Co-ordinate activation of lipogenic enzymes in hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma is a very common neoplastic disease in countries where hepatitis viruses B and/or C are prevalent. Small hepatocellular carcinoma lesions detected by ultrasonography at an early stage are often hyperechoic because they are composed of well-differentiated cancer cells that are rich in triglyceride droplets. The triglyceride content of hepatocytes depends in part on the rate of lipogenesis. Key lipogenic enzymes, such as fatty acid synthase, are co-ordinately regulated at the transcriptional level. We therefore examined the mRNA expression of lipogenic enzymes in human hepatocellular carcinoma samples from 10 patients who had undergone surgical resection. All of the samples exhibited marked elevation of expression of mRNA for lipogenic enzymes, such as fatty acid synthase, acetyl-CoA carboxylase and ATP citrate lyase, compared with surrounding non-cancerous liver tissue. In contrast, the changes in mRNA expression of SREBP-1, a transcription factor that regulates a battery of lipogenic enzymes, did not show a consistent trend. In some cases where SREBP-1 was elevated, the main contributing isoform was SREBP-1c rather than SREBP-1a. Thus, lipogenic enzymes are markedly induced in hepatocellular carcinomas, and in some cases SREBP-1c is involved in this activation.
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1. Introduction

Hepatocellular carcinoma is a very common neoplastic disease in countries where hepatitis viruses B and/or C are prevalent. In Japan, 90% of hepatocellular carcinomas develop in patients with chronic liver diseases such as liver cirrhosis and chronic hepatitis [1]. Periodic imaging using ultrasonography in patients with chronic liver diseases has resulted in an increased rate of detection of small hepatocellular carcinomas of 2 cm diameter or less [2,3]. These are often hyperechoic lesions, which are composed of well-differentiated cancer cells rich in triglyceride droplets [4,5].

The triglyceride content of hepatocytes depends in part on the rate of fatty acid synthesis [6–8]. The fatty

acid biosynthetic pathway, composed of some 25 enzymes, is now elucidated in detail [9]. Among these enzymes, the following are of particular importance: fatty acid synthase (FAS), the main synthetic enzyme that catalyses the condensation of malonyl-CoA to produce the 16-carbon saturated fatty acid palmitate; acetyl-CoA carboxylase 1 (ACC1), which synthesises malonyl-CoA from acetyl-CoA; ATP citrate lyase (ACL), which synthesises acetyl-CoA from citrate provided through glycolysis. These enzymes are known to be co-ordinately induced when lipogenesis is needed, and this induction of enzymes is primarily at the transcriptional level [10]. It has therefore been presumed that these genes have a regulatory sequence in their promoter that interacts with common trans-acting factors such as sterol regulatory element-binding protein (SREBP)-1 [11,12].

SREBPs are members of the basic helix–loop–helix leucine zipper family of transcription factors that regulate fatty acid and cholesterol synthesis (reviewed in [13–15]). Unlike other members of the family, SREBPs are synthesised as precursors bound to the endoplasmic reticulum and nuclear envelope, and are released from the membrane into the nucleus as mature proteins by cleavage processes. To date, three isoforms of SREBP, -1a, -1c and -2, have been identified and characterised. The predominant SREBP-1 isoform in liver and adipose tissue is SREBP-1c. Whereas SREBP-2 plays a crucial role in regulation of cholesterol synthesis, SREBP-1c co-ordinately upregulates the transcription and expression of lipogenic enzymes such as FAS and ACL, leading to elevated triglyceride synthesis (reviewed in [12,16–18]). It is remarkable that SREBP-1c controls not only the synthetic rate of triglycerides but also their content in the liver [7,8,19].

As well-differentiated hepatocellular carcinomas often have abundant triglyceride droplets and SREBP-1c is a determinant of triglyceride content in hepatocytes, we hypothesised that the activation of SREBP-1 and thereby lipogenic enzymes might be involved in the early stage of cancer development in hepatocytes. To test this hypothesis, we examined the expression levels of SREBP-1 and its downstream lipogenic enzymes in surgically resected hepatocellular carcinomas.

2. Materials and methods

2.1. Patients

Ten consecutive patients who had undergone surgical resection of hepatocellular carcinoma at Tokyo University Hospital from January to June 1999 were included in the study. Informed consent was obtained from the patients. All cases were diagnosed as hepatocellular carcinoma by pathohistological examination.

2.2. Northern blotting

Small pieces of hepatocellular carcinoma and non-cancerous liver tissue were snap frozen in liquid nitrogen immediately after surgical resection. Necrotic portions, identified macroscopically, were excluded. Total RNA from these samples was extracted using TRIzol reagent (Invitrogen), and 10 μg RNA was run on a 1% agarose gel containing formaldehyde and transferred to a nylon membrane. The cDNA probe for SREBP-1 was cloned as described previously [6]. The cDNA probes for FAS, ACC1, ACL, stearoyl-CoA desaturase (SCD) and malic enzyme (ME) were prepared by cloning RT-PCR products from human liver total RNA into pGEM-T easy vectors (Promega). The PCR primers used for FAS were 5′-TCGGAGAACTTGCAG-GAGTT-3′ and 5′-CCAGCCTCAAGAACTGCAC-3′; for ACC1, 5′-CACATAAGGTCCAGCATGTCTG-3′ and 5′-CCAGGCACTGGCACATAGT-3′; for ACL, 5′-TCTCTCTGCAGCCATGTCG-3′ and 5′-GGAGA-TAAAACTGGCCAGAATTTC-3′; for SCD, 5′-TGCTGCAGGACGATATCTCTAG-3′ and 5′-CAG-GGCACTGACAAAATAGTAGAAT-3′; for ME, 5′-CATCTGAACTCTGACTTTGACAGGT-3′ and 5′-CCGTAGTCCAATGTAGAGTGGATC-3′. The probes were labelled with [α-32P]dCTP using Mega-prime DNA Labeling System kit (Amersham Biosciences). The membranes were hybridised with the radiolabelled probe in Rapid-hyb Buffer (Amersham Biosciences) at 65 °C. The membranes were washed in $0.1 \times$ SSC, 0.1% SDS at 65 °C. Blots were exposed to Kodak XAR-5 film and the BAS2000 Bio Imaging Analyzer (Fuji Photo Film, Tokyo, Japan).

2.3. Immunohistochemistry

The expression of FAS was immunohistochemically detected in formalin-fixed, paraffin-embedded sections as described previously [20]. As the primary antibody, rabbit polyclonal antibody to FAS (IBL, Fujioka, Japan) was used at 2 μg/ml IgG.

2.4. RNase protection assay

RNase protection assay was performed as described previously [21]. Briefly, after linearisation of the template plasmid DNA with *Hin*dIII, antisense RNA probes for SREBP-1a and -1c were transcribed with [α-32P]CTP using bacteriophage T7 RNA polymerase (MAXIscript *in vitro* transcription kit, Ambion) and purified by electrophoresis on 5% polyacrylamide gel. Then aliquots of probes and total RNA (10 μg) from each sample were mixed and subjected to the RNase protection assay using HybSpeed RPA kit (Ambion). After digestion with RNase A/T1, protected fragments were separated on 8 M urea/10% polyacrylamide gels.