# Loss of a consensus heparin binding site by alternative splicing of latent transforming growth factor-β binding protein-1

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**Abstract Latent transforming growth factor-β binding protein-1 (LTBP-1), plays an important role in controlling localisation and activation of transforming growth factor-β (TGF-β). We show that alternative splicing generates a form of mRNA which lacks bases 1277–1435 (termed LTBP-1Δ53). The 53 amino acids encoded by these bases include the eighth cysteine of the first cysteine repeat and a consensus heparin binding sequence. Sequencing of genomic clones showed that alternative splicing resulted from the use of an intra-exonic 3′ splice acceptor site. The loss of the heparin binding site implies that LTBP-1Δ53 will bind to the extracellular matrix less efficiently than LTBP-1.**



*Key words:* Latent transforming growth factor-β binding protein; Heparin binding; Cysteine rich repeat; Alternative splicing

## 1. Introduction

There are numerous reports showing a role for TGF-β in a wide variety of normal cellular processes, including cell growth [1], cell differentiation [2–4], matrix biosynthesis [5], angiogenesis [6,7], bone formation [8,9] and embryonic development [10]. TGF-β has been shown to play a role in wound healing [11], fibrosis [12] and immunosuppression [13,14], and has also been implicated in atherosclerosis [15–17] and cancer [18–20]. There are three mammalian isoforms of TGF-β (TGF-β1 to TGF-β3) which are similar to each other ($>70\%$ amino acid homology) and highly conserved between species. The TGF-β isoforms are exported by cells in various latent complexes which lack any known biological activity. A small latent complex ($\sim 100$ kDa) consisting of the active TGF-β homodimer (25 kDa) is non-covalently associated with a disulfide-bonded homodimer of a latency associated peptide (LAP). LAP is the amino-terminal remnant of the TGF-β precursor and is sufficient to confer latency. Large latent TGF-β complexes are formed by the association of the small latent complex with a latent TGF-β binding protein (LTBP) to form a prototypical complex of 270 kDa. There are four LTBP genes (LTBP-1 to -4) which have all been cloned. LTBP-1 forms a large latent complex disulfide bonding to LAP [21,22]. Recently, an additional protein termed the latent TGF-β complexed protein-1 (LTCP-1) was also found to bind to the large latent TGF-β in CHO cell cultures [23].

Cell culture experiments suggest that LTBP-1 may play an important role for some types of cells in the assembly and rapid secretion of TGF-β1 [24] and have implicated LTBP-1 in the regulation of TGF-β activation [25,26]. Immunohistochemical and immunoelectron microscopy studies have localised LTBP to the extracellular matrix and it has also been suggested that LTBP is a structural component of the matrix [27–29].

LTBP-1 expressed in different cell types is heterogeneous in size ranging from 125 kDa in platelets [30] to 190 kDa in fibroblasts [31]. This heterogeneity may be accounted for, at least in part, by the existence of alternatively spliced short (LTBP-1S) and long (LTBP-1L) forms of LTBP-1 [32]. LTBP-1S has multiple EGF-like repeats, some of which contain consensus calcium binding sites, three eight cysteine repeats of a novel type, an RGD motif and an eight amino acid sequence homologous to the laminin B2 chain [31]. LTBP-1L contains additional 346 N-terminal amino acids with one additional EGF-like repeat [32].

LTBP-1 eight cysteine repeat regions are homologous to the cysteine repeats in fibrillin-1 which also contain eight cysteines. The NMR structure determined for one of the fibrillin cysteine repeats suggested that the eight cysteines form four pairs of stabilising disulfide bonds [33]. Mutations which replace cysteine residues in the cysteine repeats of fibrillin-1 occur in Marfan's syndrome [34–36]. Mutations in the EGF-like domains may also lead to Marfan's syndrome, possibly because the loss of calcium binding sites increases the susceptibility of fibrillin-1 to proteolysis [34,37]. Calcium binding has been shown to protect both fibrillin-1 and LTBP-1 against proteolysis [37,38].

