Liver enriched transcription factors and differentiation of hepatocellular carcinoma

Y Hayashi, W Wang, T Ninomiya, H Nagano, K Ohta, H Itoh

Abstract

The development of a complex organism relies on the precise temporal and spacial expression of genes in many different cell types. The unique phenotype of hepatocytes arises from the expression of genes in a liver specific fashion, which is controlled primarily at the level of mRNA synthesis. By analysing DNA sequences implicated in liver specific transcription, it has been possible to identify members of the nuclear proteins, such as the liver enriched transactivating factors, hepatic nuclear factor 1 (HNF-1), HNF-3, HNF-4, HNF-6, CCAAT/enhancer binding protein (C/EBP), and D binding protein (DBP), which are key elements in the liver specific transcriptional regulation of genes. Each of these factors is characterised by DNA binding domains that bind to unique DNA sequences (cis-acting factors) in the promoter or enhancer regions of genes expressed in terminally differentiated hepatocytes (such as, albumin, α1-antitrypsin, transthyretin, α-fetoprotein). The determination of the tissue distribution of these factors and analysis of their hierarchical relations has led to the hypothesis that the cooperation of liver enriched transcription factors with the ubiquitous transactivating factors is necessary, and possibly even sufficient, for the maintenance of liver specific gene transcription. With the increase in information about transcriptional regulation, it should be possible to evaluate fully the clinicopathological usefulness of transcription factors in the diagnosis and treatment of hepatocellular carcinoma.

Keywords: liver enriched transcription factor; hepatic nuclear factors; CCAAT/enhancer binding protein; D binding protein

The tissue specific expression of genes is based on the presence of cis-acting sequences in their promoter and enhancer regions that interact with sequence specific nuclear transcription factors, which potentiate or depress transcriptional initiation. Therefore, the establishment and maintenance of a cell type specific, differentiated phenotype relies on the presence and activity of an array of tissue enriched DNA binding proteins with transactivating activity. In general, the different stages of hepatocyte differentiation have been characterised by the transcripts detected, and the expression of such transcripts is believed to be governed by the sets of transcription factors that are activated. Hepatic differentiation is presumed to be the result of the combined action of members of liver enriched transcription factors (LETFs), including CCAAT/enhancer binding protein (C/EBP), D binding protein (DBP), hepatic nuclear factor 3 (HNF-3), HNF-1, HNF-4, and HNF-6. These LETFs regulate each other and form a transcriptional hierarchy that is involved both in the determination and the maintenance of the hepatic phenotype. Hepatic carcinogenesis is characterised by the sequential appearance of preneoplastic and neoplastic populations of cells that show, in comparison with their normal counterparts, alterations in their proliferative activity and a large number of liver specific proteins. The phenotypical heterogeneity of hepatocellular carcinoma foci and nodules indicates the existence of distinct subpopulations of lesions. The proliferative regions of several genes, including those encoding albumin, α1-antitrypsin, transthyretin, fibrinogen, and certain members of the cytochrome P450 family that are highly expressed in the liver, have been shown to contain consensus sequences that bind different LETFs and the relative abundance of these factors determines the level of gene expression. Some studies have investigated whether the changes in these LETFs might also occur in hepatocarcinogenesis.

Hepatocyte nuclear factor-1 (HNF-1) family

The HNF-1 family includes HNF-1α (also called LFB1 or AFP) and HNF-1β (also called variant HNF-1). These proteins are characterised by a homeobox containing, DNA binding domain that is well conserved throughout evolution and a POU domain that confers sequence specificity. As a result of a similar dimerisation domain in their N-terminal regions, HNF-1α and HNF-1β proteins can dimerise on their own to form homodimers or they can form heterodimers with each other. The functions of different transactivation domains localised at their C-terminal regions show that HNF-1α has a higher potency of transactivation than HNF-1β (fig 1). HNF-1α and HNF-1β genes are located on different chromosomes—chromosomes 12 and 17, respectively. HNF-1β is expressed early on during embryonic development, in the endoderm of the foregut, whereas HNF-1α is activated later, upon condensation of the hepatic parenchyma, and its expression decreases in the adult liver (fig 2).

