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Cloning, Structural Organization and Chromosomal Mapping of Rat Costimulatory Molecule 4-1BBL

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Abstract 4-1BBL (TNFSF9) is a member of the tumor necrosis factor (TNF) ligand superfamily, which is expressed on some activated antigen presenting cells and B cells. We isolated a rat cDNA clone encoding the rat homologue of the human 4-1BBL (GenBank accession No. AY259541). The deduced rat 4-1BBL protein, consisting of 308 amino acids with a molecular weight of 33,469 Da, was a typical type II transmembrane glycoprotein, the same as human and murine 4-1BBL. "SDAA" in the cytoplasmic domain of rat 4-1BBL was deduced to act as the phosphorylation site for casein kinase I ("SXXS" motif), which is present in the cytoplasmic domains of human and murine 4-1BBL, and all other TNF ligand family members known to utilize reverse signaling. The two introns of 4-1BBL were also cloned (GenBank accession No. AY332409). Rat 4-1BBL is much more homologous with murine 4-1BBL than with human 4-1BBL, in both nucleotide and amino acid sequences. Rat 4-1BBL was expressed in all tested tissues: brain, lung, colon, liver, thymus, testicle, kidney, adrenal, stomach, spleen and heart. The chromosomal location of rat *4-1BBL* was first identified by bioinformatics, then by fluorescence *in situ* hybridization at 9q11 (GenBank accession UniGene No. Rn.46783). Rat, murine and human *4-1BBL* genes are evolved from a common gene. The identification and characterization of the rat counterpart of human 4-1BBL will facilitate studies of the biological function of this molecule.

Key words TNF superfamily; rat; 4-1BBL; cloning; mapping

Over the past several years, costimulatory molecules belonging to the tumor necrosis factor (TNF) receptor and TNF ligand superfamilies have been identified and characterized [1,2]. These include CD27/CD27L, CD30/CD30L (CD153), CD40/CD40L (CD154), OX40 (CD134)/OX40L and 4-1BB (CD137)/4-1BBL. These molecules are type II transmembrane glycoproteins, except for LT-α which is the only entirely secreted tumor necrosis factor-like protein. The members of the TNF ligand superfamily share common biological activities, but some properties are shared by only some ligands, while others are unique. The diverse biological activities triggered through tumor necrosis factor receptor (TNFR) are mainly related to the regulation of cellular activation, including immune responses and inflammatory reactions, as well as the pathology of a series of human diseases.

The murine 4-1BBL cDNA was isolated from a murine thymoma cell line in 1993 using an expression screening approach [3]. In 1994, Alderson *et al.* [4] utilized cross-species hybridization to isolate a cDNA encoding human 4-1BB (hu4-1BB). A fusion protein of the extracellular portion of hu4-1BB coupled to the Fc region of human IgG1 (hu4-1BBL.Fc) was then used to identify and clone the gene for human 4-1BBL from an activated $CD4^+$ T cell clone using a direct expression cloning strategy. Human and murine *4-1BBL* genes are located on human chromosome 19p13 and mouse chromosome 17D, respectively. Human 4-1BBL is expressed primarily on professional antigen presenting cells (APCs), such as

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mature dendritic cells, activated B cells and activated macrophages [5,6], and is also expressed inducibly in T lymphocytes [7] and constitutively in monocytes [4]. 4-1BB is expressed on CD4 and CD8 T cell lines [8]. Agonistic antibodies and the ligand of 4-1BB enhance lymphocyte activation [6,7,9–11], and the 4-1BB protein has an opposite effect in that it inhibits proliferation of activated T lymphocytes and induces programmed cell death [3,6,7,9,10]. This means that a reverse signal is transmissive through the ligand [12].

In exploring the physiological and pathological functions of 4-1BBL, the rat system has some advantages over the human and murine systems. Superior models of autoimmune diseases, such as collagen-induced arthritis, experimental autoimmune uveoretinitis and experimental allergic encephalomyelitis, have been developed [13,14]. Some models of human diseases, such as the human T-cell lymphotropic virus-1-associated pathologies [15], have only been set up in the rat system.

In order to facilitate the study of 4-1BBL, we cloned rat *4-1BBL* using Internet resources and characterized its expression and chromosome mapping in the rat system.

