

Underexpressed Coactivators PGC1 α AND SRC1 Impair Hepatocyte Nuclear Factor 4 α Function and Promote Dedifferentiation in Human Hepatoma Cells^{*[5]}

Received for publication, April 27, 2006, and in revised form, August 4, 2006. Published, JBC Papers in Press, August 4, 2006, DOI 10.1074/jbc.M604046200

Celia P. Martínez-Jiménez^{*1}, M. José Gómez-Lechón[‡], José V. Castell^{‡§}, and Ramiro Jover^{‡§2}

From the [‡]Unidad de Hepatología Experimental, Centro de Investigación, Hospital Universitario La Fe, 46009 Valencia, Spain and

[§]Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Universidad de Valencia, 46010 Valencia, Spain

Hepatocyte nuclear factor 4 α (HNF4 α) plays critical roles during liver development and in the transcriptional regulation of many hepatic genes in adult liver. Here we have demonstrated that in human hepatoma HepG2 cells, HNF4 α is expressed at levels as high as in human liver but its activity on target genes is very low or absent. We have discovered that the low expression of key coactivators (PGC1 α , SRC1, SRC2, and PCAF) might account for the lack of function of HNF4 α in HepG2 cells. Among them, PGC1 α and SRC1 are the two most important HNF4 α coactivators as revealed by reporter assays with an ApoCIII promoter construct. Moreover, the expression of these two coactivators was found to be down-regulated in all human hepatomas investigated. Overexpression of SRC1 and PGC1 α by recombinant adenoviruses led to a significant up-regulation of well characterized HNF4 α -dependent genes (*ApoCIII*, *ApoAIV*, *PEPCK*, *AldoB*, *OTC*, and *CYP7A1*) and forced HepG2 cells toward a more differentiated phenotype as demonstrated by increased ureogenic rate. The positive effect of PGC1 α was seen to be dependent on HNF4 α . Finally, insulin treatment of human hepatocytes and HepG2 cells caused repression of PGC1 α and a concomitant down-regulation of *ApoCIII*, *PEPCK*, *AldoB*, and *OTC*. Altogether, our results suggest that SRC1, and notably PGC1 α , are key coactivators for the proper function of HNF4 α in human liver and for an integrative control of multiple hepatic genes involved in metabolism and homeostasis. The down-regulation of key HNF4 α coactivators could be a determinant factor for the dedifferentiation of human hepatomas.

Hepatoma cell lines and hepatocellular carcinomas (HCC)³ undergo phenotypic dedifferentiation leading to the loss or low

expression of typical hepatic functions such as plasma protein synthesis or xenobiotic detoxification (1–3). Dedifferentiation is a key early event in the pathogenesis of HCC that has been associated with an altered expression of liver-enriched transcription factors (4, 5). Similarly, studies in hepatoma cell lines have revealed that the maintenance of a differentiated hepatic phenotype is dependent on the expression of liver-enriched transcription factor (6, 7).

Current research supports the notion that hepatocyte nuclear factor 4 α (HNF4 α) is one of the most important liver-enriched transcription factors for hepatocyte differentiation. HNF4 α is a highly conserved member of the nuclear receptor superfamily that was initially identified as a factor required for liver-specific gene expression (8). HNF4 α plays critical roles not only in the specification of the hepatic phenotype during liver development but also in the transcriptional regulation of genes involved in glucose, cholesterol, fatty acids, and xenobiotic metabolism and in the synthesis of blood coagulation factors (9–12). Disruption of *HNF4 α* leads to an early embryonic lethal phenotype associated with a failure of differentiation of visceral endoderm (13). Genome-scale location analysis revealed surprising results for HNF4 α in hepatocytes. The number of genes that exhibit a binding of HNF4 α to their regulatory regions (>1500 genes) was much larger than that observed with other typical liver-specific regulators. Notably, from the genes occupied by RNA polymerase II, 42% were also bound by HNF4 α in hepatocytes (14). Therefore, HNF4 α emerges as a widely acting transactivator in the liver, consistent with the observation that the expression of this constitutively active transcription factor overcomes repression of the hepatic phenotype in dedifferentiated hepatoma cells (15).

However, the significance of a correlation between the expressions of both HNF4 α and hepatic functions is challenged by several studies showing that hepatic functions could be silent despite HNF4 α being expressed. Hepatic functions were found uncoupled or dissociated from HNF4 α in hepatoma cell lines, intertypic cell hybrids, and immortalized hepatocytes (3, 16–20). Additional evidence of dissociation between the expression of HNF4 α and selected hepatic functions was obtained by HNF4 α transfection in rat hepatoma cells lacking

^{*} This work was supported in part by a grant from the Ministry of Science and Technology (SAF 2003-09353) and from a European Union Integrated Research Project (PREDICTOMICS LSHB-CT-2004-504761). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[5] The on-line version of this article (available at <http://www.jbc.org>) contains supplemental Figs. S1–S3 and Tables S1 and S2.

¹ Recipient of a predoctoral grant from the Consellería de Educación Ciencia y Cultura, Generalitat Valenciana (Valencian Regional Government).

² To whom correspondence should be addressed: Unidad de Hepatología Experimental, Centro de Investigación, Hospital La Fe, Avenida de Campanar, 21, 46009 Valencia, Spain. Tel.: 34-96-197-30-48; Fax: 34-96-197-30-18; E-mail: ramiro.jover@uv.es.

³ The abbreviations used are: HCC, hepatocellular carcinoma; HNF, hepatocyte nuclear factor; PGC1 α , peroxisomal proliferator-activated receptor- γ coactivator-1- α ; SRC, steroid receptor coactivator; Apo, apolipoprotein;

AldoB, aldolase-B; OTC, ornithine transcarbamylase; PEPCK, phosphoenolpyruvate carboxykinase; CBP, cAMP-response element-binding protein-binding protein; L-FABP, liver fatty acid-binding protein; PCAF, P300/CBP-associated factor; m.o.i., multiplicity of infection; Ad, recombinant adenovirus; RT-PCR, reverse transcription PCR.

this factor (15, 21, 22). Moreover, indirect evidence suggests that this could also be the case in certain human HCC where HNF4 α is well preserved despite significant dedifferentiation (4). The existence of such dissociations suggests that in some instances HNF4 α could be highly expressed but not fully active.

A key element for a correct nuclear receptor function is a balanced, physiologic level of coregulators. It is well known that HNF4 α interacts with coactivators and corepressors through its activation function domains. Full activity is achieved through the interaction of HNF4 α homodimers with DNA and coactivators. Various studies have shown that HNF4 α interacts strongly with the p160 family coactivators (SRC1, 2, and 3) (23–25) and that HNF4 α activity can be enhanced by the action of CBP/P300 (26, 27). In addition, HNF4 α has been linked to nutrient metabolism in the liver through interactions with the coactivator PGC1 α (28, 29).

In the present study, we have demonstrated that HNF4 α is highly expressed but not fully active in the human hepatoma HepG2. This lack of function can be accounted for by the low levels of the coactivators SRC1 and, notably, PGC1 α , which after re-expression caused a marked improvement of the HNF4 α function and its target genes and enhanced the hepatic phenotype significantly. Expression analysis in several human hepatomas also suggests that the down-regulation of PGC1 α and SRC1 could be an important mechanism involved in hepatocyte dedifferentiation and progression of HCC.

EXPERIMENTAL PROCEDURES

Cell Culture—Human hepatoma cells (HepG2, Hep3B, Mz-Hep-1, and Chang Liver) were plated in Ham's F-12/Leibovitz L-15 (1/1, v/v) supplemented with 6% fetal calf serum and cultured to 70–80% confluence. Human hepatoma BC2 cells were cultured in a mixture of 75% minimal essential medium and 25% Medium 199, supplemented with 10% fetal bovine serum, 1 mg/ml bovine serum albumin, 0.7 μ M insulin and hydrocortisone hemisuccinate, and maintained at confluence for 3 weeks. HeLa (human cervix carcinoma) and 293 cells (AdE1A-transformed human embryonic kidney) were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and maintained as monolayer cultures. Culture medium for 293 cells was also supplemented with 3.5 g/liter of glucose. Human hepatocytes were isolated from liver biopsies (1–3 g) of patients undergoing liver surgery after informed consent. None of the patients habitually consumed alcohol or other drugs. A total of five liver biopsies (two male and three female of ages ranging from 26 to 65 years) were used. Hepatocytes were isolated using a two-step perfusion technique (30) and seeded on plates coated with fibronectin (3.6 μ g/cm²) at a density of 8×10^4 cells/cm². The culture medium was Ham's F-12/Leibovitz L-15 (1/1, v/v) supplemented with 2% newborn calf serum, 5 mM glucose, 0.2% bovine serum albumin, and 10^{-8} M insulin. The medium was changed 1 h later to remove unattached hepatocytes. After 24 h, the culture medium was changed to serum-free medium containing 10^{-8} M dexamethasone. Cultures were routinely supplemented with 50 units/ml penicillin and 50 μ g/ml streptomycin.