1 Binding sites for HNF-1 have been shown in the promoters or enhancers of genes that are
expressed almost exclusively in the liver, such as albumin, α-fetoprotein, α-fibrinogen, β-fibrinogen, α1-antitrypsin, transthyretin, aldolase B, and the hepatitis B virus large surface protein. The study of the rat hepatoma cell, H4II, and its derivatives show that HNF-1α expression parallels the hepatic phenotype of the cells. HNF-1α is expressed only in fully differentiated cells and is absent in dedifferentiated variants or extinguished somatic hybrids. On the other hand, HNF-1β retains its expression in dedifferentiated variants and somatic cell hybrids. In hepatocarcinogenesis, the transcriptional alterations of HNF-1 can be demonstrated by the reverse transcription–polymerase chain reaction (RT–PCR) and the RNA protection assay, revealing that the HNF-1α:HNF-1β ratio is higher in well differentiated human hepatocellular carcinoma and lower in poorly differentiated human hepatocellular carcinoma, the latter being lower by 20–30%; in addition, HNF-1β mRNA remains unchanged in mouse liver tumours. The concentration of HNF-1α protein is also reduced in rat liver tumours and HNF-1 binding activity is reduced by lowered concentrations of HNF-1α protein in the transition from well differentiated to poorly differentiated human hepatocellular carcinoma. Both HNF-1α and HNF-1β are also found in other tissues including kidney, stomach, and intestine. The loss of HNF-1α has been shown during renal carcinogenesis, which is usually accompanied by dedifferentiation processes, including the loss of tissue specific gene expression. The promoter analysis of HNF-1α shows that in addition to HNF-1α autoregulation, HNF-4 is an essential activator of HNF-1α gene expression (fig 3).

**Hepatocyte nuclear factor 4 (HNF-4)**

HNF-4 is expressed in liver, kidney, and intestine in the adult, and activates a diverse set of liver genes such as transthyretin and α1-antitrypsin in early liver development, often interacting in synergy with adjacent binding factors. It belongs to the orphan steroid hormone nuclear receptor superfamily and is characterised by two highly conserved domains (fig 1). The DNA binding domain in the N-terminal half consists of two “zinc finger” motifs followed by an extensive C-terminal ligand binding domain, which performs a variety of functions, including transcription, ligand binding, and protein dimerisation. During mouse development, HNF-4 is expressed in the primary endoderm at 4.5 days and then restricted to the visceral endoderm from 5.5 days (fig 2). In cell genetic analysis of hepatocyte differentiation, HNF-4 shows a capability to transactivate endogenous HNF-1α and liver genes such as α1-antitrypsin and to induce redifferentiation of a dedifferentiated hepatoma cell line, H5, by stable transfection of exogenous HNF-4. H5 cells express neither HNF-1α nor HNF-4; however,
Liver enriched transcription factors in hepatocellular carcinoma

Initial activation factors?

? HNF-3β in primitive streak

? HNF-3α HNF-1β and HNF-4 in definitive endoderm

HNF-6 Expression of liver specific genes in liver primordium

HNF-1α in Liver organogenesis

Expression of C/EBPα and C/EBPβ

DBF in mature liver

Figure 2 Hierarchy of expression of liver enriched transcription factors (LETFs) in liver development. Boxes indicate the sequential developmental stages.

The presence of C/EBPα and HNF-3 has been revealed, implying that these factors alone are not sufficient to maintain expression of marker genes of hepatic differentiation. After HNF-4 transduction, cells express the previously silent HNF-1α and show activation of some hepatic proteins, but not of the endogenous HNF-4. This study implies a transcriptional hierarchy from HNF-4, to HNF-1α, to target genes (fig 3). Furthermore, the effects of HNF-4 expression extend to the re-establishment of differentiated hepatic epithelial cell morphology, such as inducing: (1) re-expression of E-cadherin, which is a prerequisite for the formation of the junctional complex, including desmosomes and tight junctions; (2) simple epithelial polarity; (3) slow, “pile up” growth habit. It is reported that a factor, “seven up”, which is the drosophila homologue of HNF-4, is involved in terminal fat cell differentiation in drosophila. These data indicate that the HNF-4 gene might be a tumour suppressor gene that plays important roles in differentiation and antiproliferation. The expression of HNF-4 decreases during hepatocarcinogenesis and a coordinate variation of HNF-4 and HNF-1α binding activity have been demonstrated. The upstream regulation of HNF-4 is still unclear, although HNF-4 transactivation is blocked by the intracellular accumulation of the HNF-1α protein (fig 3). Experiments have shown that the tyrosine or serine/threonine phosphorylations of HNF-4 negatively modulate its DNA binding and transactivation, and results suggest that HNF-4 might be regulated during cellular growth. The action of HNF-4, an orphan receptor, might be influenced by a potential ligand (fig 3).

