HNF1α Inactivation Promotes Lipogenesis in Human Hepatocellular Adenoma Independently of SREBP-1 and Carbohydrate-response Element-binding Protein (ChREBP) Activation **

Received for publication, November 20, 2006, and in revised form, March 5, 2007 Published, JBC Papers in Press, March 22, 2007, DOI 10.1074/jbc.M610725200

Sandra Reboiussou1‡‡, Sandrine Imbeaud†, Charles Balabaud†††*, Virginie Boulanger†, Justine Bertrand-Michel‡‡, François Terce‡‡, Charles Affray†, Paulette Bioulac-Sage‡‡‡, and Jessica Zucman-Rossi‡‡‡

From the 1INSERM, U674, Génomique fonctionnelle des tumeurs solides, 75010 Paris Cedex, France, the 2Université Paris 7 Denis Diderot, Institut Universitaire d’Hématologie, Centre d’Étude du Polymorphisme Humain (CEPH), 75010 Paris Cedex, France, the 3Array s/IMAGE, Genexpress, Functional Genomics and Systems Biology for Health – UMR 7091, CNRS, Université Paris 6 Pierre et Marie Curie, 94800 Villejuif, France, the 4INSERM, U889, 33076 Bordeaux, France, Université Victor Segalen Bordeaux 2, IFR66, 33076 Bordeaux, France, the 5**Centre Hospitalier Universitaire (CHU) de Bordeaux, Hôpital Saint-André, Service d’Hépatologie, 33076 Bordeaux, France, the 6CHU Bordeaux, Hôpital Pellegrin, 33076 Bordeaux Cedex, and the 7Lipidomic Platform, INSERM, IFR30, Génépolle Toulouse, CHU Purpan, 31052 Toulouse Cedex, France

Biallelic inactivating mutations of the transcription factor 1 gene (TCF1), encoding hepatocyte nuclear factor 1α (HNF1α) were identified in 50% of hepatocellular adenomas (HCA) phenotypically characterized by a striking steatosis. To understand the molecular basis of this aberrant lipid storage, we performed a microarray transcriptome analysis validated by quantitative reverse transcription-PCR, Western blotting, and lipid profiling. In mutated HCA, we showed a repression of gluconeogenesis coordinated with an activation of glycolysis, citrate shuttle, and fatty acid synthesis predicting elevated rates of lipogenesis. Moreover, the strong down-regulation of liver fatty acid-binding protein suggests that impaired fatty acid trafficking may also contribute to the fatty phenotype. In addition, transcriptional profile analysis of the observed deregulated genes in non-HNF1α-mutated HCA as well as in non-tumor livers allowed us to define a specific signature of the HNF1α-mutated HCA. In these tumors, lipid composition was dramatically modified according to the transcriptional deregulations identified in the fatty acid synthetic pathway. Surprisingly, lipogenesis activation did not operate through sterol regulatory element-binding protein-1 (SREBP-1) and carbohydrate-response element-binding protein (ChREBP) that were repressed. We conclude that steatosis in HNF1α-mutated HCA results mainly from an aberrant promotion of lipogenesis that is linked to HNF1α inactivation and that is independent of both SREBP-1 and ChREBP activation. Finally, our findings have potential clinical implications since lipogenesis can be efficiently inhibited by targeted therapies.

Hepatocyte nuclear factor 1-α (HNF1α)3 is a transcription factor that controls the expression of liver-specific genes, such as β-fibrinogen, α1-antitrypsin, and albumin (1). Heterozygous germline mutations in the gene encoding HNF1α (TCF1 (transcription factor 1)) are responsible for an autosomal dominant form of non-insulin-dependent diabetes mellitus called maturity onset diabetes of the young type 3 (MODY3), in which subjects usually develop hyperglycemia before 25 years of age (2). More recently, we identified HNF1α as a tumor suppressor gene involved in human liver tumorigenesis since we found biallelic inactivating mutations of this gene in 50% of hepatocellular adenomas (HCA) and in rare cases of well differentiated hepatocellular carcinomas developed in the absence of cirrhosis (3). HCA are rare benign primary liver tumors closely related to oral contraceptive intake (4).

Recently, in a comprehensive analysis of genotype-phenotype correlations in a large series of 96 HCA, we showed that HNF1α mutations define a homogeneous group of tumors phenotypically characterized by the recurrent presence of a marked steatosis (5). To get insight into the underlying molecular mechanisms that drive the fatty phenotype in human HNF1α-mutated HCA, we performed a transcriptome analysis using cDNA and Affymetrix microarrays. Gene expression profiles were compared between non-tumor livers and HNF1α-mutated HCA. Among the differentially expressed genes, we focused our analysis on genes involved in lipid homeostasis, and we searched for a possible alteration in fat transport, degradation, and synthesis processes. Transcriptomic deregulations were further validated in an additional series of tumors, but also

---

3 The abbreviations used are: HNF1α, hepatocyte nuclear factor 1-α; HNF4α, hepatocyte nuclear factor 4-α; ACL, ATP citrate lyase; ChREBP, carbohydrate-response element-binding protein; SREBP-1, sterol regulatory element-binding protein-1; FA, fatty acid; FAS, fatty acid synthase; MUF A, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; UFA, unsaturated fatty acids; L-FABP, liver fatty acid-binding protein; G6P, glucose-6-phosphate; GK, glucokinate; HCA, hepatocellular adenomas; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PPARγ, peroxisome proliferator-activated receptor-γ; PS, phosphatidylinositol; PI, reverse transcriptase.
HNF1α Inactivation Promotes Hepatic Lipogenesis

at the protein level, and we analyzed the precise composition of accumulated lipids.

