SHORT COMMUNICATION

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Genomic organization and mapping of the human activin receptor type IIB (*hActR-IIB*) gene

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Abstract Activins, members of a family of proteins that includes transforming growth factor-beta (TGF-beta), are gonadal polypeptide hormones that stimulate secretion of follicle-stimulating hormone (FSH). During large-scale sequencing analysis of a 1.2-Mb fragment of human genomic DNA on 3p22–p21.3, we found the gene encoding activin receptor type IIB (*hActR-IIB*). Comparison of its reported cDNA sequence with this genomic sequence showed that the *hActR-IIB* gene consists of 11 exons and spans about 30 kb of genomic DNA.

Key words Genomic structure · Activin receptor · Large-scale DNA sequencing

Activins, members of a family of proteins that includes transforming growth factor-beta (TGF- β), are gonadal polypeptide hormones that stimulate secretion of follicle-stimulating hormone (FSH) in pituitary cells (Eto et al. 1987). Activins are widely expressed during murine development; they are highly conserved among vertebrates and may be involved in induction of mesoderm (Smith et al. 1990; Thomsen et al. 1990). In humans, expression of activin mRNA in the bone marrow and spleen, and secretion of this hormone by peripheral blood monocytes, suggest that activin acts as a natural regulator of erythropoiesis in the bone marrow (Eramaa et al. 1992).

The function of activin is mediated by binding to serine/threonine kinase receptors type I and type II (Massague

1992). The type II receptor has two homologues, ActR-II and ActR-IIB; both are transmembrane proteins, each with an extracellular ligand-binding domain and an intracellular kinase domain related to serine/threonine kinases (Mathews and Vale 1991; Attisano et al. 1992). The human activin receptor type IIB (*hActR-IIB*) cDNA, first cloned by Hilden et al. in 1994, encodes 512 amino acids containing an extracellular ligand-binding domain, a hydrophobic transmembrane domain, and an intracellular serine/threonine kinase domain (Hilden et al. 1994).

During a DNA-sequencing analysis of nearly 685 kilobases of human chromosome 3p21.3, a fragment that includes the region that is homozygously deleted in a lung-cancer cell line, we detected five genes by cDNA screening combined with two computer programs, GRAIL2 (Xu et al. 1994) and HEXON (Solovyev et al. 1994). As four of them (*trans-Golgi p230*, *Integrin α RLC*, *HYA22*, *villin-like*) were removed by the homozygous deletion, we considered them to be candidates for a putative tumor suppressor gene, but we found no somatic mutations in any of the lung or renal-cell carcinomas we examined (Ishikawa et al. 1997). Then, we considered that the homozygous deletion might exert a positional effect by influencing expression of genes in the near vicinity. We therefore extended the DNA sequencing beyond the borders of the deletion and detected two genes; one encoded phospholipase C delta 1 (PLCD1) (Ishikawa et al. 1997). Here, we report the genomic structure of the other, *hActR-IIB*.

On the basis of our contig map, two cosmid clones (575 and 424) were considered to be located about 500 kb telomeric to the telomeric breakpoint of the homozygous deletion in the lung-cancer cell line. We digested these clones by sonication and subcloned 1.5- to 6.0-kb fragments into pBluescript II SK(-). We picked 300 subclones, digested the plasmid clones with *EcoRI* and *XhoI*, and electrophoresed the products on a 0.8% agarose gel to check the sizes of inserted fragments and to eliminate clones containing vector sequences by hybridization with [³²P]dCTP-labeled cosmid vector. Selected clones were sequenced at both ends with T3 and T7 dye terminator using an ABI 377 autosequencer. DNA sequences were assembled by means of the ABI "As-

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sembler" computer software. Gaps between the assembled segments were connected by direct-cosmid sequencing using primers designed from the end sequences of the assembled segments. Within the 52,985-bp sequence (GenBank accession no. AB008681) obtained from the two cosmid clones, the GRAIL2 program predicted 13 possible exons with an "excellent" score. Homology analyses of these computer-predicted exons by means of the FASTA program revealed

that 9 of the fragments were identical to parts of the cDNA encoding human activin receptor type IIB (hActR-IIB). A comparison of genomic DNA sequences with the cDNA sequence revealed that this gene consisted of 11 exons and spanned about 30 kb (Fig. 1). The exon containing the initiation codon was contained in cosmid 575; the other 10 exons were contained in cosmid 424. Sequences of the exon-intron boundaries are given in Table 1.