C/EBP and DBP

C/EBP family members have a basic region and an adjacent leucine zipper (b/ZIP) structure, and they all bind to similar DNA sequences (fig 1). In this family, C/EBPα, C/EBPβ and C/EBPδ homodimerise or heterodimerise, and have different activation effects. They show strong similarity in their C-terminal sequences and divergence in their N-terminal transactivation domains. C/EBP proteins are expressed at a later stage in development (fig 2), and are found abundantly in liver and fat tissues, particularly in fully differentiated cells. Transduction of C/EBPα cDNA into pre-adipocytes results in the suppression of clonal cell growth and the promotion of adipogenic differentiation by inducing the expression of adipocyte specific genes such as 422 adipose P2 protein 422. Proof that the expression of C/EBPα proteins is required for adipocyte differentiation is also provided by using the antisense RNA approach. Constitutive antisense C/EBPα RNA impairs the expression of C/EBPα itself and of adipocyte specific genes. C/EBPα is also expressed strongly in the mature hepatocyte and stimulates the transcription of liver specific genes such as albumin, transthyretin, and α1-antitrypsin. C/EBPα transcription correlates closely with the passage of hepatocytes through the cell cycle of the regenerating liver. Proliferating hepatocytes in the partially hepatectomised liver demonstrate an abrupt reduction of C/EBPα mRNA within the first three hours of surgery, corresponding to the transition from the G0 to the G1 phase of the cell cycle. C/EBPα returns to a normal level by 72 hours, through a gradual increase after the S phase. All evidence indicates that C/EBPα regulates two aspects of terminal differentiation: induction of differentiation specific genes and cessation of mitotic growth. One mechanism of C/EBPα-induced growth arrest is that C/EBPα upregulates p21/SDI-1 (senescent cell derived inhibitor 1) gene expression and stabilises the post-translational p21/SDI-1 protein. p21/SDI-1 interacts with cyclin dependent kinases (CDKs), cyclins, and proliferating cell nuclear antigen (PCNA) to inhibit the activity of CDKs, and prevents DNA synthesis by inhibiting DNA polymerase. The C/EBP family plays an important role in both the inhibition of proliferative and its establishment, as well as in maintaining the differentiation of
In primary cultured rat hepatocytes that exhibit the transition between growth and differentiation, the constant expression of HNF-3α and HNF-3β is evident. HNF-3β expression increases. It is evident that HNF-3α and HNF-3β expression is necessary to maintain hepatic function. In addition, HNF-3α and HNF-3β also mediate the cell specific transcriptional control of liver specific genes that are important for the function of intestine, stomach, and pancreatic acinar cells from the foregut. HNF-3 family is expressed at earlier stages of embryonic development and is competent for differentiation even in dedifferentiated hepatocyte derived cells, when other liver transcription factors are inactive or absent. Thus, members of the HNF-3 gene family might be the primary factors in the hierarchy of the expression of liver enriched transcription factors in hepatogenesis (fig 2).