Development of Adenoviral Vectors—A recombinant adenovirus was prepared for the expression of human HNF4 α as fol-

lows: HNF4 α 2 cDNA was released from the expression vector pMT2-HNF4B (Dr. Talianidis) by EcoRI digestion and subcloned into the EcoRI site of the adenoviral shuttle vector pAC/CMVpLpA. This plasmid was cotransfected with pJM17, containing the full-length adenovirus-5 genome (dl309), into 293 cells by calcium phosphate/DNA coprecipitation. Homologous recombination between adenovirus sequences in the shuttle vector pAC/CMVpLpA and in the pJM17 plasmid generates a genome of a packable size in which most of the adenovirus early region 1 is lacking, thus rendering the recombinant virus replication defective (12). The resulting virus (named Ad-HNF4 α) was plaque purified, expanded into a high concentration stock, and titrated by plaque assay as previously described (31).

A recombinant adenovirus for the coactivator SRC1 was prepared by using the AdEasyTM adenoviral vector system (Stratagene). SRC1 cDNA was released from pCR3.1-SRC1a (Dr. O'Malley) by ApaI digestion, subcloned into the pSPORT vector (Invitrogen), and ligated into the BglII and KpnI sites of the adenoviral pShuttle-CMV vector (Stratagene). To generate recombinant adenovirus, the linearized plasmid (PmeI digestion) was transferred into BJ 5183 cells that contained the pre-transferred Ad-Easy-1 vector. Colonies containing the correct recombinant adenovirus were identified using restriction enzymes and PCR with insert-specific primers. The recombinant adenovirus DNA was then linearized by PacI and transfected into human embryonic kidney 293 cells by the calcium phosphate precipitation method. After several days of culture, infected 293 cells were collected and subjected to three cycles of freezing/thawing. The generation of a high titer adenovirus stock was performed as described (31).

The adenoviral vector for the expression of PGC1 α was a kind gift from Dr. Puigserver (32). The Ad-PGC-1 vector contains, in tandem, the green fluorescent protein gene and the PGC1 α cDNA (containing FLAG and HA epitope tags) downstream of separate cytomegalovirus promoters.

Cell lines and primary hepatocytes were infected with recombinant adenoviruses for 120 min at a m.o.i. (multiplicity of infection) ranging from 1 to 40 plaque-forming units/cell. Thereafter, cells were washed and fresh medium added. 48 h post-transfection, cells were analyzed or directly frozen in liquid N₂.

Transfection and Reporter Gene Assays—The chimeric luciferase reporter construct containing three in-tandem copies of a HNF4 α response element for human ApoCIII in front of a TK promoter (pGL3-B-3xApoCIII-TK-LUC) (33) and its control reporter vector (pGL3-B-TK-LUC) were kindly provided by Dr. Talianidis. The expression vectors for transcription factors and coactivators were the following: pMT2-HNF4B (Dr. Talianidis), pcDNA3-HA-hPGC1 (Dr. Kralli), pCR3.1-SRC1a (Dr. O'Malley), pCMX-FLAG-PCAF (Dr. Talianidis), pSG5-GRIPI (Dr. Stallcup), pSG5-TIF-II (Dr. Gronemeyer), pCMX-ACTR (Dr. Evans).

Plasmid DNAs were purified with Qiagen Maxiprep kit columns (Qiagen) and quantified by A₂₆₀ and fluorescence using PicoGreen[®] (Molecular Probes). The day before transfection, cells were plated in 35-mm dishes with 1.5 ml of Dulbecco's modified Eagle's medium/Nut F12 (Invitrogen) supplemented with 6% newborn calf serum, 50 units/ml penicillin, and 50

μ g/ml streptomycin. Firefly luciferase expression constructs (pGL3-B-3xApocIII-TK-LUC and pGL3-B-TK-LUC) (0.5 μ g) were transfected with varying amounts of expression plasmids (0.2–3.0 μ g) by the calcium phosphate precipitation method as indicated in the figures. The total amount of expression vector was kept constant by adding empty expression vector. In parallel, 0.08 μ g of pRL-CMV (a plasmid expressing *Renilla reniformis* luciferase under the cytomegalovirus immediate early enhancer/promoter) was cotransfected to correct variations in transfection efficiency. Calcium phosphate/DNA coprecipitates were added directly to each culture, and cells were incubated for an additional 48 h. Luciferase activities were assayed using the Dual-Luciferase[®] reporter kit (Promega).

Quantification of mRNA Levels—Total cellular RNA was extracted with the RNeasy Total RNA kit (Qiagen), and contaminating genomic DNA was removed by incubation with DNase I Amplification Grade (Invitrogen). RNA (1 μ g) was reverse transcribed as described (34, 35). Diluted cDNA (3 μ l) was amplified with a rapid thermal cycler (LightCycler Instrument; Roche Diagnostics) in 15 μ l of LightCycler DNA Master SYBR Green I (Roche Applied Science), 5 mM MgCl₂, and 0.3 μ M of each oligonucleotide. We designed specific primer sets for 18 different cDNAs including liver genes, transcription factors, and coactivators (supplemental Table S1). Whenever possible, primer sequences were chosen to span exon boundaries. In parallel, we always analyzed the mRNA concentration of the human housekeeping porphobilinogen deaminase (hydroxymethylbilane synthase) as an internal control for normalization (supplemental Table S1). A stable expression of the housekeeping porphobilinogen deaminase gene was validated by comparison with TATA box-binding protein expression as a second constitutive control gene (Human TBP Primer Set; Invitrogen). We found that the expression ratio of these two internal control genes was practically constant in the different tissues and cells investigated. Moreover, human porphobilinogen deaminase and TATA box-binding protein do not harbor pseudogenes and show genomic stability in cancer (36). PCR amplicons were confirmed to be specific by size (agarose gel electrophoresis) and melting curve analysis. After denaturing for 30 s at 95 °C, amplification was performed in 40 cycles of 1 s at 94 °C, 5 s at 62 °C, and 15–20 s at 72 °C. The real-time monitoring of the PCR reaction and the precise quantification of the products in the exponential phase of the amplification were performed with the LightCycler quantification software according to the manufacturer's recommendations. Reproducibility of the measurements was assessed by conducting triplicate reactions.

Extraction of Nuclear Proteins and Immunoblotting—Nuclear extracts from cultured cells were prepared as described (37) and electrophoresed in an SDS-polyacrylamide gel (20 μ g of protein/lane). Proteins were transferred to polyvinylidene fluoride membranes (Immobilon; Millipore), and sheets were incubated with a goat polyclonal antibody raised against a carboxyl-terminal epitope of human HNF4 α (Santa Cruz Biotechnology). After washing, blots were developed with horseradish peroxidase-labeled IgG using an Enhanced Chemiluminescence kit (Amersham Biosciences). Equal loading was verified by Coomassie Blue staining of the membrane blots.