Materials and Methods

Animal and cDNA library

Six-week-old male Sprague-Dawley (SD) rats (*Rattus norvegicus*) were purchased from Shanghai Institutes for Biological Sciences (Shanghai, China). cDNA library of rat fetal brain was kindly provided by Dr. Long YU (Fudan University, Shanghai, China).

Rat 4-1BBL cDNA cloning and sequence analysis

Murine 4-1BBL mRNA was used to BLAST rat expressed sequence tags (ESTs) in GenBank (http://www.ncbi.nlm.nih.gov/blastn). Primers were designed by comparing murine 4-1BBL mRNA and rat ESTs. Polymerase chain reaction (PCR) was performed using a cDNA library of rat fetal brain and PCR product was purified using a PCR product purification kit (Promega, Madison, USA), then ligated to the pMD18-T vector (TaKaRa, Japan). The cDNA insert was sequenced on both strands using synthetic oligonucleotide primers and dye-labeled terminator/*Taq* DNA polymerase on automated fluorescent DNA sequence (Sangon, Shanghai, China). Alignment of amino acid sequences and estimation of homology were performed using DNAtool 6.0.122 (http://www.crc.dk/dnatools) and ClustalW (http://www.ebi.ac.uk/clustalw) softwares.

Intron cloning

Rat genome DNA was extracted from adult rat brain using phenol-chloroform. Primers were designed by comparing rat *4-1BBL* cDNA with the murine *4-1BBL* genome. PCR products were ligated to the pMD18-T vector and were sequenced.

Expression of rat 4-1BBL

Total RNA was extracted from 11 rat tissues using Trizol (Invitrogen, Carlsbad, USA) and cDNA synthesis was performed using RevertAid M-MuLV reverse transcriptase (Fermentas, Lithuania). Reverse transcription (RT)-PCR was performed with rat 4-1BBL cDNA primers and rat β-actin primers to quantify the relative abundance of 4-1BBL mRNA in each tissue. Primers of rat β-actin are as follows: 5′-TAAAGACCTCTATGCCAACAC-3′, 5′-TAAAGCCATGCCAAATGTCTC-3′.

Chromosome mapping by UniGene

Human chromosome 19 and murine chromosome 17 were used to BLAST the rat genome in GenBank, then UniGenes were analyzed (http://www.ncbi.nlm.nih.gov/blast/unigene).

Fluorescence *in situ* hybridization

Rat G-band metaphase chromosomes were prepared using marrow from an SD rat by cell cycle synchronization [16]. G-banded metaphases were located and photographed. Giemsa stain was removed with ethanol and the slides were treated with DNase-free RNase (100 μg/ml) at 37 ºC for 1 h in 2×SSC. Before hybridization the chromosomes were dehydrated through an ethanol series (70%, 95%, 100%). The 2.0 kb 4-1BBL genomic DNA fragment was generated with cDNA primers and subcloned into pMD18-T vector. DNA probes were labeled with biotin-14-dATP (Gibco BRL, Carlsbad, USA) by nick-translation, then the unincorporated nucleotides were removed by ethanol precipitation in the presence of 20 μg glycogen and stored at –20 ºC. *In situ* hybridization and detection were performed as described previously [17] with a slight modification. In brief, 50–100 ng of biotinylated probe was applied to each slide under sealed glass coverslips. Hybridization buffer contained 1.5 μl probe (50–100 ng), 1.5 μl 100 μg/ml denatured salmon sperm DNA (100 μg/ml) (Sigma, St. Louis, USA), and 12 μl hybridization solution VII (Oncor, Gaithersburg, USA). The chromosome DNA was denatured in 70% formamide in 2×SSC for 3 min, and probe was denatured at 72 ºC for 10 min. Hybridization was performed in a humidified

box at 42 ºC for approximately 16 h. Subsequently the slides were washed in 2×SSC at 72 ºC for 5 min, in 1×PBD for 2 min and in 100 μl blocking solution at 37 ºC for 10 min. The biotin-labeled probe DNA was visualized and amplified by the fluorescein-isothiocyanate (FITC)-conjugated avidin and biotinylated anti-avidin system (biotin-FITC detection kit; Oncor). Individual chromosomes were identified by counterstaining with 4,6-diamidino-2-phenylindoledihydrochloride (DAPI; Sigma). Separated images of DAPI-banded chromosomes were obtained and merged electronically using microscope Leica Q550 CW (Leica, Wetzlar, Germany) and image analysis software Leica QFISH V2.1 (Leica).