EXPERIMENTAL PROCEDURES

Patients and Samples—A series of 40 HCA, 25 non-steatotic non-tumor livers, and 11 steatotic non-tumor livers was collected in nine French surgery departments from 1992 to 2004. Liver tissues were immediately frozen in liquid nitrogen and stored at −80 °C until used for molecular studies. Among the 38 patients with an HCA, the sex ratio (male: female) was 1:9, and the mean age was 37 (median = 37 years ranging from 14 to 56). All the patients were recruited in accordance with French law and institutional ethical guidelines. The study was approved by the ethical committee of the Hôpital Saint-Louis, Paris, France. All HCA were screened for HNF1α and β-catenin mutation, pathological slides were reviewed, and all HCA were classified as described previously (5). Twenty-five HCA from 23 patients were HNF1α-mutated, 11 demonstrated steatosis in more than two-thirds of hepatocytes, eight demonstrated steatosis in one-third to two-thirds of hepatocytes, three demonstrated steatosis in less than one-third of hepatocytes, and three were not steatotic. Fifteen HCA had no mutations in HNF1α, four of them were β-catenin-mutated, five were inflammatory, and nine demonstrated steatosis. Non-tumor liver tissues were taken from patients resected with primary liver tumors developed in the absence of cirrhosis. Steatotic non-tumor livers demonstrated lipid storage either in more than two-thirds of hepatocytes (four cases) or in one-third to two-thirds of hepatocytes (seven cases), and were of various etiologies: hepatitis C virus infection (one case) dysmetabolic syndrome (six cases), alcohol (three cases), and unknown etiology (one case).

Microarray Analysis—Total RNA was extracted from frozen tissues using Qiagen RNeasy kits (Qiagen) according to the manufacturer’s instructions. RNA integrity was assessed using RNA 6000 nano chips and the Agilent 2100 bioanalyzer. RNA quality control was performed as described previously (6). Transcriptional profiling of HNF1α-mutated HCA and non-tumor liver tissues was performed using different microarray approaches. Minimum Information about a Microarray Experiment (MIAME)-compliant data (7) have been deposited in Gene Expression Omnibus (GEO) at NCBI and are available through GEO Series accession number GSE4743. The detailed procedures of analysis is provided in the supplemental experimental procedures.

Quantitative RT-PCR—Quantitative RT-PCR was performed as described previously (8) using predesigned primers and probe sets from Applied Biosystems for the detection of R18S, FABP1 (liver fatty acid-binding protein), PKC1 (phosphoenolpyruvate carboxykinase 1, soluble), PCK2 (phosphoenolpyruvate carboxykinase 2, mitochondrial), FBP1 (fructose-1,6-bisphosphatase), G6P T1 (glucose-6-phosphate transporter 1), GCK (glucokinase), GCKR (glucokinase regulatory protein), GPI (glucose-6-phosphate isomerase), ME1 (malic enzyme 1), MDH1 (NADPH malate dehydrogenase, soluble), ALCY (ATP citrate lyase), PKLR (pyruvate kinase, liver and red blood cell), PKM2 (pyruvate kinase muscle 2) detection of both PKM1 and PKM2 transcripts), ACACA (acetyl-CoA carboxylase-α), FASN (fatty acid synthase), ELOVL1 (elongation of very long chain fatty acids protein 1), ELOVL2 (elongation of very long chain fatty acids protein 2), ELOVL5 (ELOVL family member 5, elongation of very long chain fatty acids), SCD (stearyl-CoA desaturase), FADS1 (fatty acid desaturase 1), FADS2 (fatty acid desaturase 2), SREBP-1 (detection of both SREBP-1a and SREBP-1c isoforms), SREBP-1a, CHREBP, PPARG (peroxisome proliferator-activated receptor-α), PPARG, LXRα (liver X receptor α), and HNF4α. Ribosomal 18 S (R18S) was used for the normalization of expression data. The relative amount of measured mRNA in samples was determined using the 2−ΔΔCT method where ΔΔCT = (CTtarget − CTR18S)sample − (CTtarget − CTR18S)calibrator. Final results were expressed as the n-fold differences in target gene expression in tested samples when compared with the mean expression value of non-tumor tissues.

Western Blotting—Total protein extracts were obtained after homogenization in radioimmunoprecipitation assay buffer (Santa Cruz Biotechnology). Nuclear extracts were prepared using the NE-PER nuclear and cytoplasmic extraction reagent kit (Pierce), and protein concentration was determined using a Pierce BCA protein assay kit. Primary antibodies were used at the following dilutions: rabbit polyclonal anti-L-FABP 1:2000 (a gift of Dr. J Gordon), mouse monoclonals anti-fatty acid synthase (anti-FAS) and anti-SREBP-1 (detecting both the SREBP-1a and the SREBP-1c isoforms) 1:500 (Pharmingen), polyclonal rabbit anti-glucokinase (anti-GK) (Santa Cruz Biotechnology) and anti-ACL (Cell Signaling Technology) 1:200, and polyclonal rabbit anti-ChREBP 1:1000 (Novus Biologicals). We used polyclonal rabbit anti-actin (1:3000, Sigma) and polyclonal rabbit anti-lamin A/C (1:500, Cell Signaling Technology) as loading controls to normalize the signal obtained for total and nuclear protein extracts, respectively. Detection of signals was performed using the ECL SuperSignal West Pico chemiluminescent substrate (Pierce) with either anti-mouse (1:4000, Amersham Biosciences) or anti-rabbit (1:2000, Santa Cruz Biotechnology) horseradish peroxidase-conjugated IgG as second antibodies.