Hepatocyte nuclear factor 6 (HNF-6)

HNF-6 is a recently identified member of the family of liver enriched transcription factors. It contains two different DNA binding domains: the novel homeodomain and a domain homologous to the drosophila cut domain (fig 1). HNF-6 is expressed in tissues that originate from the endoderm cells lining foregut, liver, pancreas, nervous system, brain, and spinal cord. In the study of HNF-6 potential target genes during development, it has been found that there are HNF-6 binding sites in the promoter regions of HNF-3β and HNF-4, as well as in the liver specific genes transthyretin and α-FP. An overexpression of HNF-6 can stimulate the expression of HNF-3β and HNF-4 in cotransfection experiments. The onset of HNF-6 gene transcription is detected in the liver at embryonic day 9, correlating with the onset of liver differentiation (fig 2). In situ hybridisation studies of staged specific embryos demonstrate that the HNF-6 expression pattern and level are consistent with those of its target gene HNF-3β in liver and pancreas. HNF-6 expression disappears transiently from the liver between embryonic days 12.5 and 15, but is
Liver enriched transcription factors in hepatocellular carcinoma

Liver enriched transcription factors in hepatocellular carcinoma

The progress made to date suggests strongly that the combined control of liver specific gene transcription and a regulatory network among liver enriched transcription factors are involved in the fine tuning of hepatocyte differentiation. A variety of pathophysiological states in the liver are represented by abnormalities in hepatic protein synthesis. Many human hepatocellular carcinomas develop from precancerous states to carcinoma by multistep carcinogenesis, which is considered to be involved in fine tuning of hepatocyte differentiation. The liver enriched transcription factors are involved in the differentiation of less well differentiated hepatocellular carcinoma cells. These findings suggest that expression patterns diverge in pancreatic carcinoma into a well differentiated hepatoma cells.

Conclusion and perspectives

The liver enriched transcription factors is either not activated or expressed abundantly and colocalise in the exocrine acinar cells of the pancreas on day 18 of gestation and in the adult liver, although their expression patterns diverge in pancreatic epithelium. These findings suggest that HNF-6 might regulate hepatocyte specific genes and play a role in epithelial differentiation of the the gut endoderm by acting on HNF-3.

4 Withdrawn.
10 Costa RH, Lai E, Grayson DR, et al. The cell-specific enhancer of the mouse transthyretin (prealbumin) gene binds a common factor to one site and a liver-specific factor(s) at two other sites. Mol Cell Biol 1988;8:81–90.
21 Cereghini S, Yaniv M, Cortese R. Hepatocyte dedifferentiation and extinction is accompanied by a block in the synthesis of mRNA coding for the transcription factor HNF-1 LFBI, EMBO J 1990;9:2257–63.

Mol Path. First published as 10.1136/mp.52.1.19 on 1 February 1999. Downloaded from http://mp.bmj.com/ on October 31, 2023 by guest. Protected by copyright.
et al.
51 Costa RH, Grayson DR, Darnell JE. Multiple hepatocyte-
edependent programs in a variety of mouse fibroblastic cells.

34 Ktistaki E, Talianidis I. Modulation of hepatic gene expression (NF-IL6) is a member of the C/EBP family. EMBO J 1990;9:1897–906.
14 Lemaigre FP, Durviaux SM, Truong O, et al.
10 Llopis C, Suárez E,nce, development, regulation, genetic mapping offork head protein.
78 Peterson RS, Clevidence DE, Y e H, et al.
70 Ang SL, Weirds A, Wong D, et al. A transcriptional hierarch-

355 Rausa F, Samadani U, Y e H, et al.
80 Lemaigre FP, Durviaux SM, Truong O, et al.
74 DiPersio CM, Jackson DA, Zaret KS. The extracellular matrix protein PPAR-α in liver-enriched transcription factors.
75 Landry C, Clotman F, Hioki T, et al.

52 Mischoulon D, Rana B, Nancy L, et al.
84 Rim KS, Sakamoto M, Watanabe H, et al.
50 Costa RH, Grayson DR, Darnell JE. Multiple hepatocyte-
edependent programs in a variety of mouse fibroblastic cells.

81 Samadani U, Costa RH. The transcriptional activator hepatocyte nuclear factor 3 involves two transcriptional activation domains, one of which is novel and conserved with the Drosophila fork head homeotic gene fork head. Genes Dev 1991;5:416–27.
2553–60.
51 Costa RH, Grayson DR, Darnell JE. Multiple hepatocyte-
edependent programs in a variety of mouse fibroblastic cells.

14 Lemaigre FP, Durviaux SM, Truong O, et al.
109:1871–84.
72 Y e H, et al.
109:1871–84.
72 Y e H, et al.
109:1871–84.
72 Y e H, et al.