Gene Expression, Vol. 16, pp. 101–108
Printed in the USA.

1052-2166/15 $90.00 + .00
DOI: http://dx.doi.org/10.3727/105221615X14181438356292
E-ISSN 1555-3884
www.cognizantcommunication.com

Review

Role of Hepatocyte Nuclear Factor 4α (HNF4α) in Cell Proliferation and Cancer

Chad Walesky*† and Udayan Apte*

*Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS, USA
†Department of Medicine – Genetics Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Hepatocyte nuclear factor 4α (HNF4α) is an orphan nuclear receptor commonly known as the master regulator of hepatic differentiation, owing to the large number of hepatocyte-specific genes it regulates. Whereas the role of HNF4α in hepatocyte differentiation is well recognized and extensively studied, its role in regulation of cell proliferation is relatively less known. Recent studies have revealed that HNF4α inhibits proliferation not only of hepatocytes but also cells in colon and kidney. Further, a growing number of studies have demonstrated that inhibition or loss of HNF4α promotes tumorigenesis in the liver and colon, and reexpression of HNF4α results in decreased cancer growth. Studies using tissue-specific conditional knockout mice, knock-in studies, and combinatorial bioinformatics of RNA/ChIP-sequencing data indicate that the mechanisms of HNF4α-mediated inhibition of cell proliferation are multifold, involving epigenetic repression of promitogenic genes, significant cross talk with other cell cycle regulators including c-Myc and cyclin D1, and regulation of miRNAs. Furthermore, studies indicate that posttranslational modifications of HNF4α may change its activity and may be at the core of its dual role as a differentiation factor and repressor of proliferation. This review summarizes recent findings on the role of HNF4α in cell proliferation and highlights the newly understood function of this old receptor.

Key words: c-Myc; Proliferating cell nuclear antigen (PCNA); Diethylnitrosamine; Hepatocytes; Hepatocellular carcinoma (HCC)

HNF4α, THE QUINTESSENTIAL ORPHAN NUCLEAR RECEPTOR

Hepatocyte nuclear factor 4α (HNF4α, NR2A1) is a highly conserved member of the nuclear receptor superfamily. It was first discovered bound to sites required for the transcription of transthyretin (*TTR*) and apolipoprotein CIII (*APOCIII*) within rat liver extracts (1). HNF4α is expressed at high levels in the liver and kidney and to a lesser degree (<50%) in the small intestine, colon, and pancreatic β-cells (2,3). In mouse liver development, HNF4α is expressed in the primary and extraembryonic visceral endoderm prior to gastrulation and in epithelial cells at the onset of liver, pancreas, and intestine formation (4). HNF4α-null embryos exhibit severe visceral endoderm defects preventing gastrulation and causing failure to develop past 6.5 dpc (5).

The functional domains of HNF4α consist of an N-terminal activation domain (AF-1, also referred to as A/B domain); a zinc finger domain that serves as the DNA-binding domain (C domain); a putative ligand-binding domain (E domain); and a C-terminal domain that contains a region involved in homodimerization, activation function (AF-2), and a short repressor region (6). The HNF4α gene consists of 13 exons spanning over 70 kb and has multiple alternatively spliced variants. Multiple isoforms are proposed to exist in mammals and are thought to have different physiological roles in development and transcriptional regulation of target genes (3). Further, HNF4α has two differentially utilized promoters. During early liver development, HNF4α initiates from the P2 promoter, but as the liver differentiates, transcription of the HNF4α gene begins to favor the P1 promoter. P2 isoforms appear to activate genes involved in early liver development, such as α-fetoprotein and transthyretin, while P1 isoforms appear to activate genes involved in later hepatic differentiation, such as *apoCIII* (2,7).

Recent studies have highlighted the importance of the DNA-binding and ligand-binding domains for proper

Address correspondence to Udayan Apte, Ph.D., D.A.B.T., Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, 3901 Rainbow Blvd, MS1018, Kansas City, KS 66160, USA. Tel: +1 (913) 588-9247; E-mail: uapte@kumc.edu

binding of HNF4α to its response elements. A lack of the ligand-binding domain can reduce the affinity of HNF4α for its response elements by ~75-fold (8). HNF4α's ligand-binding domain interacts with endogenous fatty acids, such as linoleic acid. The presence of the fatty acid is thought to lend structural integrity to the protein, but its presence has not been shown to confer significant transcriptional activation (9). It is likely that the fatty acid is needed for stabilization of the protein instead of switching it off and on.

HNF4α functions by binding as a homodimer to its DNA recognition site, a direct repeat element (AGGTCA) with either a one- or two-nucleotide spacer (AGGTCAnAGGTCA or AGGTCAnnAGGTCA; designated DR1 or DR2, respectively). After binding to DNA, HNF4α recruits transcriptional coactivators and accessory proteins to positively regulate the expression of its target genes, such as GRIP1, PGC-1, CBP/p300, and SRC (10–15).

HNF4α AND CELLULAR DIFFERENTIATION

HNF4α regulates important hepatic functions, such as glycolysis, gluconeogenesis, ureagenesis, fatty acid metabolism, bile acid synthesis, drug metabolism, apolipoprotein synthesis, and blood coagulation, by regulating the transcription of many of the genes involved in each of these functions (16–20). Because of its involvement in a wide array of hepatic functions, HNF4α is known throughout the literature as the master regulator of hepatic differentiation.

Postnatal hepatocyte-specific deletion of HNF4α using an HNF4α$^{Fl/Fl}$ allele utilizing an enhancer-driven Cre recombinase under the albumin promoter (Alb-Cre) showed that a loss of HNF4α results in accumulation of lipid, reduced serum cholesterol and triglyceride levels, and increased serum bile acids (16). Further, HNF4α-null livers exhibited a decrease in classic hepatocyte gene expression such as apolipoprotein B, microsomal triglyceride transfer protein, liver fatty acid-binding protein, and bile acid transport proteins sodium taurocholate cotransportering polypeptide (*NTCP*) and organic anion transportering polypeptide 1A1 (*OATP1A1*) (16). The absence of HNF4α in this model results in metabolic disruption and increased mortality (16–19).