Studies of truncated forms of LTBP-1S indicated that the amino acids 294 to 441 are required for the binding of LTBP-1 to the extracellular matrix [39]. LTBP-1L had a greater affinity for the extracellular matrix than LTBP-1S. Increased or decreased amounts of LTBP-1 associated with the extracellular matrix have been reported in various diseases, including prostate cancer [40], human chronic pancreatitis [41], colorectal adenomas [42], ovarian neoplasms [43], rheumatoid arthritis [44], muscular dystrophy [45], carcinoid heart disease [46], and glomerulosclerosis [47]. These changes in LTBP-1 amounts may result from either changes in protein production, protein stability or in the affinity of the protein for the extracellular matrix. Here we have examined whether there are alternatively spliced forms of LTBP-1 with sequence differences which might have a major effect on the ability of LTBP-1 to bind to the extracellular matrix.

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*Abbreviations:* TGF-β, transforming growth factor-β; LTBP, latent transforming growth factor-β binding protein; LR-PCR, long range polymerase chain reaction; EGF, epidermal growth factor; CHO, chinese hamster ovary; DEPC, diethyl pyrocarbonate; VEGF, vascular endothelial growth factor; PDGF, platelet derived growth factor


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## 2. Materials and methods

### 2.1. Reverse transcription (RT)-PCR and PCR

Total RNA was extracted from normal human breast (HBR) cells or rat tissues using RNeasy kit (Qiagen) according to the manufacturer's instructions. One μg of total RNA in 11 μl of DEPC treated water was denatured for 10 min at 65°C and then chilled on ice. To the denatured RNA was added 4 μl of 5×avian myelomavirus reverse transcriptase (AMV-RT) buffer (Promega), 0.5 μl (2 units) of AMV-RT (Promega), 2 μl of 2 mM dNTPs, 1 μl of 100 mM dithiothreitol, 0.5 μl of RNAsin (Promega) and 1 μl of random hexamer primers (Pharmacia). The samples were incubated at 42°C for 2 h and placed on ice to terminate the reaction. For PCR amplification, 5 μl of cDNA was mixed with 4 μl of 10×*Taq* Polymerase buffer (Pharmacia), 5 μl of 2 mM dNTPs, 2.5 μl of each primer (10 pmoles), and 21 μl of water and overlaid with 50 μl of mineral oil. The reaction was hot started by heating to 95°C for 90 s then reducing the temperature to 80°C and adding 10 μl, containing 1 μl of 10×*Taq* Polymerase buffer, 0.5 μl of (2.5 units) *Taq* Polymerase (Pharmacia), and 8.5 μl of water. The thermal cycling profile was 1 min at 95°C, 1 min at 60°C followed by 90 s at 72°C for 35 cycles. The PCR products were separated by agarose gel electrophoresis and visualised with ethidium bromide. Bands were purified using a gel extraction kit (Qiagen) and cloned into pGem-T-Easy (Promega). DNA sequencing was performed in ABI Prism automated DNA sequencer using the dye terminator procedure. Plasmid DNA was amplified as described above except that plasmid DNA was used in place of cDNA.

### 2.2. LR-PCR

Cosmid DNA was amplified using LA Taq PCR kit Ver. 2 (Takara). One μl of cosmid DNA (500 ng/50 μl), 1 μl of each primer (10 pmoles), 8 μl of dNTP (2.5 mM each) and 20 μl of water was heated to 97°C for 90 s, cooled to 80°C, and a start mixture containing 0.5 μl of LA Taq (2.5 units), 5 μl of 10×LA PCR Buffer II ($Mg^{2+}$ plus), and 14.5 μl of water, was added. The PCR parameters for long range amplifications were denaturation for 20 s at 98°C, annealing and extension for 20 min at 68°C for 14 cycles. For the following 16 cycles the conditions were the same except that the extension time was increased by 15 s each cycle.

