Vol. 159, No. 1, 1989
February 28, 1989

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 312-316

Alternative Splicing of Human Insulin Receptor Messenger RNA

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Received January 9, 1989

SUMMARY The polymerase chain reaction has been used to examine alternative splicing of human insulin receptor (hINSR) mRNA. Alternative splicing of a 36 base pair exon, exon 11, generates hINSR transcripts encoding receptor isoforms which differ in sequence at the C-terminal end of the insulin-binding α-subunit. This process appears to be tissue-specific and, in addition, may be developmentally regulated.

The human insulin receptor (hINSR) is a heterotetrameric protein composed of two α- and two β-subunits (1-3). The extracellular α-subunit binds insulin and the membrane-spanning β-subunit is a tyrosine protein kinase. The isolation and characterization of human insulin receptor cDNA clones by Ullrich et al.(4) and Ebina et al.(5) indicated that the α- and β-subunits were derived by proteolytic cleavage of a common precursor. Interestingly, the predicted size of the human insulin proreceptor obtained by these two laboratories, 1343 and 1355 residues, respectively, differed due to the absence or presence of a 12 amino acid segment at the C-terminal end of the α-subunit (several single amino acid differences were also noted which could represent polymorphisms or sequencing/cloning artifacts). The elucidation of the exon-intron organization of the INSR gene indicated that this 12 amino acid region of the receptor was encoded by a small 36 base pair exon, exon 11 (6), and suggested that alternative splicing of exon 11 can generate different isoforms of the INSR. Using a combination of cDNA synthesis together with the polymerase chain reaction (PCR) (7), we have determined the relative abundance

0006-291X/89 $1.50

of transcripts encoding INSR isoforms in several human tissues and cell lines. The results indicate that the abundance of the two types of transcripts varies between different tissues as well as between normal tissues and corresponding tumor.

MATERIALS AND METHODS

General Methods. Standard procedures were carried out as described in Maniatis et al. (8). Human tissues were obtained with institutional approval. DNA sequencing was done by the dideoxynucleotide chain-termination procedure (9) after subcloning DNA fragments into the Sma I site of M13 mp19.

cDNA Synthesis and PCR. First strand cDNA was prepared using 5-20μg of total RNA, 100 fmoles of primer B (Fig. 1) and AMV reverse transcriptase. The second strand was synthesized using RNase H and E. coli polymerase I (10). The PCR was carried out for 25 cycles using Taq DNA polymerase (Perkin Elmer Cetus) together with 100 pmoles each of primer A and B (Fig. 1) essentially as recommended by Saiki et al. (7). Initial template denaturation was at 94°C for 3 minutes; a typical cycle included incubation for 2 minutes at 50°C (annealing), 3 minutes at 65°C (extension) and 1 minute at 94°C (denaturation).

After extraction with chloroform to remove the mineral oil, 20μl of the reaction was analyzed on a 2% NuSieve GTG agarose gel (FMC Bioproducts, Rockland, ME). The relative abundance of the amplified DNA fragments corresponding to the two types of hINSR transcripts was quantified by laser scanning densitometry of negatives of the ethidium bromide-stained agarose gel. The amplified DNA fragments were also isolated from the gel, subcloned into Sma I-digested M13 mp19 and sequenced.

RESULTS AND DISCUSSION

Amplification of the region of hINSR cDNA encoding amino acids 671-882 (Fig. 1) using PCR generated two fragments of 600 and 636 base pairs (bp) (Fig. 2). The sequences of these two fragments indicated that the 600 bp fragment was derived from hINSR transcripts lacking the region encoded by exon 11 (designated $hINSR^{-11}$ mRNA) and that the 636 bp fragment was from $hINSR^{+11}$ mRNA. Thus, as suggested from the cDNA cloning studies, alternative splicing of the primary transcript of the hINSR gene generates mRNAs encoding receptors whose sequences differ at the C-terminal end of the α-subunit (Fig. 1).

Comparison of the relative abundance of the two types of hINSR mRNAs in RNA preparations obtained from various tissues and cell lines indicates that the alternative splicing of exon 11 is regulated in a tissue-specific fashion (Fig. 2). The $hINSR^{-11}$ mRNA is the predominant transcript in adult brain and spleen and in IM-9 and HepG2 cells (lymphoblastoid and hepatoblastoma cell lines, respectively). In contrast, both transcripts are present in adult

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   671                    676
   Asn Gln Ser Glu Tyr Glu Asp
5'-AAC CAG AGT GAG TAT GAG GAT-3' ------------------------
           Primer A
                                  710
Phe Arg Lys Thr Phe Glu Asp Tyr Leu His Asn Val Val Phe Val
TTT AGG AAG ACG TTT GAG GAT TAC CTG CAC AAC GTG GTT TTC GTC

      Exon 11   720                                    730
Pro Arg Lys Thr Ser Ser Gly Thr Gly Ala Glu Asp Pro Arg Pro
CCC AGA AAA ACC TCT TCA GGC ACT GGT GCC GAG GAC CCT AGG CCA