Chromatin Immunoprecipitation (ChIP) Assay and RNAPol-ChIP—Cells were treated with 1% formaldehyde in phosphate-buffered saline buffer under gentle agitation for 10 min at room temperature in order to cross-link transcription factors to DNA. Thereafter, cells were collected by centrifugation, washed, resuspended in lysis buffer, and sonicated on ice for 6 $\times$ 10-s steps at 75% output in a Branson Sonicator. Cross-linking and sonication of chromatin from human liver tissue (750 mg) was carried out following a partially different protocol (38). Sonicated samples were centrifuged to clear supernatants. DNA content was carefully measured by fluorescence with PicoGreen dye (Molecular Probes) and properly diluted to obtain an equivalent amount of DNA in all samples (input DNA). For immunoprecipitation, two different antibodies for HNF4 α (sc-6556 and sc-8987; Santa Cruz Biotechnology) and a specific antibody against the RPB1 subunit of RNAPol II (sc-899) were used. The immunofractionation of protein-DNA complexes was performed by the addition of 10 μ g/ml of specific antibodies with incubation at 4 °C overnight on a 360° rotator (antibody-bound DNA fraction). For each cell preparation, an additional mock immunoprecipitation with rabbit preimmune IgG (sc-2027; Santa Cruz Biotechnology) was performed (background DNA fraction). The immunocomplexes were affinity absorbed with 10 mg of protein A/G-Sepharose (pre-washed with lysis buffer for 4 h at 4 °C under gentle rotation) and collected by centrifugation (6500 $\times$ g, 1 min). The antibody-bound and background DNA fractions were washed as described (38). The cross-links were reversed by heating the samples at 65 °C overnight. The DNA from bound, background, and input fractions was purified, diluted (1/10 bound and background fractions, 1/400 input fraction), and subjected to quantitative real-time PCR with a LightCycler instrument. Amplification was real-time monitored and allowed to proceed in the exponential phase until fluorescent signal from input samples reached a significant value. Amplified DNA was then analyzed by agarose gel electrophoresis. Amplification and quantification of ApoCIII gene sequences (–740 and –80-bp 5'-flanking regions, and exons 3 and 4) among the pull of DNA was performed with specific primers flanking these regions (supplemental Table S2). The detection of RNA polymerase II within the coding region of the ApoCIII gene allows the quantification of the actual transcriptional rate (38). To ensure reproducibility, immunoprecipitation and PCR analysis were performed in duplicate from different liver tissue samples, cultured hepatocytes, and cell lines.

Ureagenesis—The ureogenic rate was assessed in HepG2 cells incubated with 3 mM ammonium chloride by measuring the appearance of urea in the culture medium. Urea concentration was determined by the diacetylmonoxime method (30).

RESULTS

HNF4 α Levels in Different Hepatic and Non-hepatic Cell Models and in Human Liver—HNF4 α expression levels in cultured human hepatocytes and hepatoma cell lines (HepG2, Hep3B, and BC2) were similar to those of liver tissue as assessed by RT-PCR (Fig. 1A) and immunoblotting analysis (Fig. 1B). Among the several hepatomas analyzed, the highest HNF4 α expression level was found in the widely used cell line HepG2.

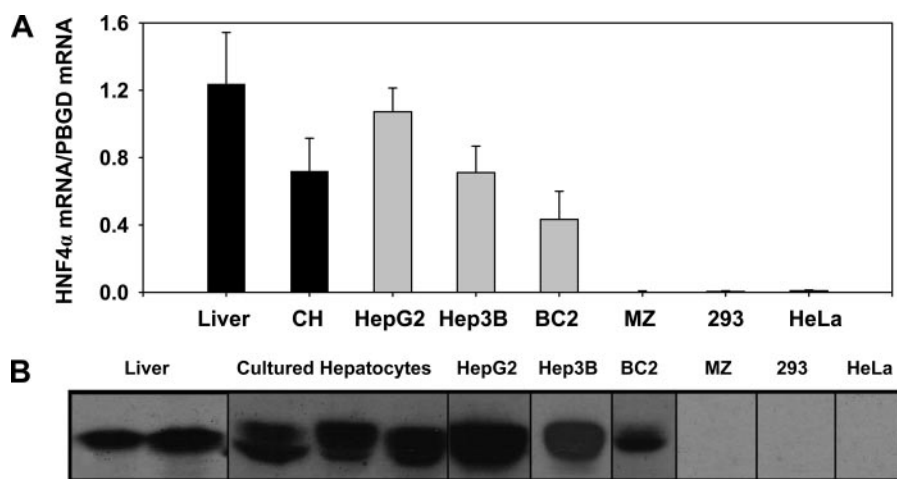


FIGURE 1. HNF4 α is expressed in human hepatoma cells at similar levels as in human liver. Total RNA and nuclear protein were purified from human liver samples, 24-h cultured human hepatocytes (CH), hepatoma cell lines (HepG2, Hep3B, BC2, and Mz-Hep-1), and non-hepatic cell lines (293 and HeLa). A, relative HNF4 α mRNA was determined by real-time quantitative RT-PCR analysis. In parallel, we also analyzed the mRNA concentration of the human housekeeping porphobilinogen deaminase (PBGD) as an internal control for normalization. Data were expressed as relative to a reference human liver sample and represent the mean $\pm$ S.D. from four-eight independent cell cultures or tissue samples. B, representative immunoblotting analysis of HNF4 α nuclear protein. Two samples from human livers and three from cultured human hepatocytes were included to illustrate inter-individual variability. Equal loading was verified by Coomassie Blue staining of the membrane blots.

The expression of HNF4 α was, however, very low or absent in non-hepatic cell lines (*i.e.* 293 and HeLa) and in the more differentiated human hepatoma Mz-Hep-1 (Fig. 1).

HNF4 α Function Is Impaired in Human Hepatoma HepG2 Cells—The expression of many hepatic genes has an absolute dependence on HNF4 α . Data obtained from HNF4 α null mice demonstrated that this transcription factor is indispensable for the constitutive expression of key hepatic genes such as apolipoproteins (A, B, and C families), *L-FABP*, *PEPCK*, *AldoB*, *OTC*, and *CYP7A1* (10, 11, 39–41). A comparative analysis of eight well characterized HNF4 α target genes in different cell types revealed high expression levels in cultured human hepatocytes and null or very low levels in HepG2 cells (supplemental Fig. S1). Among the eight mRNA measured, we specifically found that ApoCIII, AldoB, PEPCK, and OTC were essentially not expressed in HepG2 cells, whereas ApoAII, ApoAV, CYP7A1, and L-FABP showed levels of $\sim$ 20% of human liver (supplemental Fig. S1). The expression profile in HepG2 cells was closer to that of non-hepatic cell lines (293 and HeLa).

To gain a better understanding of the discrepancy between high HNF4 α levels and the low or null expression of target genes in HepG2, we performed chromatin immunoprecipitation assays and analyzed the occupancy of two different binding sites in the human ApoCIII gene by HNF4 α (Fig. 2A). In parallel, we performed RNAPol-chromatin immunoprecipitation to measure the binding of RNAPol-II to the promoter and the transcription through ApoCIII coding regions (exons 3 and 4) (Fig. 2B). We confirmed an appropriate binding of HNF4 α to the -740 and -80 -bp elements in human liver samples, as well as RNAPol-II binding to the promoter and active transcription through exons 3 and 4. Similar results were found in cultured human hepatocytes (data not shown). However, in the human hepatoma HepG2,

binding of HNF4 α to the -80 -bp element was substantially decreased (Fig. 2A) and RNAPol-II occupancy at the promoter and coding regions was almost undetectable (Fig. 2B). As a negative control, we also analyzed non-hepatic HeLa cells where HNF4 α and ApoCIII are not expressed. Our results suggest that binding and transactivation by HNF4 α is impaired in HepG2 cells.

Possible Mechanisms Underlying the Dysfunction of HNF4 α in Hepatoma Cells—HNF4 α exists in several isoforms, all of which are capable of binding to the same regulatory elements but with different transactivating properties. An imbalanced expression of HNF4 α isoforms could explain why HNF4 α is non-operative in HepG2. However, this apparently is not the case; the major HNF4 α splicing variants (α 1,

α 2, α 3, and α 7) had similar expression levels in HepG2 and in human hepatocytes (data not shown).

Another mechanism causing HNF4 α dysfunction in hepatoma cells could be an increase of negative factors that block HNF4 α activity. The small heterodimeric partner, which lacks a DNA binding domain and exhibits inhibiting interactions with HNF4 α , might be involved. Similarly, the chicken ovalbumin upstream promoter-transcription factors could act as transcription repressors for several nuclear receptors, including HNF4 α . However, the expression levels of these negative factors in HepG2 cells do not differ substantially from those in cultured hepatocytes or human liver (data not shown), suggesting that other mechanisms might be involved.

Another possible explanation for the loss of function of endogenously expressed HNF4 α could be that HepG2 cells either lack the essential coactivators needed for proper functionality or have increased levels of corepressors. We measured seven coactivators in cultured cells and liver tissue and found that four of them (SRC1, SRC2, PGC1 α , and PCAF) were down-regulated in HepG2 cells to 10–30% of the human liver levels (supplemental Fig. S2A). Other important coactivators, SRC3, CBP, and P300, did not change. We also measured two corepressors (nuclear receptor corepressor (NcoR) and silencing mediator of retinoic acid and thyroid hormone receptor (SMRT)) and found that their expression levels in HepG2 cells were similar to those of liver or cultured hepatocytes (supplemental Fig. S2A). Thus, the low level of coactivators (SRC1, SRC2, PGC1 α , and PCAF) could be a cause of HNF4 α dysfunction in HepG2 cells.