### 2.3. Southern-blot analysis and library screening

DNA was prepared using a midi-prep kit (Qiagen). Following electrophoresis, DNA was denatured and transferred to nylon membranes (Hybond, Amersham) and u.v. cross-linked [48]. $^{32}$P-labeled probes were made by random priming using an oligonucleotide labeling kit (Pharmacia). The filters were hybridised to the probe at 60°C in 3×SSC (1×SSC contains 150 mM NaCl and 15 mM sodium citrate), dextran sulfate (5%), 10×Denhardt's solution (1×Denhardt's solution contains 0.02% Ficoll 400, 0.02% polyvinylpyrrolidone and 0.02% BSA), salmon sperm DNA (250 μg/ml), and SDS (0.1%) overnight. The filters were rinsed once and washed twice at 60°C for 30 min in 1×SSC/0.1% SDS, and exposed overnight to a PhosphorImager screen (Molecular Dynamics).

A human cosmid library for chromosome 2 (LL02NC02) obtained from the UK HGMP Resource Centre on stamped filters was screened by hybridisation to a $^{32}$P-labeled probe as described above.

Table 1
Primer sequences

| Primer | Sequence |
|---|---|
| A | CAAGGTGTATGCCCTAATGGTGAGTGTTTG |
| B | CTCACTGGCCATAAATCCTGCTGGGCAAATG |
| C | GGGAGAGGTGGAGGATGAGTACTTTTAG |
| D | GCCCACACTACAACAGCAGAGCTGCTTG |
| E | CAGTGTATGTACCCTCTGTCTGTTCACCT |
| F | GAAGGTCAGGGCCTCCACTGGCTCTTC |
| G | CGCAGGCCAATCCATCACCATGTAGGTAAAG |
| H | GGTAGGATCCGGCCCAAATCCTATTTTG |
| I | GGAAACTGTGGATGCAGATGAATAG |

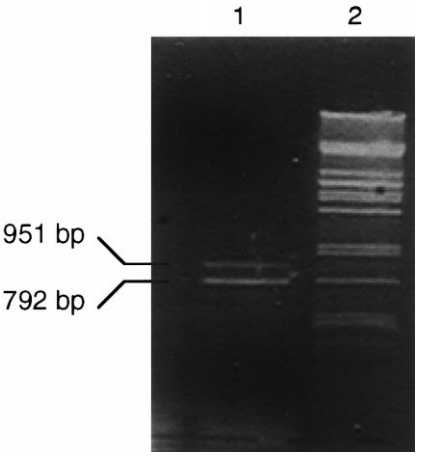


Fig. 1. Identification of two PCR products of LTBP-1 cDNA from human breast epithelial cells. Lane 1: Bands of 951 bp and 792 bp amplified using primers A and B (Table 1).

## 3. Results

RNA isolated from normal human breast epithelial cells in culture was reverse transcribed using random hexamers then amplified by PCR using primers A and B (Table 1). This reaction produced two products, one of 951 bp, the expected size for LTBP-1 and one 159 bp shorter (Figs. 1 and 2a). Both products were isolated from the gel, cloned into pGEM-T-Easy and sequenced. Comparison of the sequences with the EMBL database showed that both products were fragments of LTBP-1 cDNA but that the shorter fragment lacked 159 bp corresponding to nucleotides 1277 to 1435. This deletion corresponds to loss of a 53 amino acid sequence (Fig. 2b). Examination of the coding sequence showed that the region deleted in the LTBP-1 contained the eighth cysteine of the first cysteine repeat and a consensus heparin binding site (1330–1357, Fig. 2c). We have defined this short form of LTBP-1, LTBP-1Δ53.

To confirm the existence of alternative spliced forms the genomic structure of this region of LTBP-1 was defined. A cosmid library of chromosome 2 was screened using a probe corresponding to nucleotides 1009–1959. Three cosmids (AE25e19, AE68b4 and AE24g16) were subsequently isolated and restriction mapping showed that they contained inserts of approximately 42 kb, 15 kb and 12 kb, respectively. To determine the intron-exon boundaries in the spliced region of LTBP-1, cosmid AE25e19 was sequenced from primers A, C, D, E, F, G and H (Table 1). The sequences showed that bases 989 to 1111, 1112 to 1276 and 1277 to 1504 form discrete exons (Fig. 2b). LA-PCR analysis using primers A, C, D and E generated fragments of approximately 10 kb and 4 kb, corresponding to the introns separating exons A and B and B and C, respectively.

