One novel transcript of human hereditary multiple exostoses 2 (EXT2)*

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Abstract The encoding sequence of human hereditary multiple exostoses gene EXT2.1 is 30 bp longer than EXT2, and they differ in a sequence of 90 base pairs. In order to clarify EXT2.1 structure, this 90 bp sequence was analyzed with the Human Sequence Draft, a database provided by Celera Genomics. The result shows that EXT2.1 is a novel transcript of EXT2 gene, suggesting a rare event of alternative splicing.

Keywords: hereditary multiple exostoses, EXT2, EXT2.1, alternative splicing.

Hereditary multiple exostoses (HME) is an autosomal dominant disorder charaterized by multiple exostoses most commonly arising from juxtaepiphyseal region of the long bone[1]. EXT2 gene on Chromosome 11 was identified by Stickens et al., which is a tumor suppressor[2]. The protein encoded by EXT2 gene is an endoplamic reticulum-localized type Ⅱ transmembrane glycoprotein that possesses or is tightly associated with glycosyltransferase activities involved in the polymerization of heparan sulfate[3,4].

The alignment of EXT2 genes of *Drosophila melanogaster*, *Caenorhabditis elegans* and *Arabidopsis thaliana* shows a high homology among them, especially in the conserved carboxyterminal region. *Drosophila* homolog of EXT2, dubbed "toutvelu", is required for diffusion of the signaling protein hedgehog[5]. Rib-2, a *Caenorhabditis* homolog of human EXT2 gene, is an only gene of *C. elegans* which encodes α1, 4-N-acetyglucosaminyltransferase involved in the biosynthetic initiation and elongation of heparan sulfate[6]. The role of *Arabidopsis* homolog of EXT2 is unknown yet.

EXT2 gene, like EXT1 gene, is ubiquitously expressed. Stickens et al. have reported that EXT2 gene is differently expressed in bone and cartilage during mouse embryogenesis[7], which means that EXT2 gene is involved in the development of embryo.

Clines et al. found that the EXT2 gene consists of 14 exons (plus 2 alternative exons), covering an estimated 108kb, encoding a protein of 718 amino acids[8]. Deng et al. cloned the EXT2.1 from a human placenta cDNA library (Accession number: U72263), and found that EXT2.1 gene had a 90 bp insertion located at downstream of exon 7 of EXT2, which replaced 60 nucleotides at 5′ end of exon 8 of EXT2. This replacement resulted in a peptide of 728 aminao acids[9]. This finding strongly suggested that EXT2.1 is the result of a novel alternative splicing.

1 Materials and methods

The inserted sequence of 90 nucleotides was used for analysis, which is: CTCTTCATGGAACCAGTCAGGAGAGAGAACTGGTCAGCTGCTAATCACCAAATGAACTCCCTGATCTGGCCTAGGGAACAGTGGGATTCA. Using the Human Sequence Draft provided by Celera Genomics (http://www.celera.com), and the BLASTN software of National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov), the contig that covers EXT2 was obtained.

2 Results and discussion

A contig of genomic sequence, about 140kb in size, was found by searching in the database of Human Sequence Draft, which covers 12 exons of EXT2 gene, from exon 2 to exon 13. The homologous

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alignment was performed between the 90 bp sequence and the contig sequence by Pairwise Blast on NCBI web site. We found that the 90 bp sequence was located in intron 7 of EXT2 gene, about 17 kb away from the 3′ end of exon 7 and about 27 kb from the 5′ end of exon 8. The result also showed that EXT2.1 was a novel transcript, with one more exon than EXT2, that is spliced out from intron 7 of EXT2 (Fig. 1). This alternative splicing led to a 60 bp deletion at the 5′ end of exon 8, and the remaining part of exon 8 in EXT2 made up exon 9 of EXT2.1. A substitution of Adenine for Guanine in exon 11 of EXT2.1 gene caused the replacement of Glycine for Aspartate at the position of amino acid 578, which might be a new single nucleotide polymorphism (SNP).

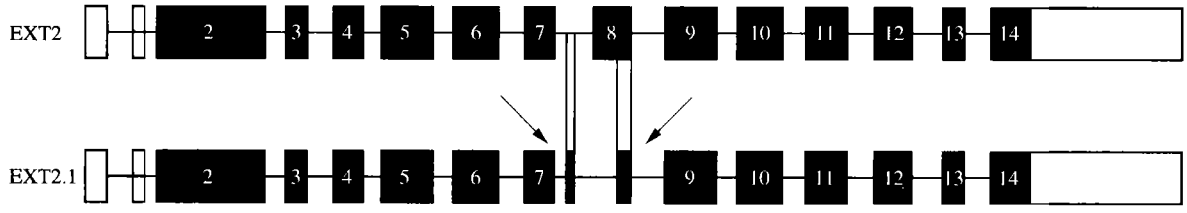

Fig. 1. Gene structures of EXT2 and EXT2.1. New formed exons are indicated by arrows.

It is known that there appear to be about 30000 ~ 40000 protein-coding genes in human genome. However, the structures and functions of the genes are more complicated due to the alternative splicing which will generate a large number of protein products. According to literature, it is common that one gene may generate more protein products by alternative splicing, in order to function in different tissues or different developmental stages.

EXT2.1 is a novel transcript of EXT2 gene, which was cloned from human placenta. The expression of EXT2.1, unlike EXT2, was only found in placenta[9]. According to the function of EXT2, we consider that EXT2.1 is involved in biosynthesis of heparan sulfate in placenta. As mentioned above, EXT2 gene is differently expressed during embryogenesis[7], so we may not exclude the possibility that EXT2.1 also functions in the development of embryo. More experiments need to be carried out to clarify the role of EXT2.1 in the placenta.

Generally, exons are spliced out from introns, or skipped by alternative splicing mechanism. Our observation is different, in that part of the sequence of intron 7 and part of exon 8 of EXT2 formed a new exon respectively after splicing. It seems a rare event of alternative splicing, but the reason is unknown yet.

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