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SHORT COMMUNICATION

Yosuke Harada · Kouichi Ozaki · Mikio Suzuki
Tsutomu Fujiwara · Ei-ichi Takahashi
Yusuke Nakamura · Akira Tanigami

Complete cDNA sequence and genomic organization of a human pancreas-specific gene homologous to *Caenorhabditis elegans sel-1*

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Abstract We have isolated the complete cDNA of a human *SEL-1L* gene, termed *TSA305*, that is abundantly expressed only in the pancreas. The cDNA contained an open reading frame of 2382 nucleotides, encoding a deduced protein of 794 amino acids whose predicted sequence showed 46% identity and 64% similarity with SEL-1 of *Caenorhabditis elegans*. SEL-1 is thought to be a negative regulator of the NOTCH, LIN-12, and GLP-1 receptors, which are required for differentiation and maturation of cells as well as cell–cell interactions during development in *C. elegans*. The degree of homology among these proteins suggests that the *TSA305* gene product may be a member of the SEL-1 family and therefore involved in downregulation of mammalian Notch signaling. Direct sequencing revealed at least 20 coding exons in *TSA305*. We localized the gene to chromosome bands 14q24.3–q31 by radiation hybrid (RH) mapping and fluorescence *in situ* hybridization (FISH). The *IDDM11* locus has been mapped in this region, and *TSA305* may represent a candidate gene for predisposition in some families whose insulin-dependent diabetes is not linked to the HLA locus.

Key words Pancreas-specific gene · SEL-1 · SEL-1L · Notch signaling · *IDDM11* · 14q24.3–q31

Introduction

The *sel-1* gene of *Caenorhabditis elegans* encodes a predicted extracellular protein identified as an extragenic suppressor of a *lin-12* hypomorphic mutant (Grant and Greenwald 1996). The *C. elegans lin-12* gene encodes a member of the LIN-12/NOTCH receptor family (Greenwald and Rubin 1992; Artavanis-Tsakonas et al. 1995). The members of this family include *C. elegans* GLP-1 and *Drosophila* Notch. Two vertebrate homologs, murine *int-3* and human *TAN-1*, have been implicated in some cancers (Ellisen et al. 1991; Jhappan et al. 1992; Robbins et al. 1992). Abnormalities in genes that are expressed only in certain tissue(s) might lead to organ-specific diseases. For example, because insulin-dependent diabetes mellitus (IDDM) is caused by the destruction of the insulin-producing β-cells in the islets of Langerhans in the pancreas, defects in genes that regulate pancreatic islet development and insulin biosynthesis probably contribute to the pathogenesis of this disorder.

Among gastrointestinal malignancies, pancreatic cancers have the poorest prognoses, being the fourth or fifth leading cause of cancer-related deaths in Japan and Western countries (Poston et al. 1991). Determining the genes that are involved in such tissue-specific disorders may ultimately allow development of useful diagnostic tools and more effective therapies. The differential display technique (DD) provides a rapid and efficient way to isolate genes that are differently expressed among various tissues. We have isolated several human pancreas-specific genes in this way, and demonstrated that DD gives access to transcripts as rare as 1 in 1 million molecules of total mRNA (Ozaki et al. 1996, 1998). In the present study, a continuation of that effort, we identified a pancreas-specific transcript and subsequently cloned its full-length cDNA, which encoded a protein similar to SEL-1 of *C. elegans*. We mapped the pancreas-specific gene to the distal portion (q24.3–q31) of the long arm of human chromosome 14, a region containing one (*IDDM11*) of several loci associated with insulin-dependent diabetes mellitus. Toward exploring the regulatory role of this gene and identifying mutations that might affect its function with respect to diabetes and cancer, we determined all exon–intron junctions and analyzed its pattern of expression in normal human tissues.