Alignment of the cDNA sequences from the two spliced forms of LTBP-1 with the genomic sequence showed that 159 bases absent in the short form of the gene did not exist as a discrete exon but were the 5′ part of exon C. This observation suggested that splicing was the result of alternative use of two exons, one a duplicate of 69 bases at the 3′ end of exon C or that exon C contained an internal splice junction. Sequence analysis showed that exon C contained a complete internal splice acceptor site corresponding to bases 1404 to

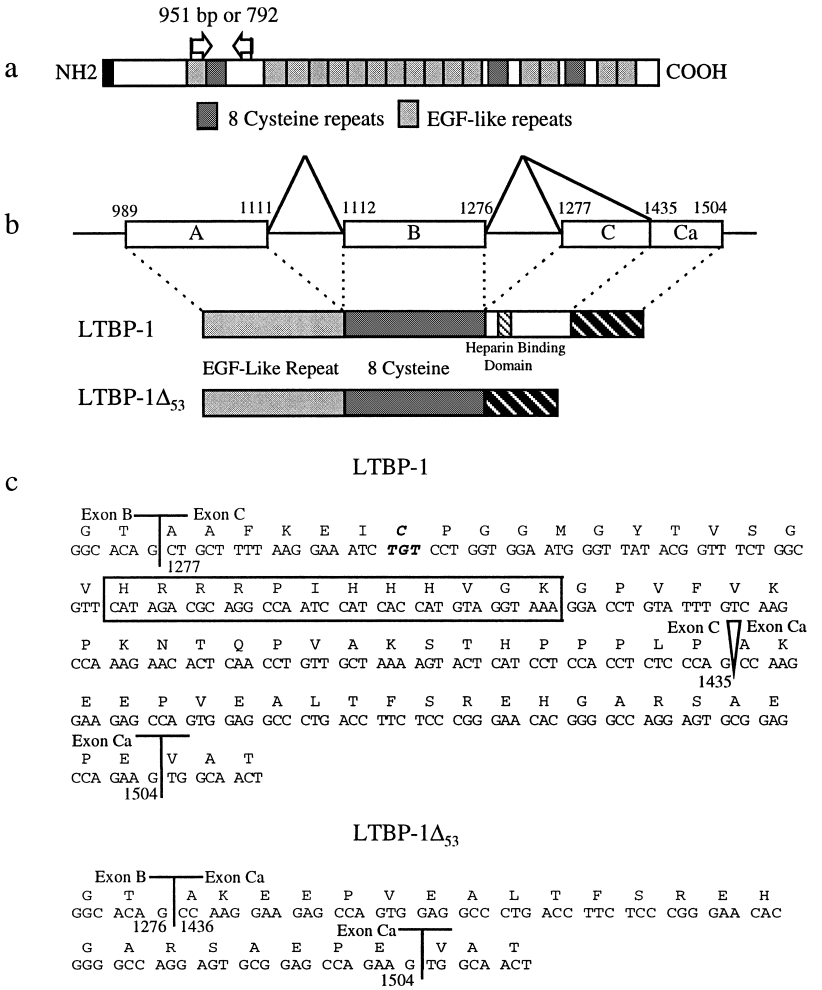


Fig. 2. LTBP-1. a: Structure of LTBP-1 protein showing the region encoded by the sequence amplified as in Fig. 1 marked by arrows. b: Three exons of LTBP-1: exon C undergoes alternative splicing with loss of a region encoding a putative heparin binding domain and the eighth cysteine of a cysteine repeat. c: DNA and amino acid sequence of the alternatively spliced region. The vertical arrow indicates the intra-exonic splice site. The cysteine residue encoded by exon C is italicised: the putative heparin binding domain is boxed. Both are deleted in the alternative LTBP-1Δ53 form. Numbers refer to the DNA sequence of Kanzaki et al. [31].

1435 of the LTBP-1 cDNA, the use of which would generate LTBP-1Δ53 (Fig. 2c, Fig. 3). LR-PCR using primers F and G with genomic DNA isolated from human lymphocytes generated a single product (132 bp), supporting the suggestion that the 3′ end of exon C has not been duplicated elsewhere in the gene and that two isoforms are generated by the use of alternative 3′ splice acceptor sites.