Results

Cloning of rat 4-1BBL cDNA

In order to identify rat 4-1BBL, murine 4-1BBL mRNA was used to BLAST the rat ESTs in GenBank. Two highly homologous rat ESTs were obtained (GenBank accession No. BF565084 and BF394106) and coincidently the two ESTs properly spanned the murine 4-1BBL coding region. Primers possibly spanning the possible coding region were designed according to the two ESTs. Electrophoresis of the PCR product on 1% agarose gel showed a 1000-bp band as expected. The sequencing result was then linked with the two ESTs, and a 1328-bp fragment of rat 4-1BBL cDNA was obtained. The sequence of the cDNA clone shown in **Fig. 1(A)** contained a single large open reading frame (ORF) encoding a protein of 308 amino acid residues. Hydrophilicity analysis predicted a single hydrophobic domain encompassing AA83 to AA102 and no signal sequence. Thus, like murine and human 4-1BBL, this protein was predicted to be a type II membrane protein with its carboxy-terminal domain extracellular. Like murine 4-1BBL (containing three potential N-linked glycosylation sites: AA139, AA161, AA293), the extracellular domain of rat 4-1BBL also contains three potential N-linked glycosylation sites: AA138, AA160 and AA292, while human 4-1BBL does not. The nucleotides of the possible coding region of rat 4-1BBL has 88% and 37% homology with that of murine and human 4-1BBL, respectively, in which the cytoplasmic domain is 85%, 30%; the transmembrane domain is 81%, 33% and the extracellular domain is 89%, 46% homology with murine and human 4-1BBL, respectively. Alignment of the predicted protein product of this rat cDNA with that of murine 4-1BBL and human 4-1BBL is shown in **Fig. 1(B)**. Rat 4-1BBL is more closely related to murine 4-1BBL with 83% homology and to a lesser extent to human 4-1BBL with 31% homology, in which the cytoplasmic domain is 76%, 32%, the transmembrane domain is 45%, 40% and the extracellular domain is 87%, 28% homology with murine and human 4-1BBL, respectively. The molecular weight of the deduced rat 4-1BBL protein is 33,469 Da, and its isoelectric point is 5.82.

Cloning of rat *4-1BBL* introns

Comparing the rat 4-1BBL cDNA and the murine *4-1BBL* genomic gene, the rat *4-1BBL* gene was deduced to have two introns just the same as murine *4-1BBL*. Sequencing results displayed two sequences of 335 bp and 687 bp, respectively, corresponding to murine intron 1 (389 bp) and intron 2 (754 bp), whereas those of human are 1489 bp and 1783 bp, respectively. Two rat *4-1BBL* introns followed the gt-ag rule (**Fig. 2**). To test the accuracy of the results, PCR was performed with the primers which were used in cloning rat 4-1BBL cDNA and with rat genome DNA as the model. PCR results showed a band of about 2000 bp as expected (389+754+1000=2043). The intron nucleotide sequences showed 86% and 85% identity in intron 1 and intron 2, respectively, between rat and murine 4-1BBL; and 8% (intron 1) and 26% (intron 2) between rat and human. The identity from start codon to stop codon was 86% between rat *4-1BBL* (1946 bp) and murine *4-1BBL* (2073 bp), and 13% between rat *4-1BBL* and human *4-1BBL* (4027 bp). So rat *4-1BBL* is more homologous with murine *4-1BBL* than with human *4-1BBL*, in both nucleotide and amino acid sequences. Human 4-1BBL protein is poorly homologous with rat 4-1BBL protein and murine 4-1BBL protein (both 31%). Their genes are all composed of three exons and two introns, in which the lengths of exon 2 are short: 31 bp (human), 37 bp (rat and murine); and the lengths of exon 3 are similar: 461 bp (rat), 464 bp (human and murine). The exon 2 is 50%–60% idential among the three genes, the same as exon 3. Exon 2 and exon 3 mainly encode the transmembrane and the extracellular domains. Compared with the identities of nucleotide sequences of the cytoplasmic domain of the three molecules, which is encoded by exon 1 (34% between human and murine, 22% between human and rat), the identities of nucleotide sequences of transmembrane and extracellular domains are higher.