The p160 steroid receptor coactivator gene family contains three homologous members (SRC1, 2, and 3) that operate as transcriptional coactivators for nuclear receptors and other transcription factors. It has been shown that these coactivators have an overlapping activity, and it may be spec-

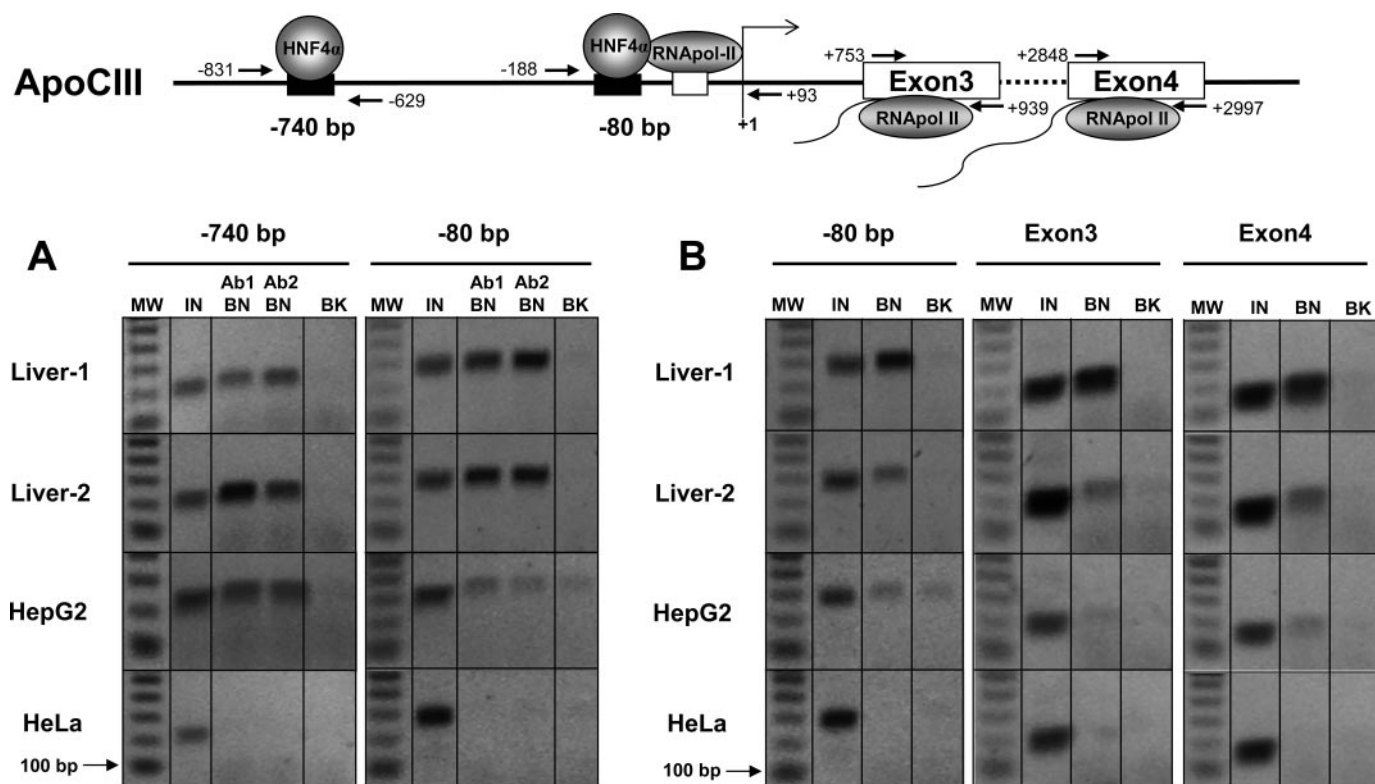


FIGURE 2. HNF4 α is partially bound to its target DNA response elements, but transactivation is impaired in human hepatoma HepG2 cells. Formaldehyde cross-linked chromatin from human liver tissues (*Liver-1* and *Liver-2*), HepG2, and HeLa cells were incubated with antibodies against human HNF4 α (Ab1 and Ab2) (A) or RNApol-II (B). Immunoprecipitated DNA (antibody-bound DNA fraction, BN) was analyzed by PCR with primers specific to the -740 and -80 -bp 5'-flanking regions and to exons 3 and 4 of the human ApoCIII gene. Parallel PCR reactions were performed with both input DNA (input DNA fraction, IN) and mock-immunoprecipitated DNA (background DNA fraction, BK). Marker, 100-bp DNA ladder.

ulated that a decrease in one SRC form (e.g. SRC1 or 2) could be compensated by an increase of the expression of the other family members (e.g. SRC3). However, absolute quantification of mRNA levels demonstrated that SRC1 and SRC2 are the most abundant forms in human liver and cultured hepatocytes and their decrease in HepG2 cells cannot be compensated by SRC3 expression levels (supplemental Fig. S2B).

Relevance of Coactivators for HNF4 α -mediated Activity in Reporter Gene Assay—In HepG2 cells, the luciferase (LUC) activity of a construct containing three copies of a HNF4 α response element (pGL3-B-3xApocIII-TK-LUC) was no different from that of a control construct lacking HNF4 α binding sites (pGL3-B-TK-LUC), which supports a lack of function of endogenous HNF4 α in hepatoma cells (supplemental Fig. S3A). This is reinforced by the fact that the transfection of HNF4 α caused a modest dose-dependent increase in luciferase activity, which suggests that other missing factors may limit the response (supplemental Fig. S3A). The transfection of coactivators SRC1 and, notably, PGC1 α caused a significant increase in the 3xApocIII-TK-LUC reporter activity in HepG2 cells (2.3- and 25.0-fold, respectively; supplemental Fig. S3A), whereas a much lower effect was noted in HeLa cells lacking endogenous HNF4 α (1.1- and 3.3-fold increase, respectively; supplemental Fig. S3A). Other coactivators (SRC2, SRC3, and PCAF) produced no substantial change in promoter activity. This experimental evidence suggests that endogenous HNF4 α in HepG2 cells is functional but its activity is limited by the low concentration of SRC1 and PGC1 α . This possibility was further

demonstrated by cotransfection experiments. We found a synergistic effect of HNF4 α and PGC1 α in HeLa cells, where the cotransfection of both factors was needed to largely improve the HNF4-dependent reporter gene activity (26.0-fold increase; supplemental Fig. S3B). However, cotransfection of both factors in HepG2 cells caused a very similar response to that obtained with the sole transfection of PGC1 α (supplemental Fig. S3B), likely because in HepG2 cells the coactivation by exogenous PGC1 α is attained via endogenous HNF4 α .

Down-regulation of PGC1 α and SRC1 Is a Common Event in Human Hepatomas—We measured the mRNA levels of PGC1 α and SRC1 in five different human hepatoma cell lines derived from hepatocellular carcinomas, and we compared them with liver levels. We found that PGC1 α was consistently down-regulated in all hepatomas with levels of $\sim 20\%$ of human liver (Fig. 3A). SRC1 mRNA expression was also consistently lower in all hepatoma cell lines, although in this case the relative levels were between 20–50% of human liver (Fig. 3B). These results suggest that underexpressed coactivators could be a common feature of hepatomas and hepatocellular carcinomas.

