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cDNA Sequences for Asialoglycoprotein Receptor from Human Fetal Liver

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Abstract : The asialoglycoprotein receptor (ASGPR) was the first described mammalian lectin that mediates the specific binding and internalization of galactose/N-acetylgalactosamine-terminating glycoproteins by hepatic parenchymal cells. H1 and H2 are known as essential subunits of the functional ASGPR. There were close similarities in ASGPR H2 subunits between cultured cell line HepG2 and normal human liver cells including identical sequences at both termini. It was therefore expected that there may be some similarities between the subunits from normal liver cells and fetal liver cells. The two subunits of human fetal liver ASGPR, designated *FL-H1* and *FL-H2*, were cloned from cDNA library by PCR and the sequences were compared with the known H1 and H2 sequences of HepG2, and the *H1* sequence of nomal human liver cells. The results showed that *FL-H1* was identical to *H1* of HepG2. Whereas *FL-H2* contains a 15-bp miniexon, but missing 57-bp at the near upstream from the membrane-spanning domain compared to *H2* of HepG2 and normal human liver cells indicating that *FL-H2* resulted from a differential splicing compared to HepG2 and normal liver cells.
Keywords : hepatitis B virus, HepG2, miniexon, polymerase chain reaction, preS1

The asialoglycoprotein receptor (ASGPR) was the first described mammalian lectin (Morell *et al.*, 1968) that mediates the specific binding and internalization of galactose/N-acetylgalactosamine-terminating glycoproteins by hepatic parenchymal cells (Ashwell and Harford, 1982; Stockert and Morell, 1983; Schwartz, 1984). Following ligand binding to this cell surface receptor, the receptor-ligand complex is internalized and transported by a series of membrane vesicles and tubules to an acidic sorting organelle where receptors and ligands dissociate (Geuze *et al.*, 1983). The receptor returns to the cell surface, while the ligand is transported to lysosomes where it is degraded (Hubbard *et al.*, 1979; Schwartz *et al.*, 1982; Ciechanover *et al.*, 1983). It has recently been suggested that the hepatic ASGPR is able to bind natural hepatitis B virus (HBV) specifically by its preS1 region and that this attachment might provide a clue to understand the hepatic endocytosis of HBV (Treichel *et al.*, 1994). The human hepatic ASGPR is constructed of two polypeptides of related amino acid sequences, namely H1 and H2 (Baenziger and Maynard, 1980; Lederkremer *et al.*, 1991). Both polypeptides span the membrane once, with a large carboxyl-terminal exoplasmic segment containing the galactose-binding sites. The cDNAs encoding the subunits of ASGPR have been previously cloned from rat (Halberg *et al.*, 1987) and human hepatoma cell line, HepG2 (Spiess *et al.*, 1985; Spiess and Lodish, 1985). In human liver cells, however, only subunit *H2* was cloned from normal liver cells (Paietta *et al.*, 1992). There were close similarities between ASGPR *H2* subunit from both cultured cell line HepG2 and from normal liver cells. Both termini of cloned subunits *H2* (Spiess and Lodish, 1985; Paietta *et al.*, 1992b) showed identical sequences. The same was reported also in several variants (Spiess and Lodish, 1985; Lederkremer and Lodish, 1991; Paietta *et al.*, 1992a). It was therefore expected that there may be some similarities between H2 subunits from normal liver cells and fetal liver cells. In an attempt to clone the subunits of ASGPR from human fetal liver cells, we obtained the genes of two subunits from human fetal liver cDNA library. Here we report the cloning of both subunits, nucleotide sequences and alignments of their cDNAs coding for the complete ORF.

Materials and Methods

Four oligo primers were designed and synthesized from the known sequences of the two subunits from

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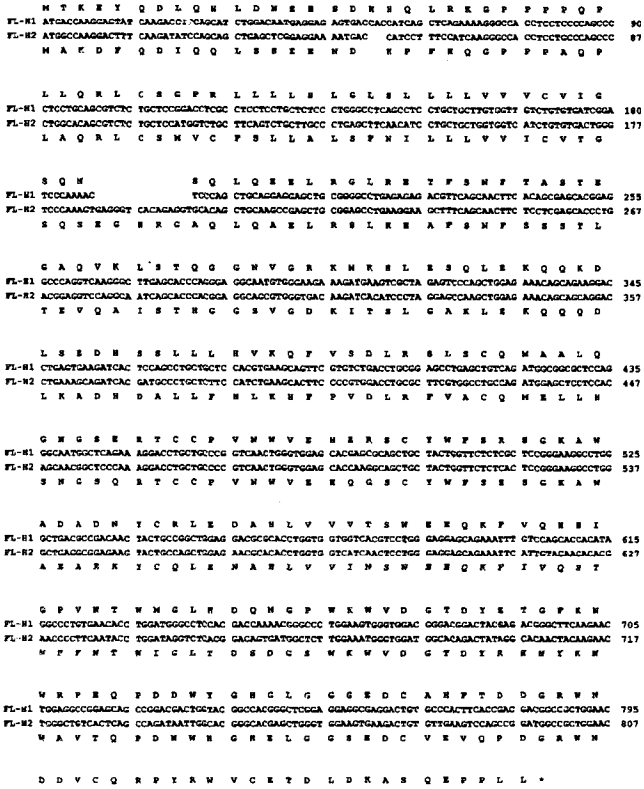

Fig. 1. Nucleotide sequences of the human fetal liver *FL-H1* and *FL-H2*-encoding cDNA ORF and the deduced amino acid sequences. The GenBank accession number for *FL-H2* cDNA is U97197.