Y. Harada · K. Ozaki · M. Suzuki · T. Fujiwara · E. Takahashi · A. Tanigami (✉)
Otsuka GEN Research Institute, Otsuka Pharmaceutical Co., Ltd., 463-10 Kagasuno, Kawauchi-cho, Tokushima 771-0192, Japan
Tel. +81-88-665-2888; Fax +81-88-637-1035
e-mail: atanigam@otsuka.gr.jp

Y. Nakamura
Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan

Materials and methods

Differential display

The differential display (DD) procedure was carried out essentially as described by Liang and Pardee (1992). Because the polymerase chain reaction (PCR) products contained plural bands of the same size, the reamplified products were electrophoresed on agarose gels containing a bisbenzimide–PEG (polyethylene glycol) conjugate, H.A.-Yellow (Hanse Analytik GmbH, Bremen, Germany); as this reagent specifically adheres to adenine/thymine (A/T) bases, target genes can be separated from background bands. The desired cDNA fragments were subcloned in the manner described previously (Ozaki et al. 1996). Nucleotide sequences were determined with an ABI 377 auto-sequencer (Applied Biosystems, Foster City, CA, USA).

Northern blot analysis

Human multiple-tissue Northern (MTN) blots I and II (Clontech, Palo Alto, CA, USA) were prehybridized and then hybridized with an α-[^{32}P]dCTP-labeled fragment (nucleotides 1–2382) of a cDNA (provisionally designated *TSA305*) that had been isolated through DD. We used the manufacturer's protocol with a random-labeling kit (BcaBEST Labeling Kit; Takara, Kyoto, Japan). Washed membranes were autoradiographed for 12 h at −80°C.

Screening of cDNA and sequencing

A human pancreatic cDNA library was constructed using oligo(dT_{16})+ random hexamer-primed pancreatic cDNA and Uni-ZAPXR (Stratagene, La Jolla, CA, USA). A total of 1×10^6 clones were screened with an α-[^{32}P]dCTP-labeled fragment (nucleotides 7514–7885) of *TSA305* cDNA. Positive clones were selected and their insert DNAs were excised *in vivo* in pBluescript II SK(−) (Stratagene) according to the supplier's recommendation. Sequencing was performed with an ABI 377 auto-sequencer.

Radiation hybrid mapping

Using primer 1 (5′-GGAGGTACTGCTGTGTAATG-3′) and primer 2 (5′-TCCACTCAACTTACATGAG-3′) generated from the 3′-noncoding region of *TSA305* cDNA, we analyzed a panel of 93 radiation hybrid (RH) clones (GeneBridge 4; Research Genetics, Huntsville, AL, USA) by PCR. Amplified products were separated according to size by electrophoresis on 1.5% agarose gels. Each hybrid clone was scored as positive or negative according to the presence or absence, respectively, of a 282-bp band. The data from the panel were analyzed by two-point maximum-likelihood analysis software, RHMAPPER, through the URL at http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl.

Cosmid, BAC screening, and genomic organization

To prepare a probe for fluorescence *in situ* hybridization (FISH) analysis, we isolated a cosmid (0202G02) containing sequences corresponding to the *TSA305* gene by screening a total of 153,600 cosmid clones by PCR (Watanabe et al. 1996), using primers 1 and 2 (above). Cycling conditions were 94°C for 2 min, followed by 35 cycles of denaturing at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 40 s. To determine genomic organization, a clone containing sequences corresponding to the 5′-portion of *TSA305* cDNA was isolated from the human BAC library (Research Genetics) by PCR screening using primer 3 (5′-GCGAAGGCGACAGCTCTA-3′) and primer 4 (5′-CCGAGGACGCCGAGGCCAA-3′) for 35 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 40 s. The exon–intron boundaries were defined by directly sequencing the BAC (184M20) and cosmid DNAs using appropriate primers for each exon. For the region not covered by the BAC and cosmid clones, we defined exon–intron junctions by sequencing PCR products that had been amplified from human genomic DNA using exonic primers flanking the introns.