Adenovirus-mediated Re-expression of PGC1 α and SRC1 Reactivates HNF4 α Target Genes in Hepatoma Cells—Adenoviral-mediated transfection of PGC1 α in HepG2 cells caused a dose-dependent increase in most of the HNF4 α target genes, *ApoCIII*, *ApoAV*, *AldoB*, *PEPCK*, *OTC*, and *CYP7A1* (Fig. 4A). On the contrary, adenoviral transfection of SRC1 had a less broad effect on HNF4 α target genes, where a significantly increased expression was observed only in *CYP7A1* and *PEPCK*

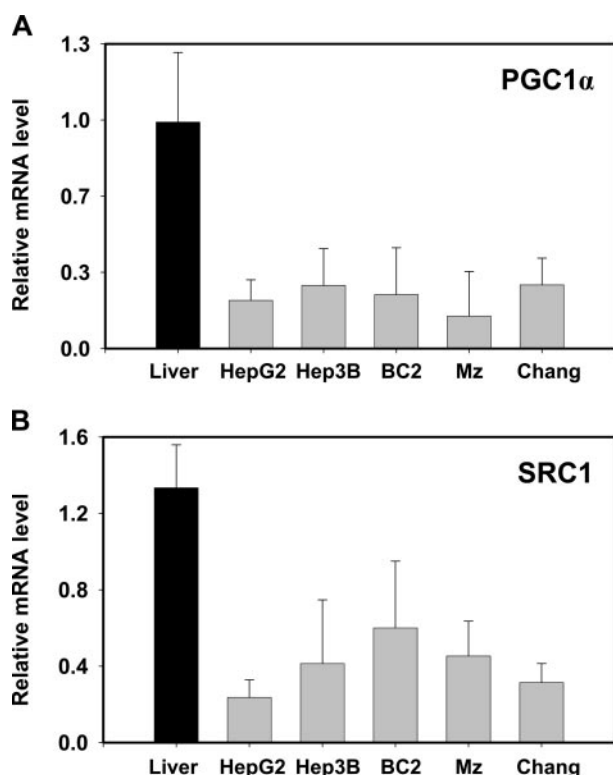


FIGURE 3. Relative mRNA levels of PGC1 α and SRC1 in five human hepatoma cell lines. Total RNA was purified from human liver tissue and five different human hepatoma cell lines. The mRNA level of coactivators PGC1 α (A) and SRC1 (B) was determined by real-time quantitative RT-PCR analysis and expressed as the relative value to a reference human liver sample. Data represent the mean $\pm$ S.D. from 4–11 independent cell cultures or tissue samples.

mRNAs (Fig. 4B). A parallel transfection with the control adenoviral vector did not modify the expression of these genes. The effect observed with Ad-PGC1 α or Ad-SRC1 in HepG2 cells did not significantly improve via the cotransfection with an adenoviral vector for HNF4 α (data not shown). Therefore, we may postulate that the re-expression of PGC1 α and SRC1 restores HNF4 α activity in human hepatoma cells, which in turn leads to a strong up-regulation of multiple hepatic-specific genes.

To further test whether the effects caused by PGC1 α were mediated through HNF4 α , we carried out transfection experiments in Mz-Hep-1, a hepatoma cell line that lacks endogenous HNF4 α (see Fig. 1). Preliminary transfection experiments with Ad-HNF4 α and Ad-GFP showed that a dose of 16 m.o.i. was sufficient for a high expression level (Fig. 5A) in nearly 100% of the cultured cells (Fig. 5B). Although separate transfections of PGC1 α and HNF4 α did not cause a significant increase in HNF4 α target genes, the cotransfection of both factors triggered a dramatic increase (Fig. 5C). ApoCIII, AldoB, PEPCK, and OTC mRNA concentrations rose from marginal levels to reach expression levels comparable with those observed in adult livers (Fig. 5C). For instance, ApoCIII mRNA in Mz-Hep-1 cells transfected with HNF4 α plus PGC1 α increased from levels of around the detection limit (>36 cycles) to 15–20% of those detected in human liver (>130-fold increase).

Finally, we investigated whether a re-expression of PGC1 α in human hepatoma HepG2 cells could improve liver-specific metabolic functions associated with the differentiated phenotype. We transfected HepG2 cells with Ad-PGC1 α , and 48 h

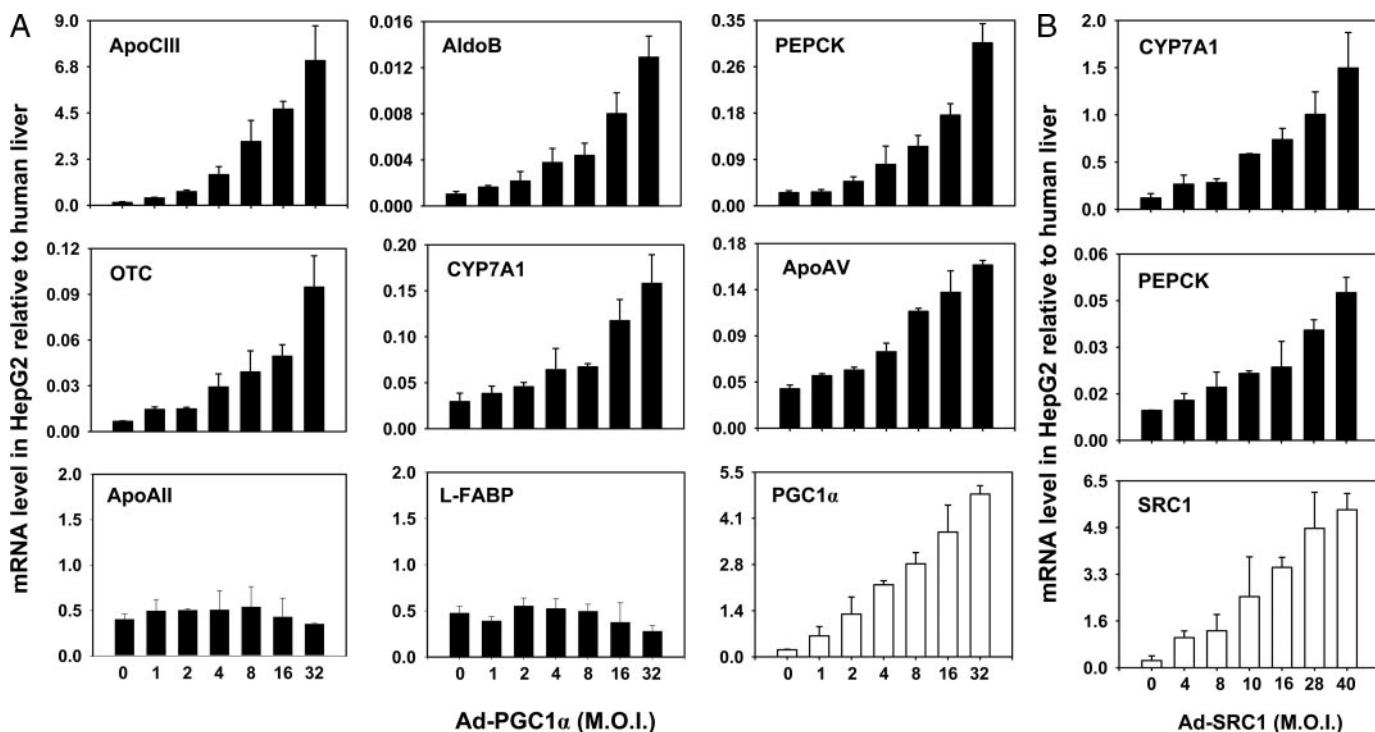


FIGURE 4. Adenoviral-mediated transfection of PGC1 α and SRC1 reactivates expression of multiple hepatic genes in HepG2 cells. Hepatoma cells were transduced with increasing doses of Ad-PGC1 α (1–32 m.o.i.) (A) or Ad-SRC1 (4–40 m.o.i.) (B), and the mRNA concentrations of eight liver genes were measured by quantitative RT-PCR 48 h later. The expressions of the transfected coactivators (open bars) were also analyzed by RT-PCR after a previous treatment of purified RNA with DNaseI. Data represent the mean $\pm$ S.D. from three–four independent cultures.