Glucose-6-phosphate Measurement—Glucose-6-phosphate (G6P) concentrations were determined enzymatically in normal and tumor samples by exploiting the selective and quantitative conversion of G6P to 6-phosphogluconic acid in the presence of NADP+ and glucose-6-phosphate dehydrogenase, as described previously (9).

Histochemical Analysis of Glycogen Storage—For this analysis, we used liver tissues frozen through immersion in isopentane cooled in liquid nitrogen. Periodic acid Schiff histochemical staining was performed on frozen section from five HNF1α-mutated HCA and five non-mutated HCA as well as their matched non-tumor livers.

Lipid Profiling—Detailed protocols used for lipid profiling are provided in the supplemental experimental procedures.

Statistical Analysis—All the values reported are mean ± S.D. Statistical analysis was performed using GraphPad Prism version 4 software, and significance was determined using the non-parametric Mann-Whitney test for unpaired data. Difference was considered significant at p < 0.05.
RESULTS

Gene Expression Profiles in HNF1α-mutated HCA—Our cDNA and Affymetrix microarray experiments analyzed the expression of a total of 15,000 different genes comparing, respectively, eight HNF1α-mutated HCA with their corresponding non-tumor liver and five HNF1α-mutated HCA with four non-tumor livers. Computational analysis of both experiments identified 375 and 222 genes, respectively, significantly down- and up-regulated in HNF1α-mutated HCA. Among these genes, a large fraction was related to the normal hepatocyte function including carbohydrate and lipid metabolism, detoxification, and synthesis of secreted proteins such as complement and coagulation factors (supplemental Table 1). Among differentially expressed genes, promoter regions of 33 were previously identified to bind HNF1α in primary human hepatocytes using chromatin immunoprecipitation-on-chip (ChIP-on-chip) technology (10). All but four of these genes were down-regulated in HNF1α-mutated HCA (supplemental Table 1), which is in accordance with the well known transactivating function of HNF1α. In addition, we identified 82 genes showing a common pattern of expression with liver from lnf1α-null mice (11) (supplemental Table 1). Interestingly, 19 of these common deregulated genes were involved in gluco- lipidic metabolism and particularly in bile acid metabolism, cholesterol, and fatty acid synthesis (supplemental Table 1).

Fatty Acid Transport and Oxidation—In HNF1α-mutated HCA, we identified an important modification in the expression profiles of apolipoprotein genes. Particularly, components of the high density lipoprotein particles encoded by APOM, LPLA2, APOF, LPA, and APOA4 were strongly down-regulated, whereas APOL3 was up-regulated (supplemental Table 1). In contrast, the expression of critical genes for hepatocyte very low density lipoprotein assembly and secretion such as APOB and MTP was not affected. We also found a dramatic decrease in the mRNA of FABP1 encoding the liver fatty acid-binding protein (L-FABP). This result was confirmed by quantitative RT-PCR (~68-fold change, Fig. 1A) and by Western blotting analysis that showed an absence of L-FABP in mutated HCA (Fig. 1B). In contrast, we did not find any change in the expression of genes encoding mitochondrial and peroxisomal β-oxidation enzymes. The expression level of genes encoding plasma membrane transporters such as CD36 was also normal in mutated HCA as well as the LDLR gene. However, we identified a number of deregulated genes related to the lipogenesis pathway (Figs. 2A and 3A).

Glucose-6-phosphate in HNF1α-mutated HCA (Fig. 2B). According to a predicted increased glucose phosphorylation rate, we showed a significantly higher level of glucose-6-phosphate in HNF1α-mutated HCA (Fig. 2D). Moreover, periodic acid Schiff staining revealed a glycogen overload in these tumors consistent with G6P increase (Fig. 2E). We also found an up-regulation of GPI mRNA (4-fold, Fig. 2B), which encodes the glucose phosphate isomerase, a second glycolytic enzyme. In contrast, PKLR mRNA, which encodes the liver-specific pyruvate kinase that catalyzes the last irreversible step of glycolysis, was 3-fold decreased in HNF1α-mutated HCA (Fig. 2F). However, PKLR down-regulation may be balanced by a 2-fold increase of PKM2 gene that encodes the muscle-specific pyruvate kinase (Fig. 2F).

Activation of the Citrate Shuttle—Besides the observed gluconeogenesis inhibition and glycolysis activation that predict an overproduction of pyruvate, a substrate for the mitochondrial synthesis of acetyl-CoA, the citrate shuttle system that exports acetyl-CoA into the cytosol was also activated in HNF1α-mutated HCA (Fig. 2A). Indeed, we found a 12-fold overexpression of ME1 mRNA, which encodes the malic enzyme, and a moderate 2-fold increase of MDH1 transcript, and ACLY mRNA encoding the ATP citrate lyase (ACL) was 3.7-fold overexpressed in HNF1α-mutated HCA (Fig. 2B). As ATP citrate lyase is crucial to provide the unique cytoplasmic source of the acetyl-CoA lipogenic precursor, we confirmed by Western blotting the ACL protein overexpression (Fig. 2C).