mRNA expression of rat 4-1BBL

RT-PCR results showed that 4-1BBL mRNA was widely but differently expressed in tested tissues (**Fig. 3**). The 4-1BBL mRNA level in each tissue, compared with that of

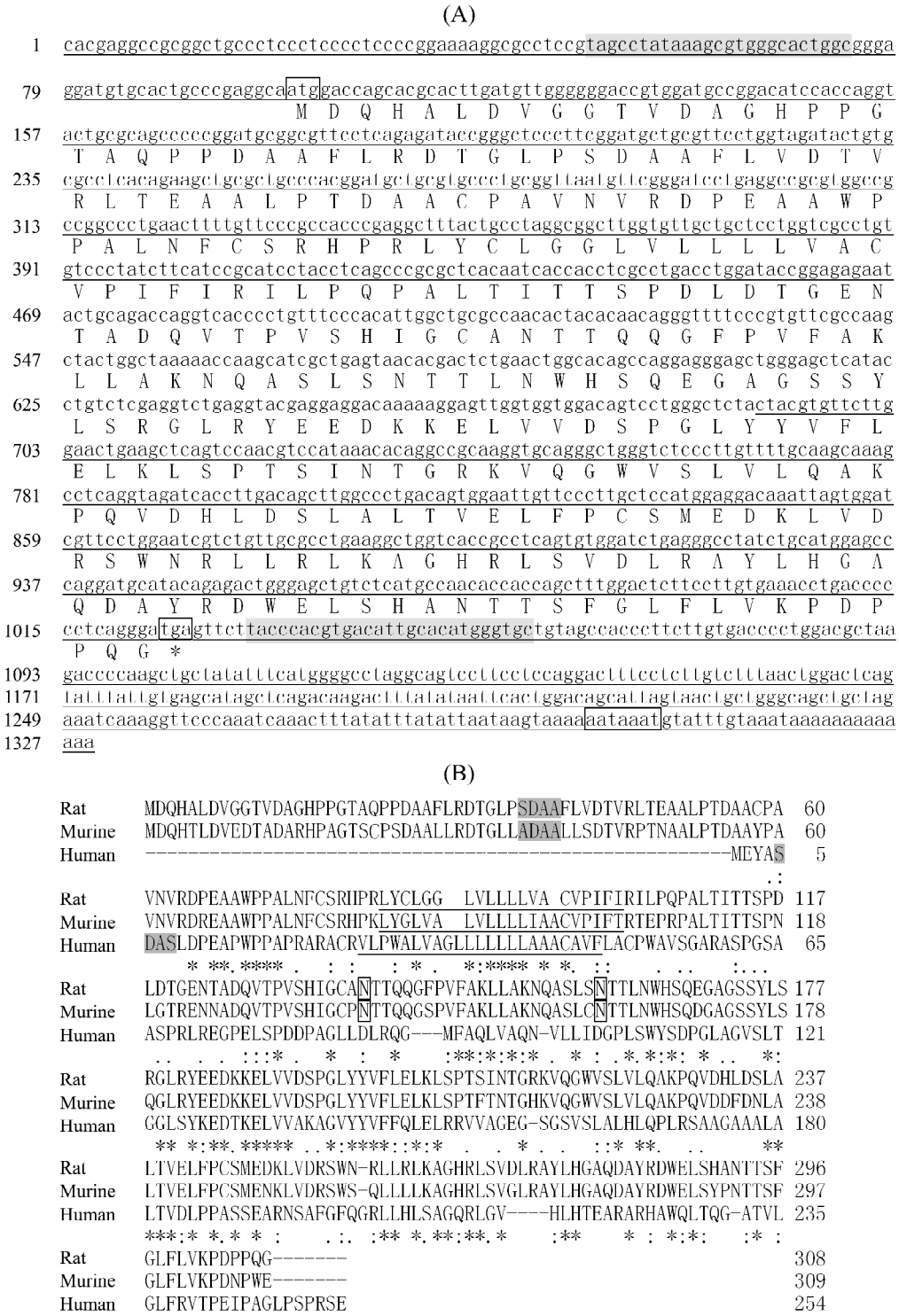

Fig. 1 Nucleotide and deduced amino acid sequences of rat *4-1BBL*

(A) The nucleotide sequence of rat 4-1BBL cDNA (GenBank accession No. AY259541) and the deduced amino acid sequence. Primers are shaded. Start codon, stop codon and potential poly(A) additional signal sequence are indicated by open boxes. The two rat expressed sequence tags (ESTs) (BF565084, BF394106) are underlined. (B) Comparison of rat, murine, and human 4-1BBL amino acid sequences. Amino acid sequence homology was analyzed by ClustalW. The putative transmembrane domains are denoted by long underlines. The potential N-linked glycosylation sites in the extracellular domains are indicated by open boxes. Casein kinase I phosphorylation sites in the cytoplasmic domain are shaded. The tumor necrosis factor (TNF)-like core domains within the extracellular region are: rat, 163–301 aa; murine, 145–302 aa; human, 88–240 aa.

cacgaggccgcggctgccctccctccccctccccggaaaaggcgcctccgtagcctataaagcgtgggcactggcgggagatgt
gcactgcccgaggcaATGGACCAGCACGCACTTGATGTTGGGGGGACCGTGGATGCCGGACATCCACCAGGTACTGCGCAGCC
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CGCCACCCGAGGCTTTACTGCCTAGGCGGCTTGGTGTTGCTGCTCCTGGTCGCCTGTGTCCCTATCTTCATCCGCATCCTACC