HNF4α AND PROLIFERATION

Evidence in Extrahepatic Tissues

The initial evidence suggesting that HNF4α is involved in cell proliferation came from the observation that HNF4α expression decreases in cancers of multiple organs that normally express HNF4α. Analysis of human renal cell carcinoma (RCC) showed a downregulation of HNF4α mRNA and protein expression along with suppression in HNF4α DNA-binding activity (21,22). As a follow-up to these studies, Lucas et al. showed that expression of HNF4α in HEK293 cells caused an inhibition in cell proliferation and characterized gene changes that correlate with the changes seen in RCCs (23). Grigo et al. narrowed the list of genes thought to be responsible for the effect of HNF4α on renal cell carcinoma to 14 genes, including *CDKN1A* (p21), *TGFA*, *MME* (*NEP*), *ADAMTS1*, *SEPP1*, *THEM2*, *BPHL*, *DSC2*, *ANK3*, *ALDH6A1*, *EPHX2*, *NELL2*, *EFHD1*, and *PROS1* (24).

Chiba et al. developed an F9 murine embryonal cell line with an inducible form of HNF4α. In this model, they provide evidence that expression of HNF4α inhibits cell proliferation. They showed that F9 cells expressing inducible HNF4α become arrested in the G_0/G_1 phase of the cell cycle due to an upregulation of *CDKN1A* (p21) in a p53-independent manner. They further confirmed their findings in rat lung endothelial cells (25).

Erdmann et al. investigated whether the presence of HNF4α could inhibit proliferation of pancreatic β-cells by overexpressing HNF4α in rat INS-1 cells, a rat insulinoma cell line. They found that overexpression of the HNF4α isoform 2 led to pronounced morphological changes and a decrease in cell proliferation (26).

Tanaka et al. first showed that P1 isoforms of HNF4α are lost in colorectal carcinomas (27). Chellappa et al. expanded on this observation by providing evidence on a possible mechanism involving Src tyrosine kinase and the observed loss of P1-HNF4α (28). In their study, they observe lost or mislocalized P1-HNF4α in ~80% of Stage C colon cancer correlating with active Src.

Does HNF4α Inhibit Cell Proliferation in the Liver?

It is well established that HNF4α is essential for normal hepatic development and maintenance of a differentiated phenotype. Mice, which lack HNF4α, fail to develop functional hepatic tissue marked by a lack of hepatic gene expression (29,30). A study performed by Mizuguchi et al. (31) suggested that the maintenance of a differentiated phenotype might inhibit hepatocyte proliferation in an in vitro system. Primary rat hepatocytes treated with 2% DMSO maintained a more normal expression of hepatic markers, including higher HNF4α expression, and exhibited decreased DNA synthesis (BrdU incorporation assay). Späth and Weiss expressed HNF4α in the rat hepatoma cell line, H5, and showed that following HNF4α expression the cells took on a more hepatocyte-like morphology along with a reactivation of hepatocyte-specific genes (32). Also, they observed a decrease in cell proliferation; however, many of their observations were made only in the presence of dexamethasone.

More recent studies using an HNF4α hepatocyte-specific KO (AlbCre; HNF4α$^{Fl/Fl}$) shed some light on

postnatal liver development and a possible role in the inhibition of hepatocyte proliferation. HNF4α KO livers showed a significant increase in liver/body weight ratio (4.0±0.3 to 7.3±0.9) (16). This observed increase was not further studied in this report, but it is some of the first evidence that HNF4α may inhibit hepatocyte proliferation and maintain a normal liver/body weight ratio.

Lazarevich et al. first described a link between HNF4α's known functions in promoting differentiation and how this may be inhibited in times of increased proliferation (33). It is well accepted that there is a link between a cell's state of differentiation and its propensity for proliferation. Generally, a cell that is well differentiated does not have a propensity to proliferate (34). The study by Lazarevich shows that a slow-growing hepatocellular carcinoma (HCC) can progress to a fast-growing, dedifferentiated HCC; this correlates with a repression of HNF4α. They found that a forced expression of HNF4α in the fast-growing HCC causes redifferentiation of the tumor cells toward a more hepatocyte-like phenotype. The observed change occurs rapidly, suggesting that the progression of the tumor was due to a limited number of gene changes. Further, they observe that the forced expression of HNF4α suppressed the proliferation of the fast-growing HCC cells, suggesting that HNF4α may have a tumor-suppressing effect. The authors conclude that a loss of HNF4α may be a critical event in HCC progression and mediates a dedifferentiation, loss of cell adhesion, and an increase in cell proliferation and invasiveness.

Lazarevich et al. conducted a more recent study to investigate HNF4α as a marker for epithelial tumor progression (35). In these studies, tumor progression to a dedifferentiated phenotype correlated with a reduction in HNF4α in mice. They also observed that a decrease in HNF4α gene expression correlated with an "unfavorable" prognosis of HCC in humans and that a repression of HNF4α in human pancreatic adenocarcinomas correlated with more dedifferentiated tumors. A study by Xie and colleagues found that overexpression of HNF4α induced differentiation of hepatoma cell lines, HepG2 and Hep3B. This caused an increase in cell cycle arrest (HepG2) and apoptosis (Hep3B) in these models. In vivo overexpression of HNF4α caused a decrease in tumorigenesis of the hepatoma cells and showed an antitumor effect on tumor xenografts and in diethylnitrosamine (DEN)-induced hepatocarcinogenesis (36,37).