Stimulation of the Fatty Acid Synthetic Pathway—As shown in Fig. 3A, the fatty acid synthetic pathway was also stimulated
in HNF1α-mutated HCA. We found an increase of the acetyl-CoA carboxylase transcript (ACACA, 2.6-fold) and a strong overexpression of fatty acid synthase at both the transcriptional (FASN, 7-fold) and the protein level (FAS, Fig. 3, B and C). In the downstream steps of fatty acid elongation and desaturation, we also identified an increase in the mRNA level of three genes encoding elongases including ELOVL1 (3.4-fold), ELOVL2 (2.2-fold), and ELOVL5 (2.3-fold) and of three genes encoding desaturases such as SCD (6-fold), FADS1 (3-fold), and FADS2 (4.7-fold, Fig. 3B).

Transcriptional Alteration of Glucido-lipidic Genes Defines an Expression Pattern That Is Specific of HNF1α-mutated HCA—To search whether the pattern of alteration in the expression of the glucido-lipidic genes was specific of HNF1α inactivation, we compared the expression profiles of the 19 aforementioned genes between HNF1α-mutated HCA and a...
group of non-mutated HCA containing or not containing steatosis, using quantitative RT-PCR. In this analysis, we also included steatotic non-tumor livers from different etiologies and non-steatotic non-tumor livers. We then performed a non-supervised analysis of the results using a hierarchical clustering algorithm to group the genes as well as the samples on the basis of similarity in their expression pattern. This analysis accurately classified all samples in two major clusters according to their HNF1α status (Fig. 4). Steatotic non-tumor and non-steatotic non-tumor livers were gathered with the non-HNF1α-mutated HCA. Gene cluster analysis identified two main groups corresponding to down-regulated (cluster A) and up-regulated (cluster B) genes in HNF1α-mutated HCA (Fig. 4). As expected, four out of five genes identified in cluster A had been previously characterized as HNF1α transactivated targets. Interestingly, all up-regulated genes have never been previously found regulated by HNF1α.

Within cluster B, three subgroups of genes (subgroups 1, 2, and 3) showed a highly correlated overexpression in HNF1α-mutated HCA (supplemental Fig. 1). For example, ACY1 level of expression was correlated to ACAY overexpression in 16 HNF1α-mutated HCA (Spearman r = 0.72, p = 0.001) (supplemental Fig. 1).

In non-HNF1α-mutated liver tissues, only few genes demonstrated a significant transcriptional deregulation (supplemental Fig. 2). In steatotic non-tumor livers, expression of only two genes, PCK2 and MEI, followed an expression pattern as seen in HNF1α-mutated HCA (supplemental Fig. 2). In non-HNF1α-mutated steatotic HCA, nine genes involved in gluconeogenesis, glycolysis, and citrate shuttle were significantly deregulated when compared with the non-steatotic non-tumor livers (supplemental Fig. 2). One of these, GCK, showed an inverted pattern of deregulation when compared with the HNF1α-mutated
HNF1α Inactivation Promotes Hepatic Lipogenesis

HCA. However, except for a modest elevation in ELOVL1 transcript, expression of genes related to the fatty acid synthetic pathway were unchanged in this group of steatotic adenomas. Finally, in the group of non-HNF1α-mutated non-steatotic HCA, only four genes showed a significant change in expression pattern (FBP1, GPI, ACLY, ELOVL1).

These results indicated that the patterns of expression of the 19 analyzed genes were specific to HNF1α inactivation. Down-regulations could be directly attributed to the loss of the classical HNF1α transactivation activity. In contrast, overexpressed genes demonstrated a coordinated activation, but none are known to date to be directly controlled by HNF1α.

Activation of Lipogenesis in HNF1α-mutated HCA Is Independent of SREBP-1 and ChREBP Activation—Among the lipogenic genes whose expression was increased in HNF1α-mutated HCA, 8 out of 13 were previously described as SREBP-1c targets. However, using quantitative RT-PCR, we did not find any significant variation in the mRNA level of total SREBP-1 in HNF1α-mutated HCA when compared with non-tumor livers nor in SREBP-1a (Fig. 5A). Moreover, surprisingly, Western blotting studies revealed a decrease in the nuclear active form of SREBP-1 in the tumors when compared with their corresponding non-tumor liver (Fig. 5B), and the microarray study also found an increase in INSIG1 (insulin-induced gene 1) mRNA (2-fold, supplemental Table 1) whose product is known to inhibit SREBP-1 cleavage processing. We then looked for a potential alteration of ChREBP expression, another key transcriptional activator of fatty acid synthesis in the liver. RT-PCR results showed a significant decrease in the mRNA of CHREBP in HNF1α-mutated HCA (−1.8-fold, Fig. 5A), and Western blotting studies revealed a decrease of the protein level in the nucleus (Fig. 5B). Using the same samples where we demonstrated a repression of both SREBP-1 and ChREBP, we found an obvious overexpression of the FAS and GK proteins, which are known to be transactivated by these two transcription factors expression was found in the non-HNF1α-mutated HCA tested (supplemental Fig. 3). We also checked for the transcriptional level of other transcription factors that have been implicated in the regulation of gluco-lipidic metabolism (Fig. 5A). Except for a 1.9-fold elevation of PPARγ transcript, the mRNA level of FOXO1A (forkhead box O1A), PPARα, LXRα, and HNF4α was not significantly different in HNF1α-mutated HCA when compared with non-tumor livers (Fig. 5A). Finally, the RXR isotypes genes were not differentially expressed in the microarray analysis.