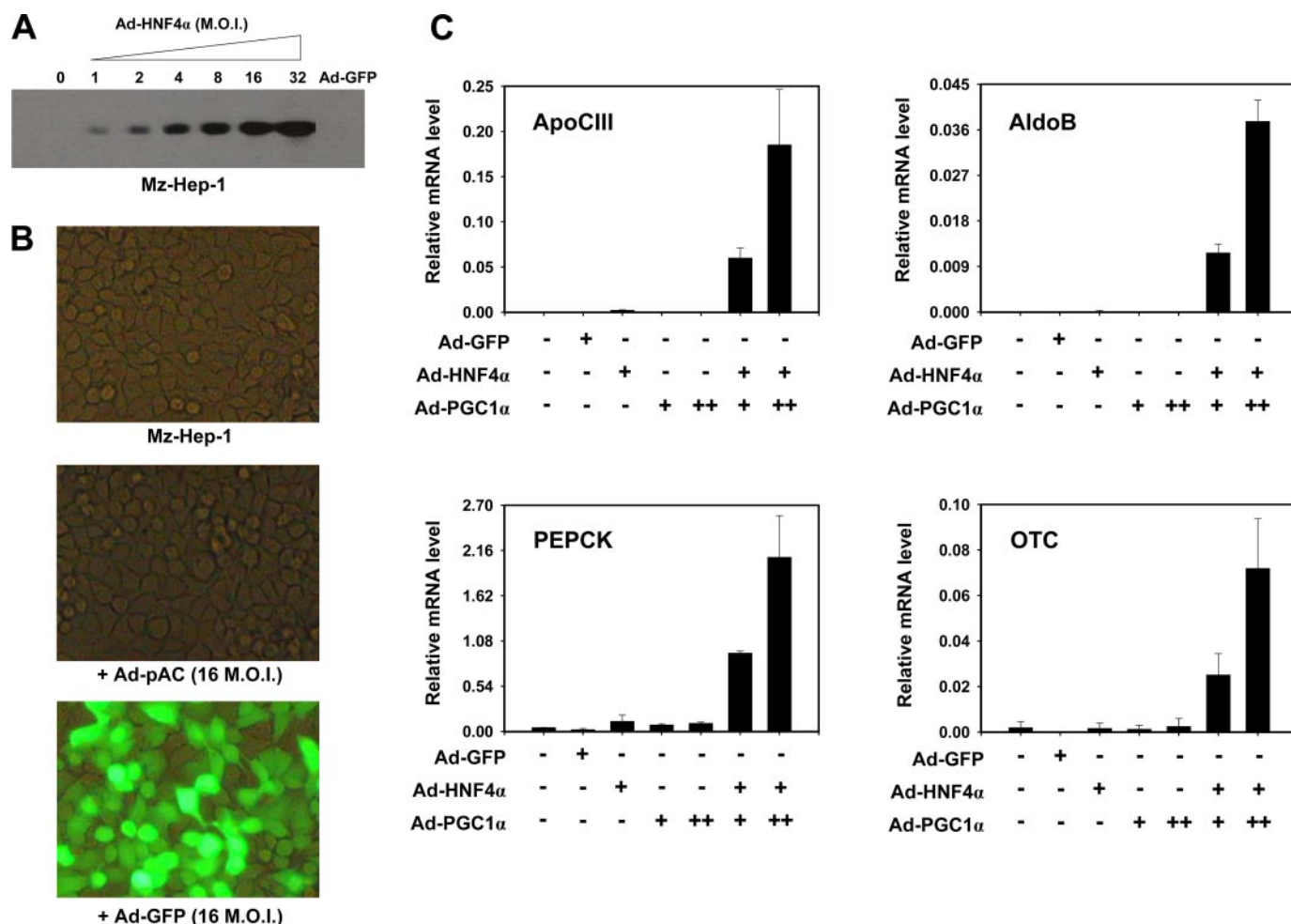


FIGURE 5. Reactivation of hepatic genes by PGC1 α in hepatoma cells requires HNF4 α . A, immunoblotting analysis of transfected HNF4 α in Mz-Hep-1 hepatoma cells. B, fluorescent microscopy analysis of Mz-Hep-1 cells infected with Ad-GFP or insertless Ad-pAC for 48 h. C, human hepatoma Mz-Hep-1 cells lacking endogenous HNF4 α were infected with Ad-GFP (16 m.o.i.), Ad-HNF4 α (16 m.o.i.), or Ad-PGC1 α (16 or 32 m.o.i.) or coinfecting in different combinations. The mRNA concentrations of ApoCIII, AldoB, PEPCK, and OTC were measured by quantitative RT-PCR 48 h later. Data represent the mean $\pm$ S.D. from three independent cultures.

TABLE 1

Effect of PGC1 α on the ureogenic rate in HepG2 cells

Human hepatoma cells were transfected with Ad-PGC1 α , and 48 h post-transfection the rate of urea synthesis was determined in cultures incubated with 3 mM ammonium chloride for 2 h (mean $\pm$ S.D., $n = 3$).

	Urea synthesis
	nmol urea/h/mg
Control	14.5 $\pm$ 2.6
Ad-PGC1 α , 4 m.o.i.	31.4 $\pm$ 10.8
Ad-PGC1 α , 32 m.o.i.	57.3 $\pm$ 16.9

post-transfection the rate of urea synthesis was determined in cultures incubated with 3 mM ammonia for 2 h. Transfection of HepG2 cells with Ad-PGC1 α at 4 and 32 m.o.i. led to an increase in the ureogenic rate of 2.2- and 4.0-fold, respectively (Table 1). These data demonstrate that activation of hepatic genes by PGC1 α in hepatoma cells leads to an improvement of hepatic metabolic functions associated with a differentiated hepatic phenotype.

Insulin Represses PGC1 α and Causes Down-regulation of HNF4 α Target Genes in Cultured Human Hepatocytes and HepG2 Cells—Our results point to PGC1 α as one of the most important coactivators for the basal expression of HNF4 α tar-

get genes in the liver. However, PGC1 α is also modulated during the feeding-fasting cycle by stimuli such as glucagon or insulin. It can therefore be suggested that physiologic modulation of PGC1 α will simultaneously influence the expression of multiple HNF4 α target genes. To investigate this possibility, we treated human hepatocytes and hepatoma HepG2 cells with insulin and measured the expression levels of both PGC1 α and HNF4 α target genes. Cells were cultured in serum- and hormone-free medium and treated with insulin for 12 h. Results were coincident in both cell systems (Fig. 6, A and B). Insulin caused a 35–55% decrease in PGC1 α mRNA and a concomitant 25–65% decrease in *ApoCIII*, *OTC*, *AldoB*, and *PEPCK* mRNAs (Fig. 6). Transfection of HepG2 cells with Ad-PGC1 α (16 m.o.i.) increased basal expression levels of target genes and prevented repression by insulin (data not shown). Altogether, our results reinforce the notion that PGC1 α plays a significant role in the transcriptional regulation of HNF4 α -dependent genes in human liver cells.

DISCUSSION

The regulation of gene transcription has generally been thought to occur via changes in amounts or activities of tran-

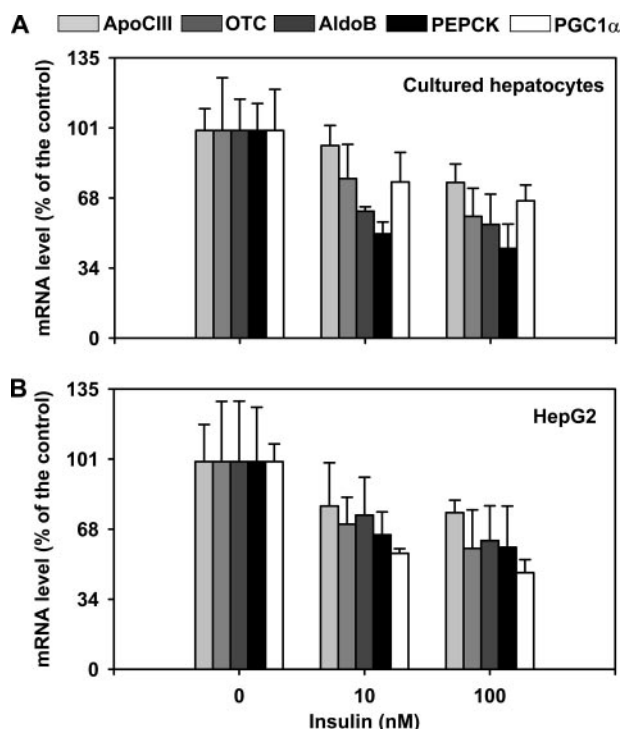


FIGURE 6. Insulin represses PGC1 α and causes the down-regulation of HNF4 α target genes in human hepatic cells. Cultured human hepatocytes (A) and HepG2 cells (B) were cultured in serum- and hormone-free medium and treated with insulin for 12 h as indicated. Total RNA was purified, and the mRNA levels of PGC1 α , ApoCIII, OTC, AldoB, and PEPCK were determined by real-time quantitative RT-PCR analysis. In parallel, we also analyzed the mRNA concentration of the housekeeping porphobilinogen deaminase for normalization. Data were expressed as a percentage of the controls and represent the mean $\pm$ S.D. from two-three independent experiments.

scription factors. However, it is now quite clear that a substantial component of gene control is directed by coactivators acting as the primary targets of differentiation or physiological signals. The down-regulation of a few coactivators can change the activity of multiple transcription factors and facilitate the progress of distinct biological programs (42). Indeed, we have shown that a lower intracellular level of coactivators (SRC1, SRC2, PGC1 α , and PCAF) in human hepatoma cells is associated with a deficient expression of hepatic genes. We have also demonstrated that the coactivator PGC1 α plays a prominent role in sustaining the basal expression of multiple distinctive hepatic genes, suggesting that this factor could have a particular relevance in the maintenance of the differentiated adult hepatic phenotype. The importance of PGC1 α in other programs of differentiation such as chondrogenesis has been demonstrated (43).