Results

Differential display

To identify novel tissue-specific genes, we compared display patterns using mRNAs that had been isolated from human brain, lung, liver, pancreas, stomach, prostate, spleen, heart, kidney, thymus, placenta, testis, skeletal muscle, and lymph node. One primer combination (arbitrary primer 5′-CTTGATTGCC-3′ and 3′-anchored oligo-dT primer 5′-GT_{15}(A/G/C)A-3′) among the 116 primer sets we examined identified a pancreas-specific DNA fragment of *TSA305* (Fig. 1A).

Northern blot analysis with cloned fragments

To confirm the pattern observed in the differential display, we examined expression of *TSA305* in various adult human tissues. Northern blots revealed a single transcript of about 7.8 kb, specifically in pancreas. Although additional transcripts of 4.0, 3.5, 1.5, and 0.8 kb were detected in pancreas, only the 7.8-kb transcript appeared in other tissues, faintly but ubiquitously, after long exposure of the X-ray film (Fig. 1B).

Cloning and sequencing of full-length cDNA

By screening a human pancreas cDNA library (1×10^6 pfu) using *TSA305* as the probe, we identified about 100 positive clones. On the basis of this result, we estimated the abundance of transcript among total pancreatic mRNAs to be

0.01%. After checking the sizes of inserts in 20 candidate clones, the clone that carried the largest cDNA insert (about 3kb) was sequenced. To amplify the missing 5′-portion, we performed 5′-RACE experiments (5′-rapid amplification of cDNA ends (Frohman et al. 1988) several times with the 5′-AmpliFinder kit (Clontech). The assembled cDNA sequence contained 7885 nucleotides (GenBank accession number, AB020335), which included 2382 nucleotides of open reading frame encoding a protein of 794 amino acids with a calculated molecular weight of 88,750Da. Using the BLAST program to search public databases, we found 97% identity to the sequence of the "*SEL-1L*" gene previously reported by Biunno et al. (1997) under accession number U11037; the major difference was that our sequence contained a long 5′-portion upstream. The BLAST search also revealed significant similarity of our predicted amino acid sequence to SEL-1, the product of the *sel-1* gene of *C. elegans* (46% identity and 64% similarity; Fig. 2). Furthermore, this gene is highly homologous (92% identity; Fig. 2) to the deduced amino acid sequence of murine SEL-1L (mSEL-1L) reported by Donoviel et al. (1998). The PSORT program (Horton and Nakai 1996) revealed a hydrophobic signal sequence near the N-terminus of the deduced amino acid sequence, and two potential transmembrane domains near the C-terminus (Fig. 3).