Recent Evidence in the Liver. Because of the importance of the presence of HNF4α in the liver during development, a true HNF4α KO results in embryonic lethality. Therefore, the Cre-Lox system was utilized to create liver-specific inducible KO of HNF4α, which has been described previously (16). This model provided some early evidence that a lack of HNF4α may result in increased hepatocyte proliferation supported by an increase in liver-to-body weight ratio present in these mice. There are two explanations for the observed increase in liver-to-body weight ratio, hypertrophy, and/or hyperplasia. The major problem in using this model to study the role of HNF4α in proliferation is that these mice display severe hepatic metabolic disruption and death by 6–8 weeks of age. Further, the deletion of HNF4α happens early in life, when the liver is still growing and differentiating. Many gene changes are occurring throughout this time period, which would make the elucidation of the mechanism extremely difficult. Advent of tamoxifen-inducible albumin Cre (Alb-Cre ERT2) provided an excellent approach to tackle the question of whether HNF4α has a direct effect on hepatocyte proliferation. We recently developed an inducible HNF4α KO mouse, which was also developed in an independent lab concurrently (Bonzo et al.). Each utilized a liver-specific, tamoxifen-inducible Cre system, Alb*ERT2cre* (38–40).

In these studies we found that deletion of HNF4α in the mature liver results in hepatomegaly and steatosis, which were observations made previously in the Alb-Cre HNF4α KO mice (16). We also provide evidence that hepatomegaly is due to hyperplasia because of an increase in cell proliferation markers proliferating cell nuclear antigen (PCNA) (38–40) and Ki-67 (38,40), along with an observed increase in liver-to-body weight ratio (38–40). Taken together, these studies provide evidence that the presence of HNF4α inhibits hepatocyte proliferation.

We also investigated the effect of HNF4α deletion in a chemical-induced model of HCC (38). In this study, we treated mice with the known hepatic carcinogen diethylnitrosamine, or DEN, at postnatal day 15 in order to initiate transformation of hepatocytes. At 8 months of age, we deleted HNF4α, waited two more months, and observed what effects a loss of HNF4α would have on the progression of the already initiated HCC. We found that a loss of HNF4α in combination with DEN caused a large expansion in tumor number and tumor size with an almost twofold increase in liver-to-body weight ratio. We also highlighted the upregulation of Cyclin D1 and the known oncogene Myc within the HNF4α-depleted tumors.

Further, a recent study by Saha and Parachoniak et al. highlighted the importance of HNF4α in intrahepatic cholangiocarcinoma (IHCC) (41). It has been well characterized that select IHCCs are associated with gain-of-function mutations in IDH1/IDH2 (42–45). In their study, they provide a novel mechanism by which IDH mutations contribute to the formation of IHCCs. They show that an upregulation of IDH blocks liver progenitor cells from differentiating through the production of 2-HG and inhibition of HNF4α. Further, we have provided evidence, as described previously, that suppression of HNF4α causes an

increase in hepatocyte proliferation. Saha and Parachoniak et al. corroborate this finding in their IHCC model by observing increased hepatic proliferation in response to IDH-dependent downregulation of HNF4α.

These studies, along with the Xie and colleagues (36,37), Lazarevich et al. (33,46), and Tanaka et al. (27) studies, conclusively demonstrate that HNF4α functions as a tumor suppressor in the liver and is involved in inhibition of hepatocyte proliferation.

Mechanism of Increased Cell Proliferation After HNF4α Deletion. Recent data suggest the involvement of multiple mechanisms by which HNF4α may be inhibiting hepatocyte proliferation (Fig. 1). Because HNF4α functions as a transcription factor, each group characterizing the inducible HNF4α KO mice looked at global gene expression changes by microarray analysis (38–40). Each group made the observation that many of the downregulated genes are involved with differentiation, many of which are known HNF4α targets. Interestingly, many of the upregulated genes are involved with cell proliferation, cell cycle progression, and cancer.

Mechanisms of HNF4α-Mediated Gene Repression. It is probable that HNF4α's influence on hepatocyte proliferation is due to multiple signaling pathways being affected because of the large number of targets HNF4α has in the liver. It is possible that this effect is due to a direct or indirect mechanism, that is, due to direct regulation of genes by HNF4α binding or due to a secondary gene change downstream of HNF4α deletion. We approached this question by comparing global gene expression changes to HNF4α binding data (RNA-seq vs. ChIP-seq). Our data indicate that nearly half of the genes changed following a deletion of HNF4α have a putative HNF4α binding site. We have confirmed three of these binding sites by ChIP: *Ect2*, *Osgin1*, and *Hjurp*.

HNF4α is generally thought to be a positive transcriptional activator (1,20). The data presented by our group and Bonzo et al. suggest that HNF4α may repress genes involved in hepatocyte proliferation. A possible mechanism for the repression of these genes is through the recruitment of histone deacetylases. Acetylation of histones is thought to be a signal for the activation of transcription by influencing the conformation of chromatin promoting an open state that allows for the binding of transcription factors and the polymerase machinery. HNF4α may be silencing these genes by recruiting histone deacetylases and keeping the chromatin in a closed state. HNF4α has been shown in the past to interact with the known histone deacetylase recruiter, SMRT (47,48). Ungaro et al. was the first group to show that HNF4α may be using this mechanism to inhibit the transcription of the gene *PED*. They showed that HNF4α can recruit SMRT to the *PED* promoter region leading to histone deacetylation and remodeling of chromatin to a heterochromatic state (48).