PGC1 α shows a specific expression pattern restricted to tissues that have a high energy demand such as heart, brown adipose tissue, and skeletal muscle, where PGC1 α expression is induced in response to stimuli such as cold or physical exercise (44, 45). In mouse liver, PGC1 α seems to be only significantly expressed after fasting (44). However, several studies have demonstrated by Northern blot analysis constitutive PGC1 α expression in human liver (46, 47). This supports the notion that PGC1 α can also be an important coactivator for the constitutive expression of many hepatic genes in the absence of inducible stimuli.

In the liver, PGC1 α is induced in response to fasting and insulin deficiency and in isolated hepatocytes by cAMP, glucagon, and glucocorticoids, leading to the activation of all key enzymes of gluconeogenesis and the increase of hepatic glucose production (29). The opposite effects are observed with insulin, which suppresses basal PGC1 α levels in hepatoma cells (48, 49). Our results have also shown that insulin treatment of human hepatic cells causes a parallel down-regulation of PGC1 α and several HNF4 α target genes. These results reinforce the idea that PGC1 α plays a significant role in sustaining transcription by HNF4 α in human liver cells but also emphasize the importance of PGC1 α as a wide-ranging integrating coactivator in response to feeding-fasting stimuli.

In the human hepatoma cell line HepG2, we analyzed eight liver genes that have been well characterized as HNF4 α -dependent genes (10, 11, 39–41). The expression levels of these genes in HepG2 cells were consistently much lower than in human liver tissue. Transfection of the HNF4 α coactivator PGC1 α significantly improved the transcription of most of the genes analyzed (six of eight). Some of these genes were well characterized targets of PGC1 α (e.g. PEPCK and ApoAIV), but others (e.g. AldoB and OTC) have for the first time been described as *bona fide* targets of PGC1 α in this study. Our results do not support the previous notion that PGC1 α and HNF4 α have a significant activating effect only on the gluconeogenic genes PEPCK and glucose-6-phosphatase in the liver (28). We have shown that PGC1 α and HNF4 α also play an important role in the activation of genes for apolipoproteins, ureagenesis, and bile acids synthesis, which coincides with other studies showing a more general role for PGC1 α in association with HNF4 α (50–53). Nevertheless, our data, in agreement with previous studies (28), also demonstrate that not all HNF4 α -dependent genes are coactivated by PGC1 α (i.e. L-FABP and ApoAII).

The gain-of-function studies described herein provide convincing evidence that PGC1 α is a key coactivator for sustaining the expression of multiple HNF4 α -dependent genes in human hepatoma cells. The relevance of these findings to the *in vivo* situation in humans cannot be addressed, but mice with a targeted deletion of PGC1 α have been established and characterized (54, 55). These mice showed altered hepatic phenotypes such as defective hormone-stimulated gluconeogenesis (54) or mitochondrial respiratory dysfunction, reduced capacity for fat oxidation, and steatosis (55). However, a comprehensive analysis of HNF4 α target genes in PGC1 α ^{−/−} mice has not been shown.

The re-expression of SRC1 in hepatoma cells had a more limited impact on liver genes than PGC1 α . SRC1-mediated activation was restricted to PEPCK and CYP7A1, which is in agreement with previous studies (56, 57). Interestingly, we observed a marked activation of these genes by the sole transfection of SRC1 without any hormonal or physiological stimulation, suggesting that a lower expression of SRC1 in hepatoma cells could also contribute to the down-regulation of key liver genes involved in glucose and bile acid metabolism.

Besides the constitutive activation of signal transduction pathways that promote cell growth and survival, one of the most critical steps in the pathogenesis of HCC is dedifferentia-

tion and alteration of liver function. Recent studies comparing expression profiles in HCC and noncancerous liver revealed a down-regulation of typical hepatic genes such as those coding for key enzymes involved in gluconeogenesis, glycogen synthesis, amino acid and lipid metabolism (2). The underlying molecular mechanisms of the progression of HCC in most patients remain unclear although an altered expression of liver-enriched transcription factor has been demonstrated (4). In the present work, analyses of several hepatoma cell lines have shown that PGC1 α and SRC1 are consistently down-regulated, which would explain the lack of correlation between the high levels of HNF4 α and the low expression of many HNF4 target genes. Moreover, the strong dependence found between coactivator levels and hepatic gene expression prompts us to propose that a significant down-regulation of coactivators could not only switch off the regulatory balance required for the maintenance of the adult hepatic phenotype but also promote a program of cell dedifferentiation with clinical significance in the pathogenesis of HCC.

Acknowledgments—We thank C. Corchero and E. Belenchon for expert technical assistance.