To determine any tissue specific variation in the splicing pattern and the approximate amounts of the two spliced forms, RNA was isolated from a series of rat tissues and reverse transcribed from random hexamers. LTBP-1 cDNA was amplified using primers A and I, the products were separated on a 1% agarose gel and Southern blotted. The products were hybridised to an LTBP-1 cDNA probe, washed and visualised by phosphorimaging. Most tissues expressed both forms of the LTBP-1 cDNA (Fig. 4). However, in the spleen only LTBP-1 and not LTBP-1Δ53 was detected. These data suggest that in most tissues there is competition between the two splice acceptor sites but that other tissue specific factors are used to express a preferred form in a subset of tissues.

| Exon | Intron/Exon sequence | Encoded feature |
|---|---|---|
| | Intron 5′ Exon 3′ Intron | |
| A: 123 bp | gaattgtgttttcctgcagATATTAATGA.....AGTTGTGTTCgtaagtaataatcacttttt | EGF-like domain |
| B: 165 bp | ctttatctctctgcttcagCTGATCCCCC.....CCAGGCACAGgtaagacatgcccagctgta | 8 Cysteine Repeat |
| C/Ca: 228/69 bp | tttcccatcccaaatctagCTGCTTTTAA...*.....GAGCCAGAAGgtgagagcggtaatggatca | Heparin Binding Domain |
| Splice consensus sequence | (y)$_n$n(c/t)agG.....AGgtaagt | |

* Internal 3' Acceptor site: CTCATCCTCCACCTCTCCCAG

Fig. 3. Intron/exon boundaries of exons A, B and C.

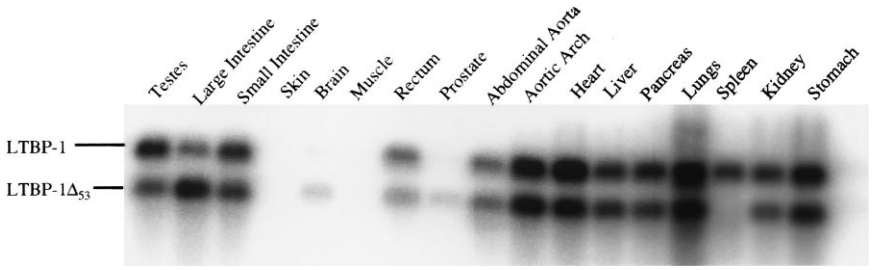


Fig. 4. Tissue distribution of LTBP-1 and LTBP-1Δ53. RNA was isolated from rat tissues, reverse transcribed, amplified using primers A and I and the product detected by Southern analysis using an LTBP-1 DNA probe.

## 4. Discussion

The data presented show that human and rat LTBP-1 can be alternatively spliced to give a new, shorter form defined here as LTBP-1Δ53 and that most normal rat tissues produce both of the splice variants. Furthermore, PCR analysis and sequencing of a cosmid containing a portion of the human LTBP-1 gene showed that the different spliced variants of LTBP-1 did not arise by splicing out an exon or alternative use of two similar exons, one with a 5′ or 3′ extension. The sequence showed that the differences arose from alternative use of two competing 3′ splice junctions. This form of splicing frequently occurs where there are two possible (C/T)AG close together as has been found for the porcine and rat pro-opiomelanocortin [49] and for an adipocyte serine protease [50]. However, for LTBP-1 there are two complete splice acceptor sites, one 159 bp downstream from the other and contained completely within the coding region of the long form of the exon. This type of splicing has been previously observed in fibronectin in which an exon contains a splice junction which can be used to generate a shorter form of the mRNA [51,52]. A recent abstract has described RT-PCR products from rat hepatocyte LTBP-1 which differed by 159 bp and may correspond to the alternatively spliced forms defined here, but the deleted sequence was not reported [53]. The relationship between the new alternatively spliced forms of LTBP-1 and the previously described LTBP-1S and LTBP-1L forms also remains to be defined.