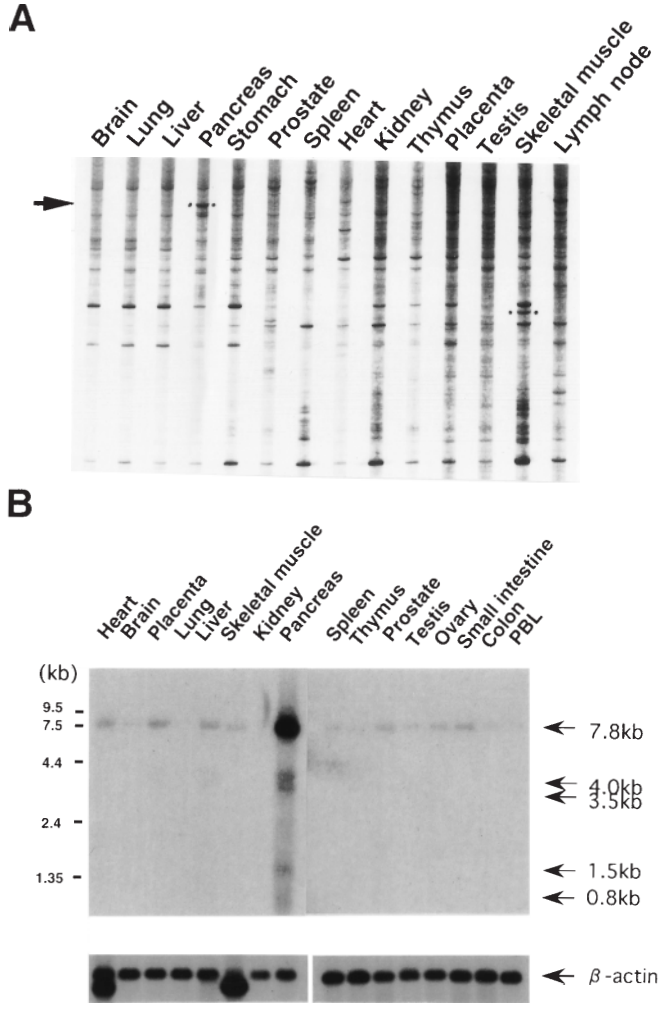

Fig. 1. **A** Differential display. The *arrow* indicates a pancreas-specific band (*TSA305*) among 14 normal human tissues. **B** Northern blotting of *TSA305*. Size markers are shown on the *left*, and the sizes of *TSA305* transcripts are indicated at *right*. Beta-actin expression was measured as control. PBL, peripheral blood lymphocytes

Chromosomal localization of *TSA305*

We localized *TSA305* by radiation hybrid (RH) mapping (Walter et al. 1994), using *TSA305*-specific primers to analyze a panel of 93RH clones by PCR to generate the following data: 00110 00100 00000 01000 00001 00000 00011 01100 01101 00000 00020 00001 00000 20000 00000 10121 000. We determined that the *TSA305* gene was most tightly linked to D14S76, a marker located at 14q24.3. We also performed FISH using cosmid clone 0202G02 as a probe. We examined 100 typical R-banded metaphase spreads, and observed doublet signals only at the q24.3–q31 bands of chromosome 14 (Fig. 4).

Genomic organization

We used BAC and cosmid clones, and PCR products amplified from human genomic DNA (see Materials and methods), to characterize the exon–intron boundaries of the *TSA305* gene (Table 1). The sequences of all splice sites conformed to the GT/AG rule (Breathnach and Chambon 1981). Our genomic analysis revealed that the gene contained at least 20 exons. Accession numbers of the *TSA305* sequence are AB024747, AB024748, AB024749, AB024750, AB024751, AB024752, AB024753, AB024754, AB024755, AB024756, AB024757, AB024758, AB024759, AB024760, AB024761, AB024762, and AB024763.

Discussion

We have described here the isolation and characterization of a human complete *SEL-1L* gene (*TSA305*), which is expressed most strongly in the pancreas. While this manuscript was being prepared, Biunno et al. (1997) reported the partial sequence of a human cDNA corresponding to *SEL-1*, its chromosomal localization, and its pattern of expression in pancreatic carcinomas. The primary sequence of their predicted product (SEL-1L) corresponded to the codons represented by amino acids 640–699 in Fig. 3. The expression profile and our RH and FISH mapping (14q24.3–q31) of the *TSA305* gene were concordant with previous results reported by the Biunno group. However, the amino acid sequence of SEL-1L seemed to lack about two-thirds of the N-terminal (amino acids 1–639) of TSA305 and part of the C-terminal region (amino acids

700–794).

The predicted gene product bears significant similarity to the *C. elegans* SEL-1 protein, which was identified as an extragenic suppressor of hypomorphic *lin-12* and *glp-1* mutants (Barth and Iva 1996). The *lin-12* gene of *C. elegans* encodes a member of the LIN-12/NOTCH family of putative receptor proteins; *lin-12* mutations affect binary cell-fate decisions in many different cell types and at many different developmental stages of the worm (Greenwald et al. 1983). SEL-1 may interact with LIN-12 and GLP-1 or their ligands to suppress the function of those proteins. Mammalian members of the LIN-12/NOTCH family include TAN-1, the human homolog of the *Drosophila* Notch, and murine INT-3. TAN-1 is often broken by chromosomal translocations in T-lymphoblastic neoplasms, and INT-3 transforms mammary epithelial cells in the mouse (Ellisen et al. 1991; Robbins et al. 1992). Although the function of the *TSA305* gene product is unknown, its similarity to SEL-1, which is capable only of modulating LIN-12 and GLP-1 activities, indicates that the human protein may play a significant role in development or progression of pancreatic carcinogenesis or normal pancreatic development.