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TTGGCTGCGCCAACACTACACAACAGgtgaggctcatcccatccctaggggaccctttttactttctggaagcatctctgcac
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GTCCATAAACACAGGCCGCAAGGTGCAGGGCTGGGTCTCCCTTGTTTTGCAAGCAAAGCCTCAGGTAGATCACCTTGACAGCT
TGGCCCTGACAGTGGAATTGTTCCCTTGCTCCATGGAGGACAAATTAGTGGATCGTTCCTGGAATCGTCTGTTGCGCCTGAAG
GCTGGTCACCGCCTCAGTGTGGATCTGAGGGCCTATCTGCATGGAGCCCAGGATGCATACAGAGACTGGGAGCTGTCTCATGC
CAACACCACCAGCTTTGGACTCTTCCTTGTGAAACCTGACCCCCCTCAGGGAtgagttcttacccacgtgacattgcacatgg
gtgctgtagccacccttcttgtgaccctggacgctaagaccccaagctgctatatttcatggggcctaggcagtccttcctc
caggactttcctcttgtctttaactggactcagtatttattgtgagcatagctcagacaagactttatataattcactggaca
gcattagtaactgctgggcagctgctagaaatcaaaggttcccaaatcaaactttatatttatattaataagtaaaaaataaa

Fig. 2 Nucleotide sequence of rat *4-1BBL* (GenBank accession No. AY332409)

Exons are in capital letters. The primers for intron cloning are underlined. The down-stream primer of intron 1 and the up-stream primer of intron 2 are complementary. gt-ag is indicated by open boxes.

rat β-actin mRNA, was analyzed using Bandleader 3.0 (http://www.bio-soft.net/draw.html); and the relative abundance from high level to low level was as follows: brain, colon, lung, thymus, adrenal, liver, testicle, kidney, stomach, spleen and heart.

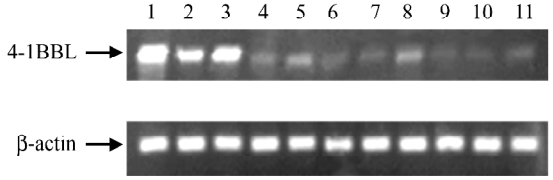

Fig. 3 Reverse transcription-polymerase chain reaction (RT-PCR) result of rat 4-1BBL mRNA

1, brain; 2, lung; 3, colon; 4, liver; 5, thymus; 6, testicle; 7, kidney; 8, adrenal; 9, stomach; 10, spleen; 11, heart.