The sequence deleted in LTBP-1Δ53 codes for 53 amino acids which do not include any consensus binding sites for calcium. Decreased resistance to proteolysis of the LTBP-1Δ53 form by the loss of calcium binding sites is therefore unlikely. However, the sequence deleted in LTBP-1Δ53 includes the eighth cysteine of the first of three cysteine repeats. These cysteine repeats are a protein motif which LTBP-1 shares with the other LTBP family members and with fibrillin. The first cysteine repeat in LTBP-1 is coded in two exons with seven of the eight cysteines in one exon and the eighth, C-terminal cysteine, encoded by the adjacent exon. This genomic structure is similar to that of the cysteine repeats in LTBP-2 [54] and in fibrillin in which four out of six repeats are encoded within two exons [55].

Structural studies of an isolated cysteine repeat from fibrillin have shown that the eight cysteines form four disulfide bonds, with the eighth cysteine bonded to the fifth cysteine. In Marfan's syndrome mutations have been detected in 19 distinct cysteine residues in fibrillin-1 [34]. Two of these mutations involve loss of the eighth cysteine of a repeat, one arising from a point mutation [36], the other resulting from exon skipping [56,57]. In LTBP-1Δ53 there is no neighbouring cysteine to replace the residue deleted by alternative splicing and it is therefore likely that the structure of this region of the protein will be altered.

A consensus heparin binding site (1330–1357) is also deleted in LTBP-1Δ53. Deletion mapping has shown that some or all of residues 294 to 441, which contain the consensus heparin binding site, are required for the binding of LTBP-1 to the extracellular matrix. Heparin binding sites are present in several growth factors and regulatory molecules (e.g. vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF)) and have been implicated in localising these molecules to the extracellular matrix. It may be noted that alternative splicing of VEGF mRNA also generates proteins with ($VEGF_{189}$) or without ($VEGF_{121}$, $VEGF_{165}$) heparin binding sites and the form of VEGF without a heparin binding site does not bind efficiently to the extracellular matrix [58]. The consensus heparin binding site in LTBP-1 may therefore serve the same role and it is plausible that LTBP-1Δ53, which lacks the consensus heparin binding site, will have a different profile of interactions with the extracellular matrix from LTBP-1. It remains to be determined whether expression of LTBP-1Δ53 is correlated with disease.

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## References

[1] Laiho, M., DeCaprio, J.A., Ludlow, J.W., Livingston, D.M. and Massagué, J. (1990) Cell 62, 175–185.
[2] Niles, R.M., Thompson, N.L. and Fenton, F. (1994) In Vitro Cell. Dev. Biol. 30A, 256–262.
[3] Cusella De Angelis, M.G., Molinari, S., Le Donne, A., Coletta, M., Vivarelli, E., Bouche, M., Molinaro, M., Ferrari, S. and Cossu, G. (1994) Development 120, 925–933.
[4] Filvaroff, E.H., Ebner, R. and Derynck, R. (1994) Development 120, 1085–1095.
[5] Bassols, A. and Massague, J. (1988) J. Biol. Chem. 263, 3039–3045.