Total Lipid Composition Is Modified in HNF1α-mutated HCA—We compared lipid profiles of four non-steatotic non-tumor livers with that of five HNF1α-mutated HCA. Total fatty acid content was 5.8-fold increased in HNF1α-mutated HCA (Fig. 6A), and the fatty acid profile was modified. Saturated (SFA) and monounsaturated (MUFA) fatty acids were 7- and 8-fold increased, respectively (Fig. 6A), as a consequence of the accumulation of all SFA and MUFA species detected (Figs. 3A and 6, C and D). In contrast, polyunsaturated fatty acids (PUFA) were raised only 2-fold (Fig. 6A) with a preferential accumulation of fatty acids containing 18 carbon atoms including the two essential fatty acids linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) (Fig. 6E). Consequently, the SFA/PUFA and MUFA/PUFA ratios in mutated HCA were significantly higher than in non-tumor livers (Fig. 6B). We also showed that C14–18 fatty acids were much more increased than C20–C23 fatty acids, as indicated by a 5-fold higher C14–18/C20–C23 ratio (Fig. 6B). We also identified a mild elevation in free cholesterol content in HNF1α-mutated HCA (36 versus 26.2 nmol/mg of proteins, Fig. 6F); however, we did not find any change neither in total cholesterol esters and diglyceride contents (Fig. 6F) nor in specific molecular species related to these two neutral lipid classes (data not shown). In contrast, total and all species of triglycerides were dramatically increased in HNF1α-mutated HCA (Fig. 6, F and G). This overload was inversely proportional to the

![FIGURE 5. Expression of transcription factors involved in the regulation of liver gluco-lipidic metabolism. A, mRNA level was assessed using quantitative RT-PCR. Results are expressed as the n-fold difference in gene expression relative to the mean expression value of non-tumor livers. Data are mean ± S.D. *, **, and ***, difference between groups at p < 0.05, 0.01, and 0.001, respectively. B, protein levels of SREBP-1 (precursor form 125 kDa and mature form 68 kDa) and ChREBP (95 kDa) were analyzed by Western blotting in total and nuclear protein fraction from three HNF1α-mutated HCA and ChREBP were also assessed in the total protein fraction from the same samples. β-Actin and lamin A/C were used as loading controls for total and nuclear extracts, respectively.](image-url)
FIGURE 6. Quantification of total fatty acids and neutral lipids. All the results are expressed as mean ± S.D. *, difference between groups at p < 0.05. A, total FA content. B, ratio of total fatty acids. C, SFA quantification. D, MUFA quantification. E, PUFAs quantification. F, quantification of neutral lipids classes. CE, cholesterol esters; DG, diglycerides; TG, triglycerides. G, measurement of triglyceride families defined according to their total number of carbon atoms.
HNF1α Inactivation Promotes Hepatic Lipogenesis

**TABLE 1**
Quantification of phospholipid classes
PL, phospholipid; SM, sphingomyelin; ND, not detected. Values are mean ± S.D.* and ***, difference between groups at p < 0.05 and 0.001, respectively.

|                | Total PL | Non-tumor livers* | HNF1α-mutated HCA | Total PL | Non-tumor livers | HNF1α-mutated HCA | % of total phospholipids | % of total fatty acids |
|----------------|----------|-------------------|-------------------|----------|-----------------|-------------------|-------------------------|----------------------|
| Non-tumor livers | 392.13 ± 75.35 | 868.38 ± 234.67*** | 55.21 ± 10.84 | 54.15 ± 4.36 | 54.08 ± 3.07 |
| HNF1α-mutated HCA | 215.55 ± 57.87 | 418.92 ± 98.32*** | 48.68 ± 4.06 | 76.66 ± 9.5 | 11.99 ± 0.68*** |
| PE             | 131.26 ± 41.33 | 385.76 ± 119***   | 33.07 ± 9.78 | 44.23 ± 4.64* | 3.32 ± 1.07* |
| SM             | 18.44 ± 4.66   | 29.92 ± 15.41     | 4.74 ± 0.93 | 3.32 ± 1.07* | 3.32 ± 1.07* |
| PS             | 3.5 ± 6.49     | ND                | 0.97 ± 1.8 | ND | ND |
| PI             | 23.18 ± 12.4   | 33.89 ± 25.52     | 6.01 ± 3.2 | 3.77 ± 2.88 | 3.77 ± 2.88 |

* *n = 8.

**TABLE 2**
Fatty acid composition in each class of phospholipid
Values are percentages of total fatty acids. Values are mean ± S.D. UI, unsaturation index = Σ (% of each unsaturated fatty acid × number of double bonds of the same fatty acid)/100. *, difference between groups at p < 0.05.