The deduced TSA305 protein contained a potential hydrophobic sequence near the N-terminus and two transmembrane domains near the C-terminus (see Fig. 3). Hydrophobic amino-terminal regions are thought to be secretory signals (von Heijne 1986). However, the protein may be anchored at the plasma membrane by its C-terminal hydrophobic sequences. Five N-glycosylation sites (NX[S/T]) were scattered in the predicted extracellular region (amino acids 1–700). A type 2 fibronectin collagen-binding domain was found at position 127–168 in the predicted primary sequence of the *TSA305* gene product. This domain exhibits substantial homology to metalloproteinase-9 (MP-9) and matrix metalloproteinase-2 (MMP-2) of rabbit osteoclasts (Tezuka et al. 1994), to gelatinase of chicken embryo fibroblasts (Aimes et al. 1994), and to murine type IV collagenase (Reponen et al. 1992) (see Fig. 5). The sequence of this domain is conserved between human TSA305 and its murine homolog, but not in SEL-1 (see Fig. 2).

Proteolytic degradation of various constituents of the extracellular matrix and basement membranes plays an important role in tissue-restructuring processes such as cell migration, morphogenesis, wound healing, angiogenesis, and tumor invasion (Liotta et al. 1991). Type IV collagen is the major structural component of basement membranes (Martin et al. 1988). Therefore the collagenolytic activity of type IV collagenases such as MMP-9 and gelatinase B is viewed as a critical component of the metastatic process. However, as TSA305 does not retain the catalytic domain present in type IV collagenases, its collagen-binding domain may function simply through binding to the surrounding extracellular matrix.

According to the results of our Northern blot analysis, a 7.8-kb transcript of *TSA305* was present predominantly in pancreas, although it appeared in all human tissues examined after long exposure of the X-ray film. This result suggests that the principal role of TSA305 may be specific to the pancreas, especially because several smaller transcripts (4.0, 3.5, 1.5, and 0.8 kb) were detected only in pancreatic mRNA. When we used a probe constituting approximately 3 kb of the 3′-region, Northern analysis revealed only a single 7.8-kb transcript (data not shown). Therefore the smaller transcripts may be created by alternative splicing in the 3′-nontranslated regions, or they may be derived from unknown genes that are highly homologous to *TSA305*.

Our mapping experiments localized *TSA305* to chromosome bands 14q24.3–q31, where variant allele(s) responsible for one type of insulin-dependent diabetes mellitus (*IDDM11*) have been mapped. Significant linkage heterogeneity between HLA-defined subsets of families has suggested that *IDDM11* may be an important susceptibility

Table 1. Exon/intron junction sequences of the *TSA305* gene

Exon	Splice acceptor	Splice donor	Exon length (bp)
1		GCGTCCTCGG/**gt**cagtatcc	>70
2	tcctttgc**ag**/ATGAAGAAGG	AGATTCCAAG/**gt**atgtacta	38
3	atattctc**ag**/ACTACTTTGA	CGGAAACCAG/**gt**agtctgga	232
4	gtcattcc**ag**/CTTTGACCGC	TTTTGTGAAA/**gt**aagtattg	168
5	ttgtttttt**ag**/CTGAAGAAGA	AAAAAAGAGA/**gt**aggtagca	106
6	acatttga**ag**/AGCATATCGG	GGGACAGACT/**gt**aagtacat	163
7	tctatttc**ag**/GCTCTTGGCT	TCAGGCAAAG/**gt**aatactat	54
8	tctttcac**ag**/GCTCTTGTAT	CATGGTTTTG/**gt**aagtagac	60
9	tgttttac**ag**/GGTTACAGAT	GCCAATCATG/**gt**atctatgt	83
10	tctatttc**ag**/TTGCTAGTGA	ACAAGCACAG/**gt**acgtgttt	155
11	tcattttc**ag**/GTTGGTCTTG	GAATCATCAG/**gt**aactaccc	57
12	tttctcat**ag**/AGAGCATTTG	TTTGGGAAAG/**gt**actgtaca	69
13	tgttttcc**ag**/ATGTATTCGG	TGCTGACATG/**gt**aaggcttt	78
14	tgctttgc**ag**/GGCAACCCAG	ATGTACTATA/**gt**aagtaaca	151
15	tttcttac**ag**/ATGGCATTGG	TGCAGTGGAG/**gt**aaggtctt	149
16	cctcacac**ag**/TTGTTTAAGA	CTTGATCAGA/**gt**aaggttca	166
17	tttcctgt**ag**/GAGAAGCAAG	GCCTCTCAAG/**gt**aatgataa	75
18	tttgttta**ag**/GCTATACTGT	CATTAAACAG/**gt**gagtgtgg	173
19	tgtattat**ag**/GATATTCACC	GGAAACAAAC/**gt**aagtggtc	129
20	tttcatta**ag**/ATTCGAGATA		>207