[6] Dickson, M.C., Martin, J.S., Cousins, F.M., Kulkarni, A.B., Karlsson, S. and Akhurst, R.J. (1995) Development 121, 1845–1854.
[7] Pepper, M.S., Vassalli, J.D., Wilks, J.W., Schweigerer, L., Orci, L. and Montesano, R. (1994) J. Cell. Biochem. 55, 419–434.
[8] Finkelman, R.D., Eason, A.L., Rakijian, D.R., Tutundzhyan, Y. and Hardesty, R.A. (1994) Plast. Reconstr. Surg. 93, 732–738.
[9] Locklin, R.M., Williamson, M.C., Beresford, J.N., Triffitt, J.T. and Owen, M.E. (1995) Clin. Orthop. Relat. Res. 313, 27–35.
[10] Silberstein, G.B., Flanders, K.C., Roberts, A.B. and Daniel, C.W. (1992) Dev. Biol. 152, 354–362.
[11] Puolakkainen, P.A., Twardzik, D.R., Ranchalis, J.E., Pankey, S.C., Reed, M.J. and Gombotz, W.R. (1995) J. Surg. Res. 58, 321–329.
[12] Epstein, F.H. (1994) N. Engl. J. Med. Nov. 10, 1286–1292.
[13] Kehrl, J.H., Roberts, A.B., Wakefield, L.M., Jakowlew, S., Sporn, M.B. and Fauci, A.S. (1986) J. Immunol. 137, 3855–3860.
[14] Kehrl, J.H., Wakefield, L.M., Roberts, A.B., Jakowlew, S., Alvarez-Mon, M., Derynck, R., Sporn, M.B. and Fauci, A.S. (1986) J. Exp. Med. 163, 1037–1050.
[15] Grainger, D.J., Kemp, P.R., Metcalfe, J.C., Liu, A.C., Lawn, R.M., Williams, N.R., Grace, A.A., Schofield, P.M. and Chauhan, A. (1995) Nature Med. 1, 74–79.
[16] Grainger, D.J., Kirschenlohr, H.L., Metcalfe, J.C., Wissberg, P.L., Wade, D.P. and Lawn, R.M. (1993) Science 260, 1655–1658.
[17] Ross, R. (1993) Nature 362, 801–809.
[18] Kingsley, D.M. (1994) Genes Dev. 8, 133–146.
[19] Massague, J. (1996) Cell 85, 947–950.
[20] Moses, H.L., Yang, E.Y. and Pietenpol, J.A. (1990) Cell 63, 245–247.
[21] Gleizes, P.E., Beavis, R.C., Mazzieri, R., Shen, B. and Rifkin, D.B. (1996) J. Biol. Chem. 271, 29891–29896.
[22] Saharinen, J., Taipale, J. and Keski-Oja, J. (1996) EMBO J. 15, 245–253.
[23] Olofsson, A., Hellman, U., Ten Dijke, P., Grimsby, S., Ichijo, H., Moren, A., Miyazono, K. and Heldin, C.H. (1997) Biochem. J. 324, 427–434.
[24] Miyazono, K., Olofsson, A., Colosetti, P. and Heldin, C.H. (1991) EMBO J. 10, 1091–1101.
[25] Flaumenhaft, R., Abe, M., Sato, Y., Miyazono, K., Harpel, J., Heldin, C.H. and Rifkin, D.B. (1993) J. Cell Biol. 120, 995–1002.
[26] Sato, Y., Okada, F., Abe, M., Seguchi, T., Kuwano, M., Sato, S., Furuya, A., Hanai, N. and Tamaoki, T. (1993) J. Cell Biol. 123, 1249–1254.
[27] Taipale, J., Miyazono, K., Heldin, C.H. and Keski-Oja, J. (1994) J. Cell Biol. 124, 171–181.
[28] Dallas, S.L., Miyazono, K., Skerry, T.M., Mundy, G.R. and Bonewald, L.F. (1995) J. Cell Biol. 131, 539–549.
[29] Nakajima, Y., Miyazono, K., Kato, M., Takase, M., Yamagishi, T. and Nakamura, H. (1997) J. Cell Biol. 136, 193–204.
[30] Miyazono, K., Hellman, U., Wernstedt, C. and Heldin, C.H. (1988) J. Biol. Chem. 263, 6407–6415.
[31] Kanzaki, T., Olofsson, A., Moren, A., Wernstedt, C., Hellman, U., Miyazono, K., Claesson-Welsh, L. and Heldin, C.H. (1990) Cell 61, 1051–1061.
[32] Olofsson, A., Ichijo, H., Moren, A., ten Dijke, P., Miyazono, K. and Heldin, C.H. (1995) J. Biol. Chem. 270, 31294–31297.
