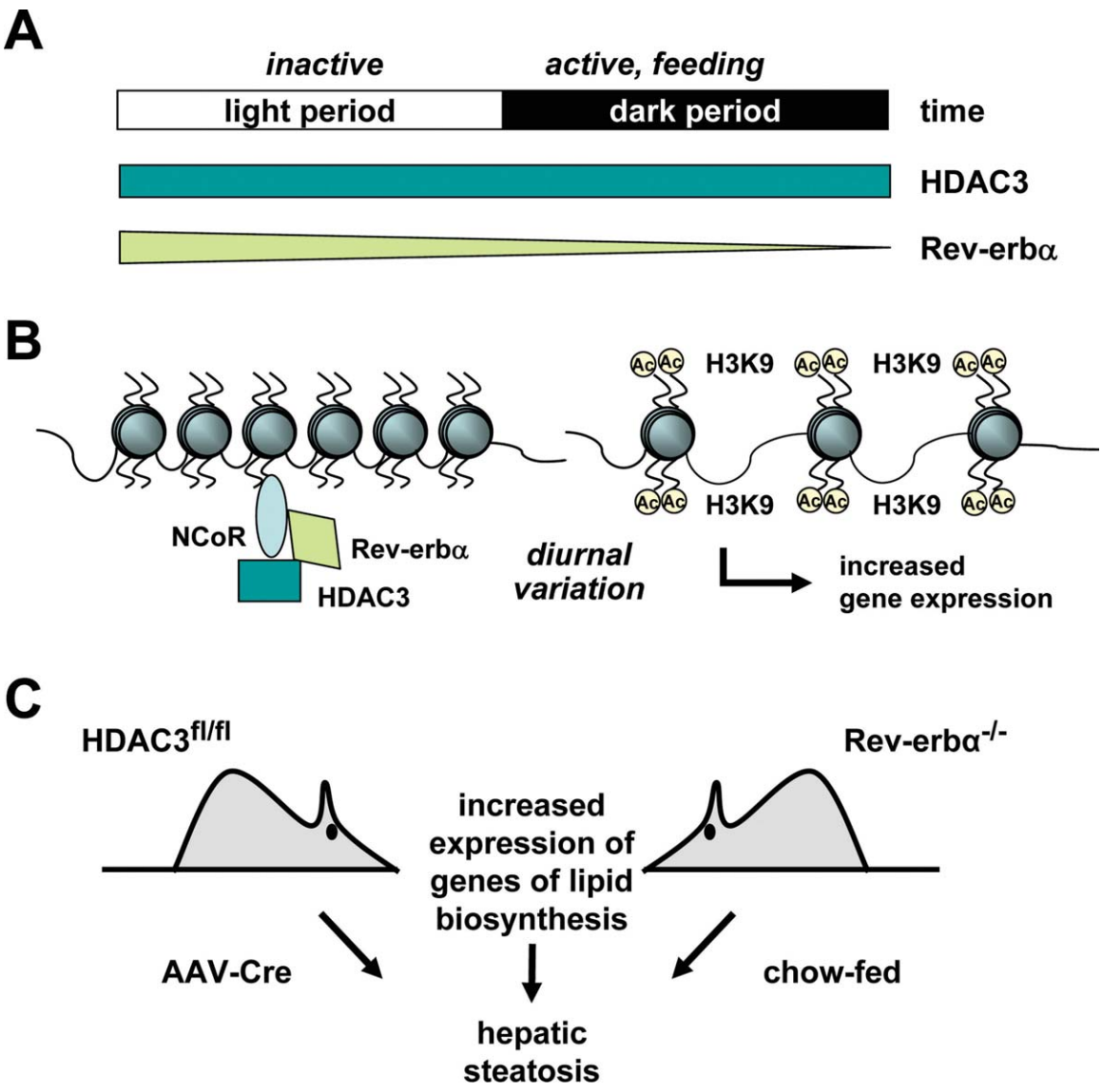

Fig. 1. Hepatic lipid homeostasis is regulated by a circadian rhythm. (A) Levels of HDAC3 and Rev-erbα expression during light (inactive phase) and dark (active phase) periods. (B) Diurnal variation of genomic recruitment of HDAC3 during the inactive (left) and active (right) periods. In the inactive phase, HDAC3 is recruited by way of the nuclear receptor corepressor (NCoR) and the circadian nuclear receptor Rev-erbα to liver metabolic genes, halting the biosynthesis of lipids. During the inactive phase, when concentrations are reduced and histone components (i.e., H3K9) are hyperacetylated, lower amounts of HDAC3 are bound to the genome, permitting lipid synthesis. (C) Chow-fed mice in which the HDAC3 gene was genetically removed in the liver (left) or which were Rev-erbα-deficient (right) showed increased expression of genes involved in lipid biosynthesis, resulting in hepatic steatosis.

suggesting that the tight and proper control of circadian clocks is a prerequisite of hepatic integrity.[15]

Thus, liver steatosis may be one of the myriad negative health effects of shift work, and, certainly, not only from the hepatologist's perspective should this be avoided. Still, if this is not feasible it seems mandatory to avoid or at least minimize the misalignment of the circadian and the hepatic clock. Of note, balanced diets containing carbohydrates/sugars and proteins were shown to be necessary for proper entrainment of the liver clock in mice.[16] Future studies have to show whether these findings may assist in the development of dietary recommendations for shift workers. In addition to the quality and quantity of food, not only for shift-workers and jet-lagged air travelers, the time of food consumption may be a risk factor for fatty liver.

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