REFERENCES

- Dong, X. Y., Pang, X. W., Yu, S. T., Su, Y. R., Wang, H. C., Yin, Y. H., Wang, Y. D., and Chen, W. F. (2004) *Int. J. Cancer* **112**, 239–248
- Xu, X. R., Huang, J., Xu, Z. G., Qian, B. Z., Zhu, Z. D., Yan, Q., Cai, T., Zhang, X., Xiao, H. S., Qu, J., Liu, F., Huang, Q. H., Cheng, Z. H., Li, N. G., Du, J. J., Hu, W., Shen, K. T., Lu, G., Fu, G., Zhong, M., Xu, S. H., Gu, W. Y., Huang, W., Zhao, X. T., Hu, G. X., Gu, J. R., Chen, Z., and Han, Z. G. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98**, 15089–15094
- Rodriguez-Antona, C., Donato, M. T., Boobis, A., Edwards, R. J., Watts, P. S., Castell, J. V., and Gomez-Lechon, M. J. (2002) *Xenobiotica* **32**, 505–520
- Xu, L., Hui, L., Wang, S., Gong, J., Jin, Y., Wang, Y., Ji, Y., Wu, X., Han, Z., and Hu, G. (2001) *Cancer Res.* **61**, 3176–3181
- Lazarevich, N. L., Cheremnova, O. A., Varga, E. V., Ovchinnikov, D. A., Kudrjavtseva, E. I., Morozova, O. V., Fleishman, D. I., Engelhardt, N. V., and Duncan, S. A. (2004) *Hepatology* **39**, 1038–1047
- Gomez-Lechon, M. J., Donato, T., Jover, R., Rodriguez, C., Ponsoda, X., Glaise, D., Castell, J. V., and Guguen-Guillouzo, C. (2001) *Eur. J. Biochem.* **268**, 1448–1459
- Griffo, G., Hamon-Benais, C., Angrand, P. O., Fox, M., West, L., Lecoq, O., Povey, S., Cassio, D., and Weiss, M. (1993) *J. Cell Biol.* **121**, 887–898
- Sladek, F. M., Zhong, W. M., Lai, E., and Darnell, J. E., Jr. (1990) *Genes Dev.* **4**, 2353–2365
- Sladek, F. M. (1993) *Receptor* **3**, 223–232
- Li, J., Ning, G., and Duncan, S. A. (2000) *Genes Dev.* **14**, 464–474
- Hayhurst, G. P., Lee, Y. H., Lambert, G., Ward, J. M., and Gonzalez, F. J. (2001) *Mol. Cell Biol.* **21**, 1393–1403
- Jover, R., Bort, R., Gomez-Lechon, M. J., and Castell, J. V. (2001) *Hepatology* **33**, 668–675
- Chen, W. S., Manova, K., Weinstein, D. C., Duncan, S. A., Plump, A. S., Prezioso, V. R., Bachvarova, R. F., and Darnell, J. E., Jr. (1994) *Genes Dev.* **8**, 2466–2477
- Odum, D. T., Zizlsperger, N., Gordon, D. B., Bell, G. W., Rinaldi, N. J., Murray, H. L., Volkert, T. L., Schreiber, J., Rolfe, P. A., Gifford, D. K., Fraenkel, E., Bell, G. I., and Young, R. A. (2004) *Science* **303**, 1378–1381
- Spath, G. F., and Weiss, M. C. (1997) *Mol. Cell Biol.* **17**, 1913–1922
- Chaya, D., Fougere-Deschatrette, C., and Weiss, M. C. (1997) *Mol. Cell Biol.* **17**, 6311–6320
- Bulla, G. A. (1999) *Nucleic Acids Res.* **27**, 1190–1197
- Bulla, G. A., and Kraus, D. M. (2004) *Biosci. Rep.* **24**, 595–608
- Butura, A., Johansson, I., Nilsson, K., Warngard, L., Ingelman-Sundberg, M., and Schuppe-Koistinen, I. (2004) *Biochem. Pharmacol.* **67**, 1249–1258
- Amicone, L., Spagnoli, F. M., Spath, G., Giordano, S., Tommasini, C., Bernardini, S., De Luca, V., Della Rocca, C., Weiss, M. C., Comoglio, P. M., and Tripodi, M. (1997) *EMBO J.* **16**, 495–503
- Bulla, G. A., and Fournier, R. E. (1994) *Mol. Cell Biol.* **14**, 7086–7094
- Bailly, A., Spath, G., Bender, V., and Weiss, M. C. (1998) *J. Cell Sci.* **111**, Pt. 16, 2411–2421
- Iordanidou, P., Aggelidou, E., Demetriades, C., and Hadzopoulou-Cladaras, M. (2005) *J. Biol. Chem.* **280**, 21810–21819
- Sladek, F. M., Ruse, M. D., Jr., Nepomuceno, L., Huang, S. M., and Stallcup, M. R. (1999) *Mol. Cell Biol.* **19**, 6509–6522
- Wang, J. C., Stafford, J. M., and Granner, D. K. (1998) *J. Biol. Chem.* **273**, 30847–30850
- Dell, H., and Hadzopoulou-Cladaras, M. (1999) *J. Biol. Chem.* **274**, 9013–9021
- Eeckhoutte, J., Formstecher, P., and Laine, B. (2001) *Mol. Endocrinol.* **15**, 1200–1210
- Rhee, J., Inoue, Y., Yoon, J. C., Puigserver, P., Fan, M., Gonzalez, F. J., and Spiegelman, B. M. (2003) *Proc. Natl. Acad. Sci. U. S. A.* **100**, 4012–4017
- Yoon, J. C., Puigserver, P., Chen, G., Donovan, J., Wu, Z., Rhee, J., Adelmant, G., Stafford, J., Kahn, C. R., Granner, D. K., Newgard, C. B., and Spiegelman, B. M. (2001) *Nature* **413**, 131–138
- Gomez-Lechon, M., and Castell, J. (2000) in *The Hepatocyte Review* (Berry, M. N., and Edwards, A. M., eds) pp. 11–17, Kluwer Academic Publishers, London
- Castell, J. V., Hernandez, D., Gomez-Foix, A. M., Guillen, I., Donato, T., and Gomez-Lechon, M. J. (1997) *Gene Ther.* **4**, 455–464
- Lehman, J. J., Barger, P. M., Kovacs, A., Saffitz, J. E., Medeiros, D. M., and Kelly, D. P. (2000) *J. Clin. Invest.* **106**, 847–856
- Ktistaki, E., and Talianidis, I. (1997) *Science* **277**, 109–112
- Perez, G., Tabares, B., Jover, R., Gomez-Lechon, M. J., and Castell, J. V. (2003) *Toxicol. In Vitro* **17**, 643–649
- Rodriguez-Antona, C., Jover, R., Gomez-Lechon, M. J., and Castell, J. V. (2000) *Arch. Biochem. Biophys.* **376**, 109–116
- Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., and Speleman, F. (2002) *Genome Biol.* **3**, RESEARCH0034.1–0034.11
- Andrews, N. C., and Faller, D. V. (1991) *Nucleic Acids Res.* **19**, 2499
- Sandoval, J., Rodriguez, J. L., Tur, G., Serviddio, G., Pereda, J., Boukaba, A., Sastre, J., Torres, L., Franco, L., and Lopez-Rodas, G. (2004) *Nucleic Acids Res.* **32**, e88
- Inoue, Y., Hayhurst, G. P., Inoue, J., Mori, M., and Gonzalez, F. J. (2002) *J. Biol. Chem.* **277**, 25257–25265
- Stoffel, M., and Duncan, S. A. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 13209–13214
- Parviz, F., Matullo, C., Garrison, W. D., Savatski, L., Adamson, J. W., Ning, G., Kaestner, K. H., Rossi, J. M., Zaret, K. S., and Duncan, S. A. (2003) *Nat. Genet.* **34**, 292–296
- Spiegelman, B. M., and Heinrich, R. (2004) *Cell* **119**, 157–167
- Kawakami, Y., Tsuda, M., Takahashi, S., Taniguchi, N., Esteban, C. R., Zemmyo, M., Furumatsu, T., Lotz, M., Belmonte, J. C., and Asahara, H. (2005) *Proc. Natl. Acad. Sci. U. S. A.* **102**, 2414–2419
- Puigserver, P., Wu, Z., Park, C. W., Graves, R., Wright, M., and Spiegelman, B. M. (1998) *Cell* **92**, 829–839
- Knutti, D., and Kralli, A. (2001) *Trends Endocrinol. Metab.* **12**, 360–365
- Knutti, D., Kaul, A., and Kralli, A. (2000) *Mol. Cell Biol.* **20**, 2411–2422
- Esterbauer, H., Oberkofler, H., Kremppler, F., and Patsch, W. (1999) *Genomics* **62**, 98–102
- Daitoku, H., Yamagata, K., Matsuzaki, H., Hatta, M., and Fukamizu, A. (2003) *Diabetes* **52**, 642–649
- Herzig, S., Long, F., Jhala, U. S., Hedrick, S., Quinn, R., Bauer, A., Rudolph, D., Schutz, G., Yoon, C., Puigserver, P., Spiegelman, B., and Montminy, M. (2001) *Nature* **413**, 179–183
- Rhee, J., Ge, H., Yang, W., Fan, M., Handschin, C., Cooper, M., Lin, J., Li, C., and Spiegelman, B. M. (2006) *J. Biol. Chem.* **281**, 14683–14690
- Prieur, X., Schaap, F. G., Coste, H., and Rodriguez, J. C. (2005) *Mol. Endocrinol.* **19**, 3107–3125

52. Louet, J. F., Hayhurst, G., Gonzalez, F. J., Girard, J., and Decaux, J. F. (2002) *J. Biol. Chem.* **277**, 37991–38000
53. Bhalla, S., Ozalp, C., Fang, S., Xiang, L., and Kemper, J. K. (2004) *J. Biol. Chem.* **279**, 45139–45147
54. Lin, J., Wu, P. H., Tarr, P. T., Lindenberg, K. S., St-Pierre, J., Zhang, C. Y., Mootha, V. K., Jager, S., Vianna, C. R., Reznick, R. M., Cui, L., Manieri, M., Donovan, M. X., Wu, Z., Cooper, M. P., Fan, M. C., Rohas, L. M., Zavacki, A. M., Cinti, S., Shulman, G. I., Lowell, B. B., Krainc, D., and Spiegelman, B. M. (2004) *Cell* **119**, 121–135
55. Leone, T. C., Lehman, J. J., Finck, B. N., Schaeffer, P. J., Wende, A. R., Boudina, S., Courtois, M., Wozniak, D. F., Sambandam, N., Bernal-Mizrahi, C., Chen, Z., Holloszy, J. O., Medeiros, D. M., Schmidt, R. E., Saffitz, J. E., Abel, E. D., Semenkovich, C. F., and Kelly, D. P. (2005) *PLoS Biol.* **3**, 672–687
56. Stafford, J. M., Waltner-Law, M., and Granner, D. K. (2001) *J. Biol. Chem.* **276**, 3811–3819
57. Xu, P. L., Liu, Y. Q., Shan, S. F., Kong, Y. Y., Zhou, Q., Li, M., Ding, J. P., Xie, Y. H., and Wang, Y. (2004) *Mol. Endocrinol.* **18**, 1887–1905

Underexpressed Coactivators PGC1 α AND SRC1 Impair Hepatocyte Nuclear Factor 4 α Function and Promote Dedifferentiation in Human Hepatoma Cells
Celia P. Martínez-Jiménez, M. José Gómez-Lechón, José V. Castell and Ramiro Jover

J. Biol. Chem. 2006, 281:29840-29849.

doi: 10.1074/jbc.M604046200 originally published online August 4, 2006

Access the most updated version of this article at doi: [10.1074/jbc.M604046200](https://doi.org/10.1074/jbc.M604046200)

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts

Supplemental material:

<http://www.jbc.org/content/suppl/2006/08/07/M604046200.DC1>

This article cites 56 references, 29 of which can be accessed free at
<http://www.jbc.org/content/281/40/29840.full.html#ref-list-1>