| Fatty acid | Non-tumor livers | HNF1α-mutated HCA | Total PL | Non-tumor livers | HNF1α-mutated HCA | % of total fatty acids | % of total fatty acids |
|------------|------------------|-------------------|----------|-----------------|-------------------|-------------------------|----------------------|
| SFA        | 49.13 ± 1.18     | 47.96 ± 1.7       | 48.96 ± 1.12 | 47.34 ± 3.26 | 54.15 ± 4.36 | 54.08 ± 3.07 |
| MUFA       | 10.71 ± 1.39     | 13.33 ± 2.01      | 7.84 ± 0.75 | 8.16 ± 1.81 | 7.66 ± 0.95 | 11.99 ± 0.68*** |
| PUFA       | 40.16 ± 1.96     | 38.71 ± 2.3       | 14.32 ± 1.6 | 44.5 ± 3.17 | 38.19 ± 2.5 | 31.93 ± 2.57 |
| MUFA/PUFA  | 0.27 ± 0.04      | 0.35 ± 0.07       | 0.18 ± 0.02 | 0.18 ± 0.04 | 0.21 ± 0.05 | 0.38 ± 0.02* |
| MUFA/SFA   | 0.22 ± 0.03      | 0.28 ± 0.05       | 0.16 ± 0.01 | 0.17 ± 0.05 | 0.14 ± 0.01 | 0.21 ± 0.02* |
| SFA/PUFA   | 1.23 ± 0.08      | 1.24 ± 0.10       | 1.14 ± 0.07 | 1.07 ± 0.15 | 1.45 ± 0.32 | 1.77 ± 0.25 |
| SFA/PUFA   | 0.97 ± 0.05      | 0.92 ± 0.06       | 0.96 ± 0.04 | 0.90 ± 0.12 | 1.20 ± 0.22 | 1.29 ± 0.17 |
| C14-C18/C20-C24 | 3.11 ± 0.49 | 5.26 ± 1.04       | 1.85 ± 0.29 | 1.48 ± 0.2 | 2.42 ± 0.59 | 2.46 ± 0.4 |
| UI         | 1.48 ± 0.11      | 1.27 ± 0.10       | 1.81 ± 0.11 | 1.97 ± 0.22 | 1.46 ± 0.21 | 1.40 ± 0.11 |

* n = 4.

In mice, the loss of HNF1α inactivation seems to be primarily due to an aberrant stimulation of lipogenic rate. In mice, the loss of HNF1α inactivation seems to be primarily due to an aberrant stimulation of lipogenic rate.
CoA carboxylase and fatty acid synthase genes (14). However, our study is the first one to demonstrate a coordinate overexpression of all lipogenic genes and to highlight the major involvement of this aberrant induction in the pathogenesis of fatty liver phenotype related to HNF1α inactivation. In the present work, we identified transcriptional deregulations of 19 genes that may explain fat overload observed in the human HNF1α-mutated HCA. Interestingly, when extracting the expression data from the transcriptome analysis performed by Shih et al. (11) in the liver from hnf1α-null mice, we found that 9 of these 19 genes were commonly deregulated in mice. Moreover, it is remarkable that the most important overlap was found for the genes involved in the fatty acid synthetic pathway (five genes out of nine).

Among the 19 deregulated genes, three of the six down-regulated genes (FABP1, G6PT1, and PCK1) were previously demonstrated to be directly transactivated by HNF1α, and the promoter region of GCKR has been shown to bind HNF1α in vivo by chromatin immunoprecipitation (10, 14–16). However, among the overexpressed genes, the majority were known to be transactivated by the membrane-bound transcription factor SREBP-1c. SREBP-1c is considered as the master regulator of fatty acid synthesis in the liver, mediating the effects of insulin on the expression of glycolytic and lipogenic genes after being activated by proteolytic cleavage (17). Surprisingly, in HNF1α-mutated HCA, SREBP-1 was repressed in most of the cases, possibly resulting from the induction of INSG1 mRNA (18). This finding contrasts with that found in hnf1α-null mice liver in which a significant increase of SREBP-1 transcript was reported (11). ChREBP is another transcription factor known to activate the expression of some lipogenic genes such as ACLY, ACACA, and FASN (19). In HNF1α-mutated HCA, we also found a reduction of the ChREBP mRNA level and nuclear protein amounts, demonstrating repression. This could occur as a consequence of the observed PUFA elevation in the tumors and particularly of the linoleic acid, which has been shown to suppress ChREBP activity by increasing its mRNA decay and altering its translocation from the cytosol to the nucleus (20). Thus, the repression of SREBP-1 and ChREBP observed in most of the analyzed HNF1α-mutated HCA could result from a negative feedback responding to lipid overload. Furthermore, although a large fraction of SREBP-1 targets was overexpressed in HNF1α-mutated HCA, some well known targets, such as the 6-phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase, were not deregulated in mutated HCA. Altogether, our results imply that SREBP-1 and ChREBP are not responsible for the coordinate induction of glycolytic and lipogenic genes observed in HNF1α-mutated HCA. PPARγ was previously proposed to play a role in hepatic induction of lipogenic genes through a mechanism that is still unclear (21–23). In the HNF1α-mutated HCA, we found an increase in PPARγ gene expression; however, among three well known targets of this transcription factor (FABP4, CD36, and UCP2), only one demonstrated a significant overexpression (FABP4 gene 5-fold, supplemental Table 1), suggesting that PPARγ activity is not obviously increased in HNF1α-mutated HCA. Thus, the mechanism by which HNF1α inactivation leads to the coordinate overexpression of the lipogenic genes remains to be elucidated. The strong correlation groups identified between overexpressed transcripts suggest that a transcriptional mechanism would be most likely at the origin of the observed deregulations, instead of a post-transcriptional regulation such as mRNA stability. Consequently, transcriptional activation of the lipogenic genes related to HNF1α inactivation may occur through the loss of a direct HNF1α repressor function involving either direct DNA binding or interaction with other transcription factors and co-activator proteins (supplemental Fig. 4A). Although, HNF1α has been mainly reported as a transcriptional activator, this model would explain some of the identified overexpressions. Indeed, a co-repressor activity for HNF1α on its own transcription has already been shown through interaction with HNF4α (24). Alternatively, the absence of a functional HNF1α protein may also alter the expression of another transcription factor involved in the control of lipogenesis (supplemental Fig. 4B). New insights into understanding the mechanisms regulating lipogenic gene expression by HNF1α could come from the analysis of gene promoter activity in cellular systems in which HNF1α activity will be modulated.