Rat *4-1BBL* chromosomal mapping by bioinformatics

Chromosome BLAST results demonstrated that human chromosome 19 matched rat chromosomes 1, 5, 7, 9, 12 and 16, and murine chromosome 17 matched rat chromosomes 1, 6, 9, 10 and 20 (**Fig. 4**). Rat *4-1BBL* gene was inferred on chromosome 1 or 9. UniGenes on human chromosome 19, murine chromosome 17, and rat chromosome 1, 9 were analyzed. We found that the UniGene of human *4-1BBL* locating on human Chr.19:38.82cR is Hs.1524, and the UniGene of murine *4-1BBL* locating on murine Chr.17:799.15cR is Mm.41171. Coincidently another UniGene (Mm.23982) was also found on murine Chr.17:799.15cR, and this UniGene corresponded to rat UniGene Rn.14560. Rat UniGene Rn.14560 was the location of rat gene crumbs 3 (*CRB3*), which is on rat chromosome 9q11cM (Chr.09:0cR) (http://www.ncbi.nlm.nih.gov/UniGene/clust.cgi?ORG=Rn&CID=14560). The chromosome locations of

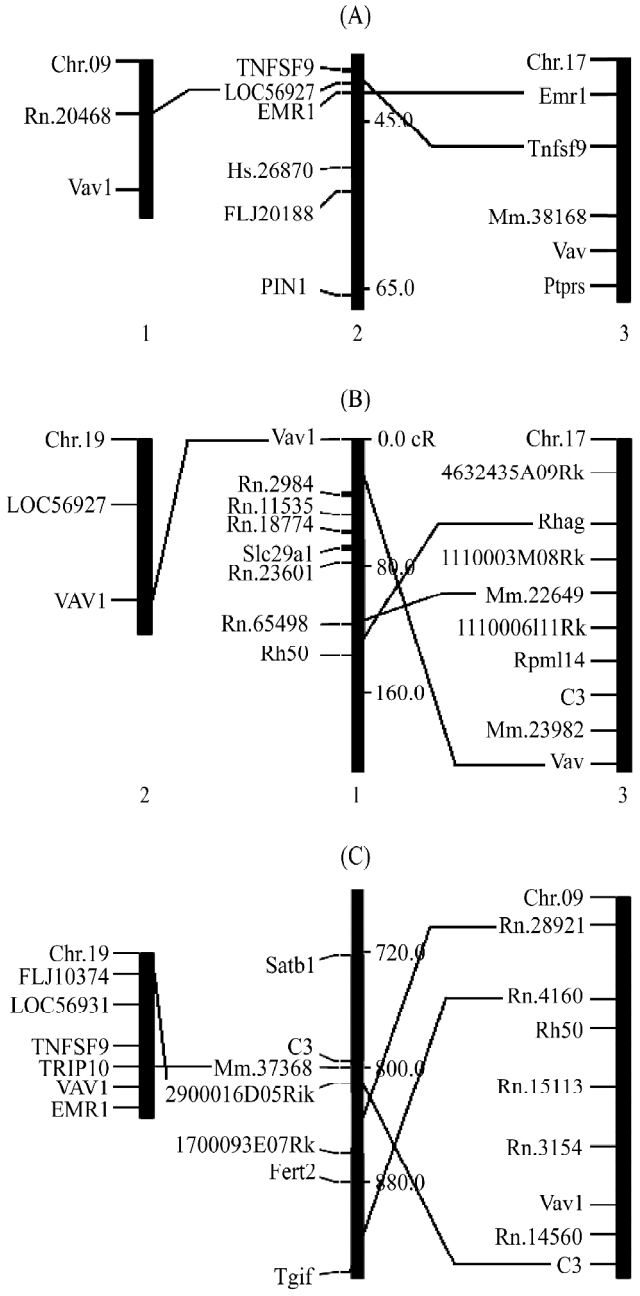

Fig. 4 Results of BLAST using chromosomes in genomes

(A) Human chromosome 19 BLAST of rat and murine genomes. (B) Rat chromosome 9 BLAST of murine and human genomes. (C) Murine chromosome 17 BLAST of rat and human genomes. 1, rat chromosome 9; 2, human chromosome 19; 3, murine chromosome 17.