[33] Yuan, X., Downing, A.K., Knott, V. and Handford, P.A. (1997) EMBO J. 16, 6659–6666.
[34] Dietz, H.C. and Pyeritz, R.E. (1995) Hum. Mol. Genet. 4, 1799–1809.
[35] Tynan, K., Comeau, K., Pearson, M., Wilgenbus, P., Levitt, D., Gasner, C., Berg, M.A., Miller, D.C. and Francke, U. (1993) Hum. Mol. Genet. 2, 1813–1821.
[36] Ades, L.C., Haan, E.A., Colley, A.F. and Richard, R.I. (1996) J. Med. Genet. 33, 665–671.
[37] Reinhardt, D.P., Ono, R.N. and Sakai, L.Y. (1997) J. Biol. Chem. 272, 1231–1236.
[38] Colosetti, P., Hellman, U., Heldin, C.H. and Miyazono, K. (1993) FEBS Lett. 320, 140–144.
[39] Nunes, I., Gleizes, P.E., Metz, C.N. and Rifkin, D.B. (1997) J. Cell Biol. 136, 1151–1163.
[40] Eklov, S., Funa, K., Nordgren, H., Olofsson, A., Kanzaki, T., Miyazono, K. and Nilsson, S. (1993) Cancer Res. 53, 3193–3197.
[41] van Laethem, J.L., Deviere, J., Resibois, A., Rickaert, F., Vertongen, P., Ohtani, H., Cremer, M., Miyazono, K. and Robberecht, P. (1995) Gastroenterology 108, 1873–1881.
[42] Van Laethem, J.L., Resibois, A., Adler, M., Peny, M.O., Ohtani, H. and Robberecht, P. (1996) Dig. Dis. Sci. 41, 1741–1748.
[43] Henriksen, R., Gobl, A., Wilander, E., Oberg, K., Miyazono, K. and Funa, K. (1995) Lab. Invest. 73, 213–220.
[44] Taketazu, F., Kato, M., Gobl, A., Ichijo, H., ten Dijke, P., Itoh, J., Kyogoku, M., Ronnelid, J., Miyazono, K. and Heldin, C.H. (1994) Lab. Invest. 70, 620–630.
[45] Yamazaki, M., Minota, S., Sakurai, H., Miyazono, K., Yamada, A., Kanazawa, I. and Kawai, M. (1994) Am. J. Pathol. 144, 221–226.
[46] Waltenberger, J., Lundin, L., Oberg, K., Wilander, E., Miyazono, K., Heldin, C.H. and Funa, K. (1993) Am. J. Pathol. 142, 71–78.
[47] Nakayama, M., Okuda, S., Tamaki, K., Shimizu, F., Kawachi, H., Ando, T., Yanagida, T. and Fujishima, M. (1997) Nephron 76, 82–89.
[48] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[49] Oates, E. and Herbert, E. (1984) J. Biol. Chem. 259, 7421–7425.
[50] Cook, K.S., Groves, D.L., Min, H.Y. and Spiegelman, B.M. (1985) Proc. Natl. Acad. Sci. USA 82, 6480–6484.
[51] Tamkun, J.W., Schwarzbauer, J.E. and Hynes, R.O. (1984) Proc. Natl. Acad. Sci. USA 81, 5140–5144.
[52] Schwarzbauer, J.E., Tamkun, J.W., Lemischka, I.R. and Hynes, R.O. Cell 35, 421–431.
[53] Gong, W.R., Roth, S., Michel, K. and Gressner, A.M. (1997) Hepatology 26, 401.
[54] Bashir, M.M., Han, M.D., Abrams, W.R., Tucker, T., Ma, R.I., Gibson, M., Ritty, T., Mecham, R. and Rosenbloom, J. (1996) Int. J. Biochem. Cell Biol. 28, 531–542.
[55] Pereira, L., D'Alessio, M., Ramirez, F., Lynch, J.R., Sykes, B., Pangilinan, T. and Bonadio, J. (1993) Hum. Mol. Genet. 2, 961–968.
[56] Liu, W., Qian, C. and Francke, U. (1997) Nature Genet. 16, 328–329.
[57] Dietz, H.C., Valle, D., Francomano, C.A., Kendzior, R.J., Pyeritz, R.E. and Cutting, G.R. (1993) Science 259, 680–683.
[58] Poltorak, Z., Cohen, T., Sivan, R., Kandelis, Y., Spira, G., Vlodavsky, I., Keshet, E. and Neufeld, G. (1997) J. Biol. Chem. 272, 7151–7158.