                                  740
Ser Arg Lys Arg Arg Ser Leu Gly Asp Val Gly Asn Val Thr Val
TCT CGG AAA CGC AGG TCC CTT GGC GAT GTT GGG AAT GTG ACG GTG

                            876                    882
                            Lys His Phe Ala Leu Glu Arg
------------------------- AAG CAC TTC GCT CTG GAA CGG
                        3'-TTC GTG AAG CGA GAC CTT GCC-5'
                                 Primer B
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Figure 1. Partial sequence of the region of the hINSR cDNA amplified using PCR. The sequences of the two PCR primers are indicated. The region encoded by exon 11 is noted and underlined. The tetrabasic proreceptor processing site is boxed. The α-subunit of the INSR includes amino acids 1-731 and the β-subunit, residues 736-1355.

liver and kidney, adipose tissue obtained from an infant, and term placenta although their relative proportions vary (Fig. 2, Table 1). It is unknown if both transcripts are or can be synthesized by the same cell in tissues containing both forms of mRNA.

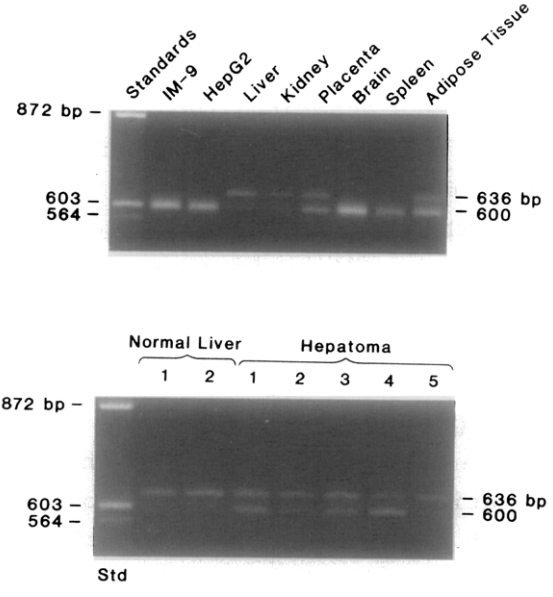

Figure 2. Agarose gel analysis of PCR products. The source of the RNA used for cDNA synthesis and PCR is indicated. The 636 bp PCR product is from $hINSR^{+11}$ mRNA and the 600 bp product from $hINSR^{-11}$ mRNA.

Table 1. Relative Abundance of Alternatively Spliced Insulin Receptor Transcripts

Tissue or Cell	hINSR^{+11} mRNA	hINSR^{-11} mRNA
IM-9	0%	100%
HepG2	0%	100%
Liver (Adult) 1	85%	15%
Liver (Adult) 2	88%	12%
Hepatoma 1	51%	49%
Hepatoma 2	58%	42%
Hepatoma 3	55%	45%
Hepatoma 4	33%	67%
Hepatoma 5	71%	29%
Kidney (Adult)	60%	40%
Placenta (Term)	45%	55%
Brain (Adult)	0%	100%
Spleen (Adult)	0%	100%
Adipose Tissue*	28%	72%

*Perirenal adipose tissue from a 9 month old female infant.

The relative abundance of hINSR^{+11} and $^{-11}$ mRNAs in two adult liver samples was almost identical, however it differed from that observed in five hepatocellular carcinomas and the HepG2 cell line (Table 1); the relative abundance in hepatomas 4 and 5 also differed from hepatomas 1-3. Although we have not been able to compare fetal and adult liver RNA preparations, we believe that the differences observed between adult liver and hepatomas suggest that the alternative splicing of hINSR exon 11 may also be developmentally regulated.

Alternative splicing is a common mechanism for generating protein isoforms and is frequently regulated in a tissue-specific or developmental fashion (11). Although common, in most instances, the functional differences among alternatively spliced isoforms are generally unknown. However, there are exceptions; alternative splicing can determine which peptide is synthesized by a specific cell (e.g. calcitonin/CGRP and preprotachykinin genes) (12, 13) as well as determine the subcellular localization of a protein (membrane-bound and secreted forms of immunoglobulin heavy chain) (14). Stable cell lines expressing both isoforms of recombinant hINSR have been described and both types of receptor are functionally active (15-17). However, the two isoforms of the hINSR have not been expressed in the same heterologous system and their properties rigorously compared especially with regard to stability or relative ability of insulin binding to activate the tyrosine protein kinase domain.

Structural and functional differences have been reported between insulin receptor preparations from different tissues (18,

19). Although some of these differences are likely a consequence of post-translational modification of the receptor, some could presumably be a consequence of the synthesis of INSR isoforms. The PCR will facilitate further studies of receptor biosynthesis at the molecular level and allow us to assess the role of alternative splicing in regulating ISNR function.

ACKNOWLEDGMENTS

We would like to thank Drs. E.T. Shapiro and A. Sakurai for providing some of the RNA preparations examined in this study, Dr. J. Whittaker for his helpful comments, and Ms. J. Dicig for her assistance in the preparation of this manuscript. This research was supported by the Howard Hughes Medical Institute.

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