human *CRB3* and murine *CRB3* were searched on the National Center for Biotechnology Information (NCBI) website, and it was found that *CRB3* was located on human 19p13.3cM (GenBank accession No. NM_174881, UniGene No. Hs.150319) and murine 17D (GenBank accession No. NM_177638, UniGene No. Mm.260076), respectively, just the same as *4-1BBL*. According to the gene linkage rule, rat *4-1BBL* may be on rat 9q11cM, just like rat *CRB3*. To prove the deduction, the chromosome arrangement of genes *CRB3*, *4-1BBL*, *C3* and another gene (in murine called *Vav*, in human and rat called *Vav1*) were analyzed, which are all located on rat chromosome 9q11, human chromosome 19p13.3, and murine chromosome 17. On human chromosome 19, the nucleotide span number of the four genes are: *CRB3*, 6414788-6418232 (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene&cmd=Retrieve&dopt=Graphics&list_uids=92359); *4-1BBL*, 6482037-6486933 (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene&cmd=Retrieve&dopt=Graphics&list_uids=8744); *C3*, 6671660-6628878 (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene&cmd=Retrieve&dopt=Graphics&list_uids=718); *Vav1*, 6723722-6808371 (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene&cmd=Retrieve&dopt=Graphics&list_uids=7409). The order of their locations is *CRB3*/*4-1BBL*/*C3*/*Vav1*. Because the murine genome was not obtained on the Internet, UniGene was used to analyze the locations of the four genes on murine chromosome 17: *Vav*, murine Chr.17:803.35cR (UniGene No. Mm.57191) (http://rgd.mcw.edu/tools/vcmap/vcmap.cgi?MapName=Murine+VirtualMap+5.0_HS&Chr=17&FirstMarker=C3&FirstPos=795.08&SecondMarker=1700093E07 Rik&SecondPos=859.3); *CRB3*, murine Chr.17:799.15cR (UniGene No. Mm.23982); *4-1BBL*, murine Chr.17:799.15cR (UniGene No. Mm.41171); *C3*, murine Chr.17:795.08cR (UniGene number Mm.1913). The order of their locations is *C3*/*CRB3*(*4-1BBL*)/*Vav*. **Fig. 4(C)** shows that the relation of the genes on rat chromosome 9 is *Vav1*/UniGene Rn.14560/*C3*, and UniGene Rn.14560 is *CRB3*. The three genes are all on rat 9q11, so rat *4-1BBL* is also located on rat 9q11, and its UniGene accession No. is Rn.46783.

Rat *4-1BBL* chromosomal mapping by fluorescence *in situ* hybridization

The biotin-labeled probe DNA was prepared with rat genome *4-1BBL* corresponding to the 2.0 kb PCR product. The signal was amplified by immunofluorescence. Positive signals were visualized on both homologous chromosomes only on 9q11 in 90% of the chromosome spreads which were investigated (**Fig. 5**). This is consistent with the result of chromosomal mapping by informatics using UniGenes.

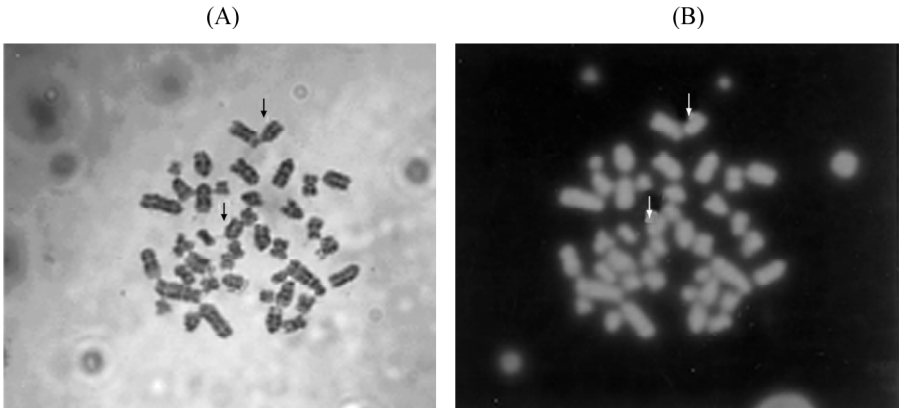

Fig. 5 Fluorescence *in situ* hybridization of rat *4-1BBL*
(A) G-band, chromosome 9 are arrowed. (B) The same metaphase with signals after hybridization (indicated by arrows).