HNF4α, Myc, and Cyclin D1. c-Myc is known to be associated with cell proliferation. It is upregulated during development and in proliferating cells and is commonly upregulated in human tumors, giving it the designation as an oncoprotein. Elevated levels of c-Myc are known to cause cell cycle progression (49,50) and cellular immortalization (51). Hwang-Versleus and Sladek first showed that c-Myc and HNF4α compete for control of the p21 promoter. In their study, they show that HNF4α

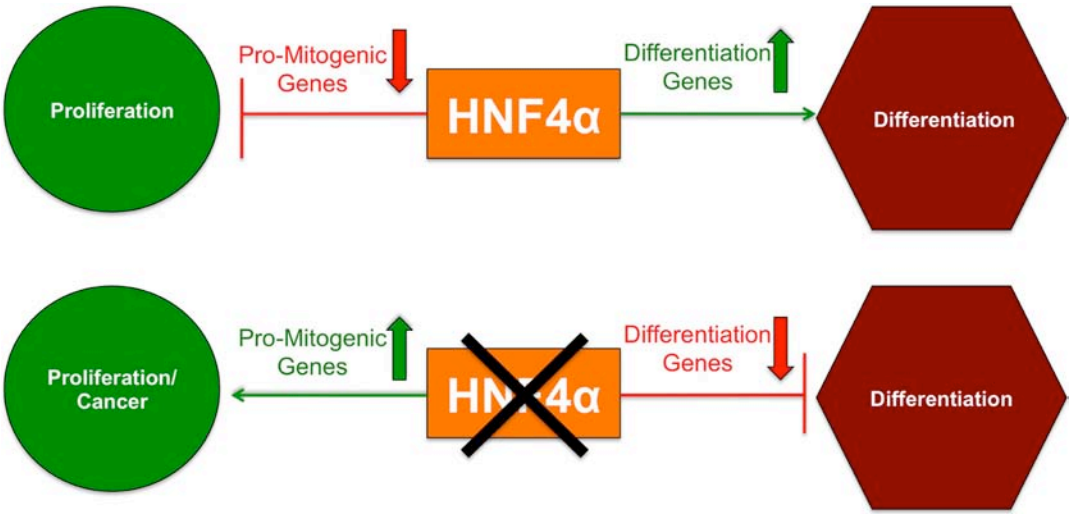

Figure 1. Scheme showing regulation of hepatocyte proliferation by HNF4α. In quiescent hepatocytes, along with stimulating expression of genes involved in hepatic differentiation, HNF4α also suppresses expression of certain promitogenic genes. Loss of HNF4α results in reprograming of the hepatocyte gene expression program leading to dedifferentiation and increased proliferation.

can upregulate p21, which is known to block cell proliferation, by direct competition and the ability to bind to c-Myc.

We observed a significant increase in c-Myc in the inducible HNF4α KO models. RNA-Seq studies revealed that the c-Myc-regulated gene network, which involves many promitogenic genes, such as *Fus*, *Set*, *Ccnb1*, *Ccnb2*, *Rrm2*, and *Myc*, was highly activated upon deletion of HNF4α. Further studies in the DEN-induced HCC model revealed a correlation between a depletion of HNF4α and an increase in c-Myc, along with an increase in cyclin D1 in tumors. These data help to support the c-Myc/cyclin D1/HNF4α loop proposed by Sladek as a possible mechanism controlling proliferation and differentiation within hepatocytes (52). Recent work by Hanse et al. (53) provided evidence on the effect cyclin D1 has on HNF4α and its transcriptional activity at select target genes. They found that cyclin D1 inhibits HNF4α's binding at select genes, causing a downregulation in gene expression. They conclude that this is due to a direct interaction between cyclin D1 and HNF4α.

HNF4α and Cyclin F. Cyclin F is expressed during the S phase and peaks during the G_2 phase of the cell cycle. It is known to interact with ribonucleotide reductase family member 2, RRM2, and is thought to promote its ubiquitylation and degradation (54). Ribonucleotide reducastes catalyze the conversion of ribonucleotides to dNTPs for synthesis of DNA during replication and repair. Cyclin F-mediated degradation of RRM2 is essential to maintain balanced levels of dNTPs. Failure to regulate dNTP levels causes genome instability and a phenotype associated with hypermutation (55). Cells in which cyclin F expression is silenced accumulate high levels of dNTPs and display an increase in mutation frequency compared with control cells (54). We observed a reduction in cyclin F expression in our HNF4α KO study. HNF4α KO tumors express lower levels of cyclin F and increased expression of RRM2. This is another potential mechanism by which HNF4α KO may promote tumorigenesis that needs further investigation.

miRNA. Hatziapostolou et al. (56) provide evidence for a possible HNF4α-driven miRNA feedback loop as a potential mechanism for inhibiting proliferation within the liver. They provide evidence for a signaling loop consisting of HNF4α, miR-124, IL6R, STAT3, miR-24, and miR-629 contributing to maintain a transformed phenotype in the liver using nontransformed, immortalized human hepatocytes. Following disruption of HNF4α, they found that the hepatocytes were transformed, had increased invasiveness, and could promote tumor formation in immunodeficient mice. The tumors showed continued suppression of HNF4α at day 55, suggesting that the inhibition of HNF4α may initiate a feedback loop that continuously suppresses the gene. They identified two miRNAs, miR-24 and miR-629, which are direct regulators of HNF4α expression. They find that miR-24 and miR-629 can be activated by STAT3, which is in turn activated by IL6R. They further characterize miR-124 as an HNF4α target that inhibits IL6R.

We, as well as Bonzo et al., investigated this mechanism in our respective models. Neither group could corroborate this result at the level of IL6R and STAT3 upregulation of activation. This could however be a limitation of the models used in either of the experiments or a species-dependent mechanism.

Recently, Yin et al. (57) identified that HNF4α can induce the expression of the miR-379-656 cluster in the DLK-DIO3 region and regulate the transcription of this region. Further, they found that miR-134, a key member of this cluster, can suppress HCC cells through a KRAS-dependent mechanism. They also show that low miR-134 levels correlate with low HNF4α levels in highly aggressive human HCCs.