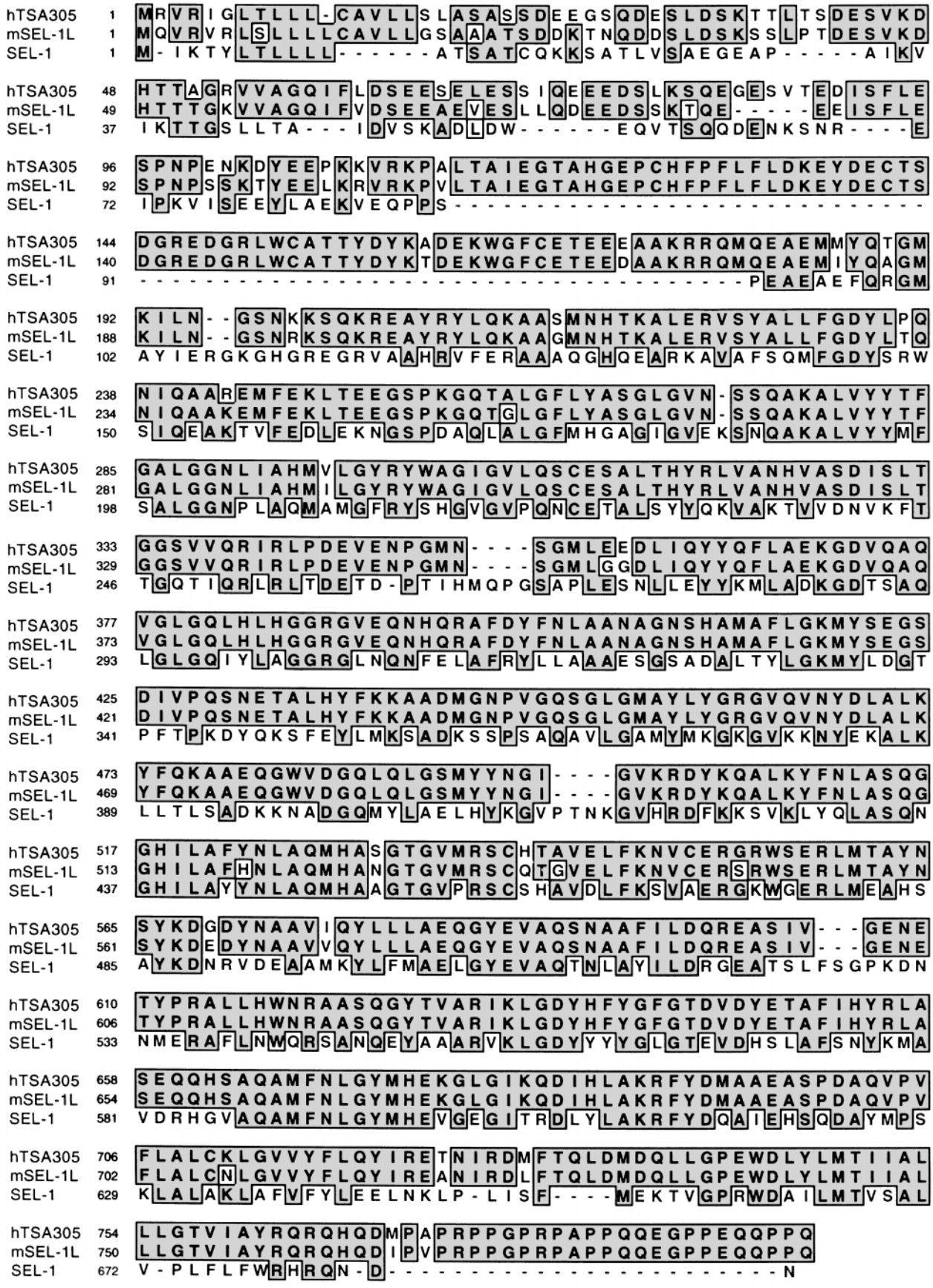

Fig. 2. Comparison of predicted amino acid sequences of human TSA305, murine SEL-1L, and *Caenorhabditis elegans* SEL-1. Identities are indicated by *boxed letters*