Discussion

As other TNFR superfamily members, 4-1BB uses adaptor molecules, which are called TNFR-associated factors (TRAFs), to transduce a downstream signal. The cytoplasmic domain of 4-1BB is able to bind specifically with TRAF1, TRAF2 and TRAF3 [10,18,19]. Accumulating evidence indicates that there exists a signal transduction pathway by way of the 4-1BB ligand as well as the 4-1BB. Cross-linking of 4-1BBL with insect cells, which express 4-1BB, induces strong B-cell proliferation synergistically with anti-IgM antibody [5]. Similarly, cross-linking of 4-1BBL by soluble 4-1BB-Fc fusion protein induces monocyte activation so that the expression of IL-6, IL-8, TNF-α and ICAM is up-regulated, whereas FcγR III expression is down-regulated [20]. Signals by way of 4-1BBL also lead to a strong production of macrophage colony-stimulating factor in monocytes, which in turn functions as a potent survival factor for monocytes [21]. In addition, the "reverse signaling" through 4-1BBL mediates monocyte proliferation by an autocrine mechanism which is still unknown yet [22]. In T lymphocytes, T-cell proliferation induced by anti-CD3 antibody is inhibited by cross-linking of 4-1BBL [12]. Furthermore, this inhibition of T-cell proliferation is accompanied by cell apoptosis. The apoptotic signal transmitted by 4-1BBL is independent of Fas [23]. Therefore 4-1BBL signaling mediates two opposite biological phenomena, cell survival and apoptosis, depending on cell type. Even though reverse signaling through TNF ligand family members has been well documented, its molecular mechanism remains undiscovered. Interestingly, a phosphorylation site for casein kinase I (SXXS) is present in the cytoplasmic domains of all TNF ligand superfamily members, which were known to utilize the reverse signaling, CD30L, 4-1BBL, CD40L and FasL, except OX40 ligand [24]. In transmembrane TNF-α, this motif has been the phosphorylation target for casein kinase, and treatment of a macrophage cell line with dimeric soluble TNFR leads to the dephosphorylation of total cellular transmembrane TNF-α. One biochemical change following the dephosphorylation is an elevation of intracellular calcium levels [24]. This motif appears as "SDAA" in the deduced rat 4-1BBL amino acid sequence, but appears as "SDAS" in human 4-1BBL and as "ADAA" in murine 4-1BBL. The difference is in that "Ser" contains -OH while "Ala" contains -H. Ser is a polar amino acid, while Ala is between polar and non-polar, and they are both uncharged. So rat "SDAA" possibly acts as the phosphorylation target for casein kinase. The existence of a phosphorylation site for casein kinase I in the cytoplasmic domain of the three molecules probably makes them function similarly in "reverse signal" transmission. Like murine 4-1BBL, rat 4-1BBL also contains additional 55 N-terminal amino acid residues belonging to the cytoplasmic domain, which is not found in the human homologue. In co-stimulation signal transmission, especially in "reverse signal" transmission, it is unknown what difference was caused by the lack of amino acid residues and potential N-linked glycosylation sites in human 4-1BBL. Study on rat 4-1BBL will make its mechanism of function and of reverse signal transmission understood.

A simple method was used here to compare the cloning of murine and human 4-1BBL. The completion of the Human Genome Project has made a lot of data available

on gene sequences, location, expression, and function analysis, which provides a short cut to clone genes with ESTs and homology analysis. Compared with murine and human 4-1BBL, the rat 4-1BBL was cloned and mapped by *in silicon* cloning and chromosome mapping, which is also a short cut. Rat 4-1BBL was firstly identified to locate at 9q11 (GenBank accession UniGene No. Rn.46783), the same as the *CRB3* gene, by bioinformatics and FISH methods. These two genes also appeared at the same site both in human (19q13) and murine (17D). The gene structure of rat, murine and human 4-1BBL are similar. *4-1BBL*, *CRB3*, *C3* and *Vav1* (*Vav*) are all lined at rat chromosome 9, murine chromosome 17 and human chromosome 19. So rat, murine and human *4-1BBL* probably evolved from a common ancestor gene. The identification and characterization of the rat counterpart of human 4-1BBL will facilitate the study of the biological functions of this molecule.

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