HepG2 cells (Spiess *et al.*, 1985; Spiess and Lodish, 1985) in order to amplify the whole two subunits of ASGPR from human fetal liver cells by PCR. The derived oligo primers, H1-For (5'-ATG ACC AAG GAG TAT CAA GAC CTT-3') and H1-Rev (5'-TTA AAG GAG AGG TGG CTC CTG-3') were completely conserved sequence segments in the N-terminal and C-terminal regions of the subunit *H1* of ASGPR. Oligo primers, H2-For (5'-ATG GCC AAG GAC TTT CAA GAT ATC-3') and H2-Rev (5'-TCA GGC CAC CTC GCC GGT GGC-3') were also completely conserved segments in both termini of the subunit *H2* of ASGPR. Using a λgt11 human fetal liver 5'-stretch plus cDNA library purchased from Clontech Laboratories, Inc (California, USA) and the four designed primers, PCR amiplification of ASGPR subunits was performed by a thermal cycler (Perkin-Elmer, GeneAmp PCR system 2400). Twenty pmol of each oligonucleotide primer was used and 35 cycles, each consisting of a 30 s melting at 95°C, 2 min annealing at 55°C, and 1 min 30 s polymerization at 72°C were performed. Amplified PCR products were resolved on a 1.5% agarose gel and the bands obtained were eluted using a QIAquick gel Extraction Kit from Qiagen Inc (De Soto Avenue, USA). Purified PCR products were directly subcloned using a pGEM-T vector system obtained from Promega (Madison, USA). Plasmids containing each insert were sequenced by the dideoxy chain termination technique (Sanger *et al.*, 1977). The nucleotide sequences of two whole subunits of ASGPR from human fetal liver, designated *FL-H1* and *FL-H2*, were determined and amino acid sequences (Fig. 1) were deduced.

Results and Discussion

Two subunits of ASGPR in human fetal liver, FL-H1 and FL-H2, were cloned by PCR. The identified sequence of each subunit was compared with the known sequences of H1 and H2 from HepG2, and the result showed that FL-H1 was completely identical to that of HepG2 H1 (Spiess *et al.*, 1985), whereas FL-H2 shows some differences.

Near the upstream of the membrane-spanning segment, 57-bp intron (Paietta *et al.*, 1992), which was retained in *H2* of HepG2 (bp 67-125), is missing in *FL-H2* as in normal liver *H2*. However, *FL-H2* contains

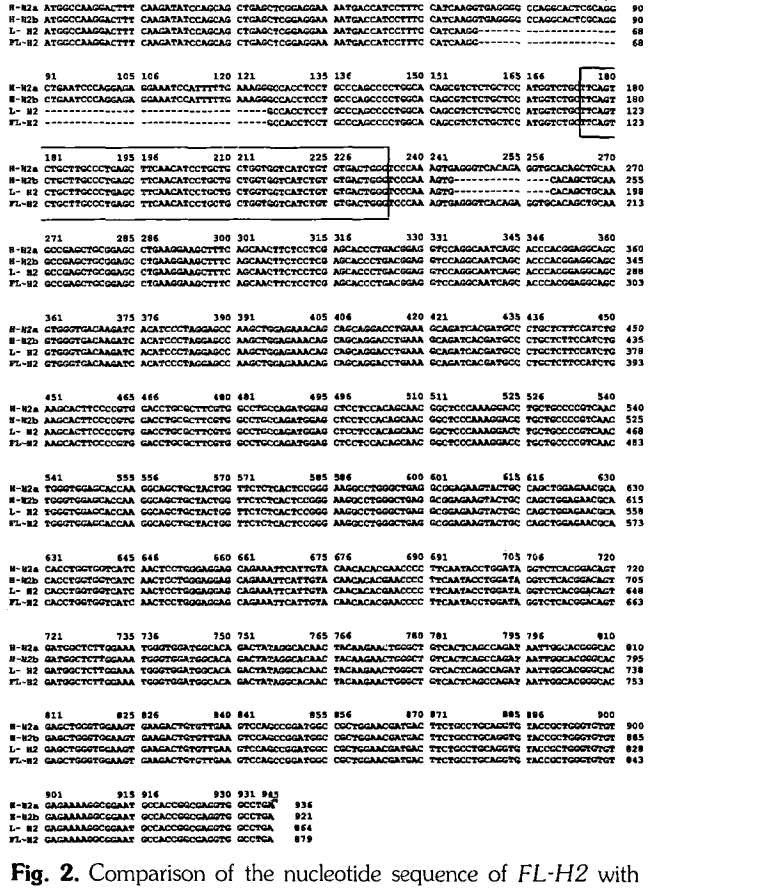

Fig. 2. Comparison of the nucleotide sequence of *FL-H2* with other *H2* subunit. The hydrophobic membrane-spanning segment is indicated by a box. Dashed lines indicate the spliced-out segments. Termination codon is indicated by an asterisk (*).

an extra 15-bp miniexon which is present in HepG2 but not in normal liver H2 (Paietta *et al.*, 1992). This miniexon is known to encode a positively charged five amino acid segment at the carboxyl-terminal side to the membrane-spanning segment in the exoplasmic domain (Spiess and Lodish, 1985). These comparisons are shown in Fig. 2. The nucleotide sequence of FL-H2 was deposited in the GenBank (accession number, U 97197).

The result therefore indicates that *FL-H2* may be generated from a single gene by another alternative splicing comparable to HepG2 *H2* (Lederkremer and Lodish, 1991). Although this 'another' alternative splicing and its exact biological function in liver cells could not be identified yet, this variant subunit, *FL-H2* could provide a clue to investigate why different forms of *H2* are present in cells.

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