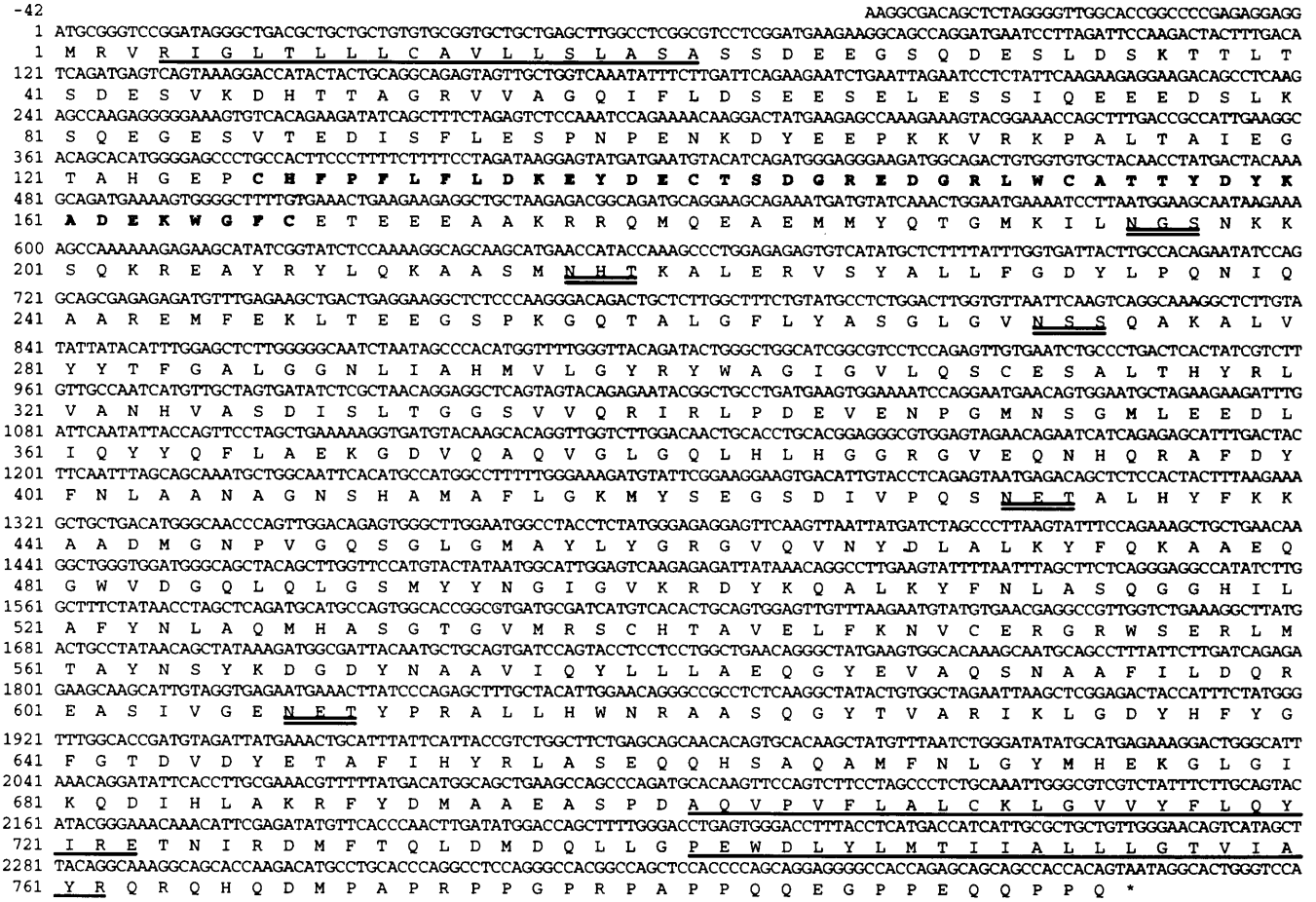

Fig. 3. Nucleotide and amino acid sequences of TSA305. Numbering begins at the position of the translation-initiation codon; the termination codon (TAA) is indicated by an *asterisk*. A hydrophobic signal sequence at the N-terminus and transmembrane regions at the C-terminus are *underlined*, and potential N-glycosylation sites are *double-underlined*. Amino acids of the collagen-binding domain are indicated by **bold type**. The nucleotide sequence will appear in the DDBJ, GenBank, and EMBL databases with accession number AB020335

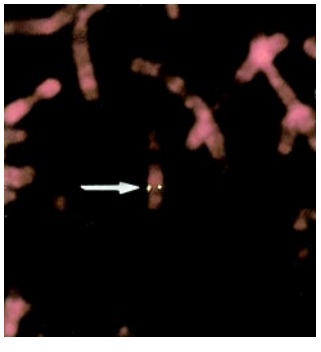

Fig. 4. Localization of *TSA305* on metaphase chromosomes by FISH. The *arrow* indicates twin signals at 14q24.3–q31