Results from lipid profiling analysis of the accumulated fat in HNF1α-mutated HCA are well correlated with the transcriptional deregulations observed in the fatty acid synthetic pathway. Indeed, the preferential accumulation of the SFA and MUFA species is in accordance with the strong overexpression of FAS and SCD and with the high elevation of ELOVL1 expression (25–27). The significant accumulation of the two essential fatty acids linoleic acid and α-linolenic acid suggests that in addition to the elevated rates of lipogenesis, other mechanisms may contribute to the fatty phenotype in HNF1α-mutated HCA. This is unlikely the consequence of impaired VLDL secretion or fatty acid entry into the tumor hepatocytes since no obvious deregulations in the expression of the genes involved in these processes was identified. However, although expression of fatty acid oxidation genes was unchanged in HNF1α-mutated HCA, it is likely that fatty acid oxidation is reduced in adenoma cells secondary to lipogenic rate increase. Indeed, this may occur through the inhibition of CPT1 activity resulting from the overproduction of malonyl-CoA by the acetyl-CoA carboxylase whose transcript was significantly up-regulated in HNF1α-mutated HCA. This, a secondary defect in β-oxidation may contribute to fat accumulation and may explain in part the observed elevation of essential fatty acids. Moreover, given the role of L-FABP in binding, trafficking, and compartmentalization of fatty acids into the hepatocyte, the dramatic decrease in L-FABP expression may also participate in the steatotic phenotype, as suggested in hnf1α-null mice liver (28). The alteration of phospholipid profile in HNF1α-mutated HCA was moderate. However, it may cause deregulation of membrane functioning in adenoma cells, leading to alteration of critical cellular processes related to tumorigenesis.

In this study, we clearly demonstrated that lipogenesis activity was increased in HNF1α-mutated HCA, and this feature has been commonly observed in tumor cells. Indeed, overexpression of the fatty acid synthase was previously identified in a wide variety of cancers as well as in precancerous lesions (29). This overexpression was often accompanied by a coordinate induction of the other lipogenic enzymes, and several studies have

**HNF1α Inactivation Promotes Hepatic Lipogenesis**
shown that tumor cell survival may depend on de novo synthesis of fatty acids. Although the fatty acid synthase has been proposed as an interesting therapeutic target for cancer treatment, more recent studies showed that the inhibition of other lipogenic enzymes such as acetyl-CoA carboxylase or ATP citrate lyase can also limit cancer cell proliferation and survival (30, 31). Recently, we proposed HNF1α as a tumor suppressor gene (3), and we demonstrated in the present work that lipogenesis activation in HNF1α-mutated HCA results from the loss of HNF1α activity. This finding may contribute to reinforce the emerging concept that induction of lipogenesis observed in tumor cells would occur downstream from oncogenic events (29) and open new avenues to understand how metabolism deregulations may contribute to benign liver tumorigenesis. Finally, our findings have potential clinical implications since lipogenesis can be efficiently inhibited by targeted therapies.

Acknowledgments—We warmly thank all the other participants to the GENTHEP (Groupe d’étude Génétique des Tumeurs Hépatiques) network. We are grateful to Emmanuelle Jeannot and Lucille Mellotte for the help in mutation screening and excellent technical assistance and Véronique Roques for helpful assistance at the Lipidomic Platform. We thank Dr J. Gordon for providing us with the L-FABP antibody and Dr. R. H. J. Bandsma for the experimental procedure of glucose-6-phosphate measurement. We also thank Alain Paris and Philippe Bois for critical reading of the manuscript.