Posttranslational Modifications of HNF4α. Posttranslational modification of proteins is an efficient biological signal for alteration of protein function playing a key role in functions such as protein activation/deactivation, protein localization, protein transportation, and transcriptional activity (58,59). Little is known about posttranslational modification of HNF4α and the involved functions. A recent study by Yokoyama et al. (60) has identified eight modification sites and created point mutations of the sites to investigate any role in transcriptional activation. In their study, they identified phosphorylation (S142, T166, S167, T432, and S436), ubiquitilation (K234 and K307), and acetylation (K458) sites. Following the introduction of point mutations at those sites, they found that a point mutation of S142D caused a downregulation of HNF4α transcriptional activity through a reduction in DNA-binding, which was consistent with previous studies (61). They also found that the point mutation K458R, which leads to an acetylation-negative HNF4α, caused a significantly high transcriptional activity. Conversely, the K458Q acetylation-mimicking mutant showed weak transcriptional activity. A study by Ktistaki et al. (62) showed that tyrosine phosphorylation can affect the subnuclear localization, DNA binding, and transactivation of HNF4α. Also Li et al. (63) showed that phosphorylation of HNF4α can reduce transcriptional activity by reducing DNA-binding through a JAK2-dependent mechanism.

In a study mentioned previously, Chellappa et al. investigated an isoform-specific loss of HNF4α in human colon cancer (28). They identified that the observed loss of P1-HNF4α is due to phosphorylation of HNF4α at three tyrosine residues by Src kinase causing a disruption in coactivator binding and a loss in transcriptional activation,

nuclear localization, and protein stability. Evidence is also provided identifying three SNPs in HNF4α that increase Src-mediated phosphorylation (L280F, P421L, and P436S).

Taken together, these studies suggest that posttranslational modifications can influence the recruitment of coactivators and corepressors in order to modify the transcriptional influence HNF4α has on genes in which it is bound. One possibility is that a given posttranslational modification may recruit coactivators that further recruit the transcription machinery, whereas another posttranslational modification may inhibit this recruitment. As mentioned previously, a new hypothesis is that HNF4α may be functioning to recruit cofactors that influence the conformation of chromatin. It is possible that posttranslational modifications are influencing which cofactors are being recruited, either coactivators or corepressors, by altering protein–protein interactions between its binding partners.

Growth Factors. Bonzo et al. looked at the expression of various growth factors, including *Areg, Bmp7, Egf, Hb-Egf, Igf-1, Scf,* and *Tgfa.* They observed a slight increase in *Tgfa* (1.75) and a reduction in *Hgf* (0.44) and *Igf-1* (0.21). The most striking difference is in *Bmp7,* which was increased over 40-fold. A recent study has shown that *Bmp7* is upregulated in certain HCCs (64). An increase in *Bmp7* was corroborated by us in which *Bmp7* was also found to be upregulated over 40-fold. Bonzo et al. came to the conclusion that *Bmp7* is not a target of HNF4α via ChIP assay. They do however comment that *Bmp7* is a known target of Myc, which has been shown to be upregulated following loss of HNF4α.

HNF4α as a Therapeutic Target. The recent evidence that HNF4α is involved in proliferation of hepatocytes, as well as other cell types throughout the body, has highlighted it as a potential target for therapeutics and/or its use as a prognostic marker. HNF4α falls into the nuclear receptor superfamily of ligand-dependent transcription factors. For a long time, HNF4α was an orphan nuclear receptor, but recent evidence has shown that fatty acids, such as linoleic acid, can bind in the ligand-binding domain (9). However, the investigators saw a moderate influence on HNF4α transcriptional activity when linoleic acid was not present. Still, this is some of the first evidence suggesting that a molecule can bind reversibly in the ligand-binding pocket of HNF4α, giving confidence that it may be able to be targeted therapeutically.

Xie and colleagues have provided evidence that forced expression of HNF4α can alleviate hepatic fibrosis and cirrhosis and block HCC development (37,65,66). Further work on how HNF4α is regulated and targets genes for activation or inhibition will be needed in order to selectively target genes in a given disease state. It would be beneficial to inhibit promitogenic genes in a disease state such as HCC in order to inhibit proliferation and promote differentiation by upregulating HNF4α itself or influencing its transactivation of select genes. This could benefit by slowing tumor growth and making tumors less invasive. On the other hand, promoting promitogenic gene expression in patients with acute liver failure may aid in the regenerative response.

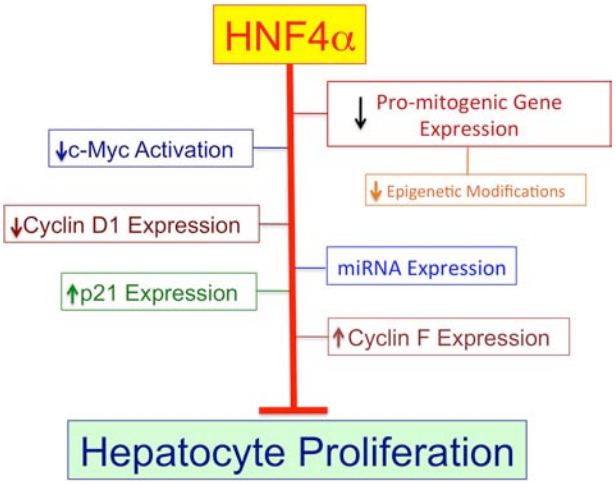

Figure 2. Proposed mechanisms by which HNF4α suppresses hepatocyte proliferation. HNF4α inhibits hepatocyte proliferation via several proposed mechanisms, including direct inhibition of promitogenic genes, inhibition of c-Myc and cyclin D1 function, miRNA regulation, and upregulation of cyclin F.