Table 1 Exon – intron boundary sequence of *hActR-IIB* gene

Exon number	Exon length (bp)	cDNA position	Splice acceptor	Splice donor	Intron number	Intron length (bp)
1	56	1–56		CTGTGCGCCG gtaagaactg	1	22 922
2	208	57–264	tggggcacag GCTCTGGGCG	GCTACGATAG gtacccaag	2	366
3	110	265–374	gtgcctccag GCAGGAGTGT	GGCCGGAAG gtaagggggc	3	170
4	152	375–526	tgtcccccag TCACGTACGA	CATCCATGAG gtgagacagt	4	82
5	144	527–670	tctcccccag GACCCTGGGC	CCCACTCCAG gtgagtgtt	5	609
6	144	671–814	ccggcctcag GACAAGCAGT	CCATGACAAG gtgagccaca	6	407
7	149	815–963	cttctctcag GGCTCCCTCA	TTGCCACAG gtacctgggt	7	1 524
8	115	964–1078	gtttccacag GGACTTTAAA	CCACGGACAG gtaacaggcc	8	742
9	139	1079–1217	cctcacatag GTAGGCACGA	GCTGCAGACG gtaagtagga	9	95
10	131	1218–1348	tttctctcag GACCCGTGGA	GAAACACCCG gtaaggggcc	10	577
11	239	1349–1587	tgctgcttag GGCCTGGCCC			

Accession number of *hActR-IIB* cDNA sequence is X77533.

Fig. 1 Location of the *hActR-IIB* gene and the two cosmid clones (575,424) relative to the five genes known to lie within the 1.2-Mb region at chromosome 3p22–p21.3 that is homozygously deleted in a lung-cancer cell line. The genomic structure of *hActR-IIB* is represented schematically; exons and introns are denoted by *boxes* and *horizontal lines*, respectively. *Filled* portions of the boxes are coding regions, and *open* areas are untranslated. *Arrows* indicate the transcriptional direction of each gene. *PLCD1*, phospholipase C delta 1

Fig. 2 Chromosomal mapping of the *hActR-IIB* gene. **a** Metaphase chromosomes stained with propidium iodide show twin-spot signals on the short arm of chromosome 3 (indicated by *arrows*). **b** G-band pattern of the same metaphase chromosomes, delineated through a UV-2A filter (Nikon), indicates that the *hActR-IIB* gene hybridized to chromosome 3p22–21.3

To confirm the localization of this gene, we performed fluorescence in situ hybridization (FISH) (Inazawa et al. 1993). Cosmid clone 424 was labeled with biotin-16-dUTP by nick-translation and hybridized to denatured metaphase chromosomes prepared by the thymidine synchronization/bromodeoxyuridine release technique for the delineation of G-banding patterns. As we expected, the hybridization signals were seen specifically on chromosomal band 3p22–21.3 (Fig. 2).

The region where we have been performing sequencing analysis, part of which is homozygously deleted in a lung-cancer cell line, shows frequent loss of heterozygosity in carcinomas of the lung, uterus, esophagus, or kidney (Yamakawa et al. 1991; Yokoyama et al. 1992; Hibi et al. 1992). Activin reportedly induces terminal differentiation in K562 cells and limits their proliferation (Yu et al. 1987), and a related protein, α -inhibin, is known to act as a tumor-suppressor gene in mice (Matzuk et al. 1992). However, their receptors may or may not have similar functions.

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