locus in diabetic families that lack a strong predisposition in the HLA region (Field et al. 1996). We are evaluating the intron–exon boundary sequences and pursuing functional analysis of TSA305 as steps toward discovering whether patients with IDDM or cancer carry mutations of the *TSA305* gene.

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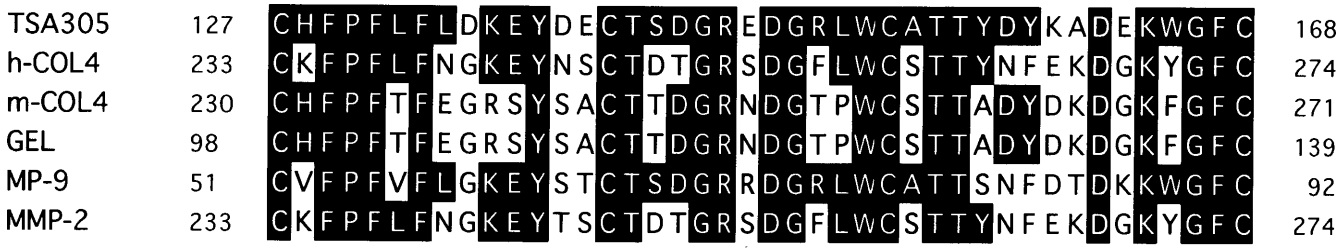

Fig. 5. The predicted amino acid sequence of the collagen-binding domain of human TSA305, aligned with the sequences of human type IV collagenase (h-COL4), a murine type IV collagenase (m-COL4), chicken gelatinase (GEL), rabbit metalloproteinase-9 (MP-9), and rabbit matrix metalloproteinase-2 (MMP-2). Conserved amino acids are highlighted in *black*

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