In closing, the recent discovery that HNF4α plays a role in cell proliferation has begun to shed light on the complex balance between organ growth and differentiation. Recent studies point to several possible mechanisms by which HNF4α may inhibit cell proliferation (Fig. 2). The mechanisms surrounding factors like HNF4α are an important area of focus for understanding the inner workings of more complex mechanisms such as liver regeneration and liver cancer pathogenesis.

ACKNOWLEDGMENTS: These studies were supported by P20 RR021940, 5T32ES007079-34, AASLD/ALF Liver Scholar Award, and 1R01DK098414.

REFERENCES

1. Sladek FM, Zhong WM, Lai E, Darnell JE. Liver-enriched transcription factor HNF-4 is a novel member of the steroid-hormone receptor superfamily. Genes Dev 1990; 4: 2353–2365.
2. Drewes T, Senkel S, Holewa B, Ryffel GU. Human hepatocyte nuclear factor 4 isoforms are encoded by distinct and differentially expressed genes. Mol Cell Biol 1996; 16:925–931.
3. Jiang S, Tanaka T, Iwanari H, Hotta H, Yamashita H, Kumakura J, Watanabe Y, et al. Expression and localization of P1 promoter-driven hepatocyte nuclear factor-4alpha

(HNF4αlpha) isoforms in human and rats. Nucl Recept 2003; 1:5.
4. Duncan SA, Manova K, Chen WS, Hoodless P, Weinstein DC, Bachvarova RF, Darnell JE, Jr. Expression of transcription factor HNF-4 in the extraembryonic endoderm, gut, and nephrogenic tissue of the developing mouse embryo: HNF-4 is a marker for primary endoderm in the implanting blastocyst. Proc Natl Acad Sci USA 1994; 91:7598–7602.
5. Chen WS, Manova K, Weinstein DC, Duncan SA, Plump AS, Prezioso VR, Bachvarova RF, et al. Disruption of the HNF-4 gene, expressed in visceral endoderm, leads to cell death in embryonic ectoderm and impaired gastrulation of mouse embryos. Genes Dev 1994; 8:2466–2477.
6. Ryffel GU. Mutations in the human genes encoding the transcription factors of the hepatocyte nuclear factor (HNF)1 and HNF4 families: Functional and pathological consequences. J Mol Endocrinol 2001; 27:11–29.
7. Torres-Padilla ME, Fougere-Deschatrette C, Weiss MC. Expression of HNF4αlpha isoforms in mouse liver development is regulated by sequential promoter usage and constitutive 3' end splicing. Mech Dev 2001; 109:183–193.
8. Chandra V, Huang P, Potluri N, Wu D, Kim Y, Rastinejad F. Multidomain integration in the structure of the HNF-4alpha nuclear receptor complex. Nature 2013; 495:394–398.
9. Yuan X, Ta TC, Lin M, Evans JR, Dong Y, Bolotin E, Sherman MA, et al. Identification of an endogenous ligand bound to a native orphan nuclear receptor. PloS One 2009; 4:e5609.
10. Wang JC, Stafford JM, Granner DK. SRC-1 and GRIP1 coactivate transcription with hepatocyte nuclear factor 4. J Biol Chem 1998; 273:30847–30850.
11. Dell H, Hadzopoulou-Cladaras M. CREB-binding protein is a transcriptional coactivator for hepatocyte nuclear factor-4 and enhances apolipoprotein gene expression. J Biol Chem 1999; 274:9013–9021.
12. Sladek FM, Ruse MD, Jr., Nepomuceno L, Huang SM, Stallcup MR. Modulation of transcriptional activation and coactivator interaction by a splicing variation in the F domain of nuclear receptor hepatocyte nuclear factor 4alpha1. Mol Cell Biol 1999; 19:6509–6522.
13. Soutoglou E, Katrakili N, Talianidis I. Acetylation regulates transcription factor activity at multiple levels. Mol Cell 2000; 5:745–751.
14. Yoon JC, Puigserver P, Chen G, Donovan J, Wu Z, Rhee J, Adelmant G, et al. Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. Nature 2001; 413:131–138.
15. Iordanidou P, Aggelidou E, Demetriades C, Hadzopoulou-Cladaras M. Distinct amino acid residues may be involved in coactivator and ligand interactions in hepatocyte nuclear factor-4alpha. J Biol Chem 2005; 280:21810–21819.