REFERENCES
1. Courtois, G., Morgan, J. G., Campbell, L. A., Fourel, G., and Crabtree, G. R. (1987) Science 238, 688–692
2. Yamagata, K., Oda, N., Kaisaki, P. J., Menzel, S., Furuta, H., Vaxillaire, M., Southam, L., Cox, R. D., Lathrop, G. M., Boriraj, V. V., Chen, X., Cox, N. J., Oda, Y., Yano, H., Le Beau, M. M., Yamada, S., Nishigori, H., Takeda, J., Fajans, S. S., Hattersley, A. T., Iwasaki, N., Hansen, T., Pedersen, O., Polonsky, K. S., Turner, R. C., Velho, G., Chèvre, J. C., Frohman, P., and Bell, G. I. (1996) Nature 384, 455–458
3. Bluteau, O., Jeannot, E., Bioulac-Sage, P., Marques, J. M., Blanc, J. F., Bui, H., Beaudoin, J. C., Franco, D., Balabaud, C., Laurent-Puig, P., and Zucman-Rossi, J. (2002) Nat. Genet. 32, 312–315
4. Edmondson, H. A., Henderson, B., and Benton, B. (1976) N. Engl. J. Med. 294, 470–472
5. Zucman-Rossi, J., Jeannot, E., Nhieu, J. T., Scoazer, J. Y., Guettier, C., Rebouissou, S., Basq, Y., Leteurtre, E., Paradis, V., Michalak, S., Wendum, D., Chiche, L., Fabre, M., Mellotte, L., Laurent, C., Partensky, C., Castaing, D., Zafrañi, E. S., Laurent-Puig, P., Balabaud, C., and Bioulac-Sage, P. (2006) Hepatology 43, 515–524
6. Imbeaud, S., Graudens, E., Boulanger, V., Barlet, X., Zaborski, P., Eveno, E., Mueller, O., Schroeder, A., and Auffray, C. (2005) Nucleic Acids Res. 33, e56
7. Brazma, A., Hingamp, P., Quackenbush, J., Sherlock, G., Spellman, P., Stoeckert, C., Aach, J., Ansorge, W., Ball, C. A., Causton, H. C., Gaasterland, T., Glinos, P., Holstege, F. C., Kim, I. F., Markowitz, V., Matese, J. C., Parkinson, H., Robinson, A., Sarkans, U., Schulze-Kremer, S., Stewart, J., Taylor, R., Vilo, J., and Yingst, M. (2001) Nat. Genet. 29, 365–371
8. Rebouissou, S., Vasilii, V., Thomas, C., Bellanene-Chantelot, C., Bui, H., Chretien, Y., Timis, J., Rosty, C., Laurent-Puig, P., Chauve, D., and Zucman-Rossi, J. (2005) Hum. Mol. Genet 14, 603–614
9. Bandmsa, R. H., Wiegman, C. H., Herling, A. W., Burger, H. J., ter Harmsel, A., Meijer, A. J., Romijn, J. A., Reijngoud, D. J., and Kuipers, F. (2001) Diabetes 50, 2591–2597
10. Odom, 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., Fradenel, E., Bell, G. I., and Young, R. A. (2004) Science 303, 1378–1381
11. Shih, D. Q., Bussen, M., Sehayek, E., Ananthanarayanan, M., Shneider, B. L., Suchy, F. J., Shefer, S., Bollili, J. E., Gonzalez, F. J., Breslow, J. L., and Stoffel, M. (2001) Nat. Genet. 27, 375–382
12. Foufelle, F., and Ferre, P. (2002) Biochem. J. 366, 377–391
13. Lee, Y. H., Sauver, B., and Gonzalez, F. J. (1998) Mol. Cell. Biol. 18, 3059–3068
14. Akiyama, T. E., Ward, J. M., and Gonzalez, F. J. (2000) J. Biol. Chem. 275, 27117–27122
15. Hiraiwa, H., Pan, C. J., Lin, B., Akiyama, T. E., Gonzalez, F. J., and Chou, J. Y. (2001) J. Biol. Chem. 276, 7963–7967
16. Yanuka-Kashles, O., Cohen, H., Trus, M., Aran, A., Benvenisty, N., and Reshef, L. (1994) Mol. Cell. Biol. 14, 7124–7133
17. Horton, J. D., Goldstein, J. L., and Brown, M. S. (2002) J. Clin. Invest. 109, 1125–1131
18. Engelking, L. J., Kuriyama, H., Hammer, R. E., Horton, J. D., Brown, M. S., Goldstein, J. L., and Liang, G. (2004) J. Clin. Invest. 113, 1168–1175
19. Iizuka, K., Bruck, R. K., Liang, G., Horton, J. D., and Uyeda, K. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 7281–7286
20. Dentin, R., Benhamed, F., Pegorier, J. P., Foufelle, F., Viollet, B., Vaulont, S., Girard, J., and Postic, C. (2005) J. Clin. Invest. 115, 2843–2854
21. Matsusue, K., Hatake, M., Lambert, G., Yin, S. H., Gavrilova, O., Ward, J. M., Brewer, B. J., Reitman, M. L., and Gonzalez, F. J. (2003) J. Clin. Invest. 111, 737–747
22. Schadinger, S. E., Bucher, N. L., Schreiber, B. M., and Farmer, S. R. (2005) Proc. Natl. Acad. Sci. U. S. A. 101, 5484–5490
23. Zhang, Y. L., Hernandez-Ono, A., Siri, P., Weisberg, S., Conlon, D., Graham, M., Moore, M., and Gonzalez, F. J. (2006) J. Biol. Chem. 281, 37603–37615
24. Kitakata, K., Yamasaki, M., Vaux, J. C., Parkinson, H., Robinson, A., Dhanak, D., Hingorani, S. R., Tuveson, D. A., and Thompson, C. B. (2005) Proc. Natl. Acad. Sci. U. S. A. 102, 6036–6041
25. Smith, S. (1994) FASEB J. 8, 1248–1259
26. Ntambi, J. M., and Miyazaki, M. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 1109–1114
27. Jacobsson, A., Westerberg, R., and Jacobsson, A. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 10981–10986
28. Gordon, J. I., Elshourbagy, N., Lowe, B. J., Liao, W. S., Alpers, D. H., and Taylor, M. (1995) J. Biol. Chem. 260, 1995–1998
29. Swinnen, J. V., Brusselmans, K., and Verhoeven, G. (2006) Curr. Opin. Clin. Nutr. Metab. Care 9, 358–365
30. Hatzivassiliou, G., Zhao, F., Bauer, D. E., Andreadis, C., Shaw, A. N., Dhanak, D., Hingorani, S. R., Tuveson, D. A., and Thompson, C. B. (2005) Cancer Cell 8, 311–321
31. Brusselmans, K., and Schrijver, E., Verhoeven, G., and Swinnen, J. V. (2005) Cancer Res. 65, 6719–6725