16. Hayhurst GP, Lee YH, Lambert G, Ward JM, Gonzalez FJ. Hepatocyte nuclear factor 4alpha (nuclear receptor 2A1) is essential for maintenance of hepatic gene expression and lipid homeostasis. Mol Cell Biol 2001; 21:1393–1403.
17. Inoue Y, Yu AM, Inoue J, Gonzalez FJ. Hepatocyte nuclear factor 4 alpha is a central regulator of bile acid conjugation. J Biol Chem 2004; 279:2480–2489.
18. Inoue Y, Peters LL, Yim SH, Inoue J, Gonzalez FJ. Role of hepatocyte nuclear factor 4 alpha in control of blood coagulation factor gene expression. J Mol Med 2006; 84:334–344.
19. Inoue Y, Yu AM, Yim SH, Ma X, Krausz KW, Inoue J, Xiang CC, et al. Regulation of bile acid biosynthesis by hepatocyte nuclear factor 4alpha. J Lipid Res 2006; 47:215–227.
20. Gonzalez FJ. Regulation of hepatocyte nuclear factor 4 alpha-mediated transcription. Drug Metab Pharmacokinet 2008; 23:2–7.
21. Sel S, Ebert T, Ryffel GU, Drewes T. Human renal cell carcinogenesis is accompanied by a coordinate loss of the tissue specific transcription factors HNF4 alpha and HNF1 alpha. Cancer Lett 1996; 101:205–210.
22. Lenburg ME, Liou LS, Gerry NP, Frampton GM, Cohen HT, Christman MF. Previously unidentified changes in renal cell carcinoma gene expression identified by parametric analysis of microarray data. BMC Cancer 2003; 3:31.
23. Lucas B, Grigo K, Erdmann S, Lausen J, Klein-Hitpass L, Ryffel GU. HNF4αlpha reduces proliferation of kidney cells and affects genes deregulated in renal cell carcinoma. Oncogene 2005; 24:6418–6431.
24. Grigo K, Wirsing A, Lucas B, Klein-Hitpass L, Ryffel GU. HNF4 alpha orchestrates a set of 14 genes to down-regulate cell proliferation in kidney cells. Biol Chem 2008; 389: 179–187.
25. Chiba H, Itoh T, Satohisa S, Sakai N, Noguchi H, Osanai M, Kojima T, et al. Activation of p21CIP1/WAF1 gene expression and inhibition of cell proliferation by overexpression of hepatocyte nuclear factor-4alpha. Exp Cell Res 2005; 302:11–21.
26. Erdmann S, Senkel S, Arndt T, Lucas B, Lausen J, Klein-Hitpass L, Ryffel GU, et al. Tissue-specific transcription factor HNF4αlpha inhibits cell proliferation and induces apoptosis in the pancreatic INS-1 beta-cell line. Biol Chem 2007; 388:91–106.
27. Tanaka T, Jiang S, Hotta H, Takano K, Iwanari H, Sumi K, Daigo K, et al. Dysregulated expression of P1 and P2 promoter-driven hepatocyte nuclear factor-4alpha in the pathogenesis of human cancer. J Pathol 2006; 208: 662–672.
28. Chellappa K, Jankova L, Schnabl JM, Pan S, Brelivet Y, Fung CL, Chan C, et al. Src tyrosine kinase phosphorylation of nuclear receptor HNF4αlpha correlates with isoform-specific loss of HNF4αlpha in human colon cancer. Proc Natl Acad Sci USA 2012; 109:2302–2307.
29. Duncan SA, Nagy A, Chan W. Murine gastrulation requires HNF-4 regulated gene expression in the visceral endoderm: Tetraploid rescue of Hnf-4(-/-) embryos. Development 1997; 124:279–287.
30. Li J, Ning G, Duncan SA. Mammalian hepatocyte differentiation requires the transcription factor HNF-4alpha. Genes Dev 2000; 14:464–474.
31. Mizuguchi T, Mitaka T, Hirata K, Oda H, Mochizuki Y. Alteration of expression of liver-enriched transcription factors in the transition between growth and differentiation of primary cultured rat hepatocytes. J Cell Physiol 1998; 174:273–284.
32. Spath GF, Weiss MC. Hepatocyte nuclear factor 4 provokes expression of epithelial marker genes, acting as a morphogen in dedifferentiated hepatoma cells. J Cell Biol 1998; 140:935–946.
33. Lazarevich NL, Cheremnova OA, Varga EV, Ovchinnikov DA, Kudrjavtseva EI, Morozova OV, Fleishman DI, et al. Progression of HCC in mice is associated with a down-regulation in the expression of hepatocyte nuclear factors. Hepatology 2004; 39:1038–1047.
34. Holland JF, Cancer medicine. In. 5th ed. Hamilton, Ontario; New York: B.C. Decker, 2000; 2546 p.
35. Lazarevich NL, Shavochkina DA, Fleishman DI, Kustova IF, Morozova OV, Chuchuev ES, Patyutko YI. Deregulation

of hepatocyte nuclear factor 4 (HNF4) as a marker of epithelial tumors progression. Exp Oncol 2010; 32:167–171.
36. Yin C, Lin Y, Zhang X, Chen YX, Zeng X, Yue HY, Hou JL, et al. Differentiation therapy of hepatocellular carcinoma in mice with recombinant adenovirus carrying hepatocyte nuclear factor-4alpha gene. Hepatology 2008; 48: 1528–1539.
37. Ning BF, Ding J, Yin C, Zhong W, Wu K, Zeng X, Yang W, et al. Hepatocyte nuclear factor 4 alpha suppresses the development of hepatocellular carcinoma. Cancer Res 2010; 70:7640–7651.
38. Walesky C, Edwards G, Borude P, Gunewardena S, O'Neil M, Yoo B, Apte U. Hepatocyte nuclear factor 4 alpha deletion promotes diethylnitrosamine-induced hepatocellular carcinoma in rodents. Hepatology 2013; 57:2480–2490.
39. Walesky C, Gunewardena S, Terwilliger EF, Edwards G, Borude P, Apte U. Hepatocyte-specific deletion of hepatocyte nuclear factor-4alpha in adult mice results in increased hepatocyte proliferation. A J Physiol Gastrointest Liver Physiol 2013; 304:G26–37.
40. Bonzo JA, Ferry CH, Matsubara T, Kim JH, Gonzalez FJ. Suppression of hepatocyte proliferation by hepatocyte nuclear factor 4alpha in adult mice. J Biol Chem 2012; 287:7345–7356.
41. Saha SK, Parachoniak CA, Ghanta KS, Fitamant J, Ross KN, Najem MS, Gurumurthy S, et al. Mutant IDH inhibits HNF-4alpha to block hepatocyte differentiation and promote biliary cancer. Nature 2014; 513:110–114.
42. Wang P, Dong Q, Zhang C, Kuan PF, Liu Y, Jeck WR, Andersen JB, et al. Mutations in isocitrate dehydrogenase 1 and 2 occur frequently in intrahepatic cholangiocarcinomas and share hypermethylation targets with glioblastomas. Oncogene 2013; 32:3091–3100.
43. Borger DR, Tanabe KK, Fan KC, Lopez HU, Fantin VR, Straley KS, Schenkein DP, et al. Frequent mutation of isocitrate dehydrogenase (IDH)1 and IDH2 in cholangiocarcinoma identified through broad-based tumor genotyping. Oncologist 2012; 17:72–79.
44. Razumilava N, Gores GJ. Cholangiocarcinoma. Lancet 2014; 383:2168–2179.
45. Voss JS, Holtegaard LM, Kerr SE, Fritcher EG, Roberts LR, Gores GJ, Zhang J, et al. Molecular profiling of cholangiocarcinoma shows potential for targeted therapy treatment decisions. Hum Pathol 2013; 44:1216–1222.
46. Lazarevich NL, Shavochkina DA, Fleishman DI, Kustova IF, Morozova OV, Chuchuev ES, Patyutko YI. Deregulation of hepatocyte nuclear factor 4 (HNF4)as a marker of epithelial tumors progression. Exp Oncol 2010; 32:167–171.
47. Ruse MD, Jr., Privalsky ML, Sladek FM. Competitive cofactor recruitment by orphan receptor hepatocyte nuclear factor 4alpha1: Modulation by the F domain. Mol Cell Biol 2002; 22:1626–1638.
48. Ungaro P, Teperino R, Mirra P, Longo M, Ciccarelli M, Raciti GA, Nigro C, et al. Hepatocyte nuclear factor (HNF)-4alpha-driven epigenetic silencing of the human PED gene. Diabetologia 2010; 53:1482–1492.
49. Amati B, Alevizopoulos K, Vlach J. Myc and the cell cycle. Front Biosci 1998; 3:d250–268.
50. Eilers M, Schirm S, Bishop JM. The MYC protein activates transcription of the alpha-prothymosin gene. EMBO J 1991; 10:133–141.
51. Penn LJ, Laufer EM, Land H. C-MYC: Evidence for multiple regulatory functions. Semin Cancer Biol 1990; 1:69–80.
52. Sladek FM. The yin and yang of proliferation and differentiation: Cyclin D1 inhibits differentiation factors ChREBP and HNF4αlpha. Cell Cycle 2012; 11:3156–3157.
53. Hanse EA, Mashek DG, Becker JR, Solmonson AD, Mullany LK, Mashek MT, Towle HC, et al. Cyclin D1 inhibits hepatic lipogenesis via repression of carbohydrate response element binding protein and hepatocyte nuclear factor 4alpha. Cell Cycle 2012; 11:2681–2690.
54. D'Angiolella V, Donato V, Forrester FM, Jeong YT, Pellacani C, Kudo Y, Saraf A, et al. Cyclin F-mediated degradation of ribonucleotide reductase M2 controls genome integrity and DNA repair. Cell 2012; 149:1023–1034.
55. D'Angiolella V, Esencay M, Pagano M. A cyclin without cyclin-dependent kinases: Cyclin F controls genome stability through ubiquitin-mediated proteolysis. Trends Cell Biol 2013; 23:135–140.
56. Hatziapostolou M, Polytarchou C, Aggelidou E, Drakaki A, Poultsides GA, Jaeger SA, et al. An HNF4α-miRNA inflammatory feedback circuit regulates hepatocellular oncogenesis. Cell 2011; 147:1233–1247.
57. Yin C, Wang PQ, Xu WP, Yang Y, Zhang Q, Ning BF, Zhang PP, et al. Hepatocyte nuclear factor-4alpha reverses malignancy of hepatocellular carcinoma through regulating miR-134 in the DLK1-DIO3 region. Hepatology 2013.
58. Sims RJ, 3rd, Reinberg D. Is there a code embedded in proteins that is based on post-translational modifications? Nat Rev Mol Cell Biol 2008; 9:815–820.
59. Witze ES, Old WM, Resing KA, Ahn NG. Mapping protein post-translational modifications with mass spectrometry. Nature Methods 2007; 4:798–806.
60. Yokoyama A, Katsura S, Ito R, Hashiba W, Sekine H, Fujiki R, Kato S. Multiple post-translational modifications in hepatocyte nuclear factor 4alpha. Biochem Biophys Res Commun 2011; 410:749–753.
61. Viollet B, Kahn A, Raymondjean M. Protein kinase A-dependent phosphorylation modulates DNA-binding activity of hepatocyte nuclear factor 4. Mol Cell Biol 1997; 17: 4208–4219.
62. Ktistaki E, Ktistakis NT, Papadogeorgaki E, Talianidis I. Recruitment of hepatocyte nuclear factor 4 into specific intranuclear compartments depends on tyrosine phosphorylation that affects its DNA-binding and transactivation potential. Proc Natl Acad Sci USA 1995; 92:9876–9880.
63. Li X, Salisbury-Rowswell J, Murdock AD, Forse RA, Burke PA. Hepatocyte nuclear factor 4 response to injury involves a rapid decrease in DNA binding and transactivation via a JAK2 signal transduction pathway. Biochem J 2002; 368:203–211.
64. Lu JW, Hsia Y, Yang WY, Lin YI, Li CC, Tsai TF, Chang KW, et al. Identification of the common regulators for hepatocellular carcinoma induced by hepatitis B virus X antigen in a mouse model. Carcinogenesis 2012; 33:209–219.
65. Yue HY, Yin C, Hou JL, Zeng X, Chen YX, Zhong W, Hu PF, et al. Hepatocyte nuclear factor 4alpha attenuates hepatic fibrosis in rats. Gut 2010; 59:236–246.
66. Fan TT, Hu PF, Wang J, Wei J, Zhang Q, Ning BF, Yin C, et al. Regression effect of hepatocyte nuclear factor 4alpha on liver cirrhosis in rats. J Dig Dis 2013; 14:318–327.