The mouse genome encodes a single homolog of the antimicrobial peptide human β-defensin 1

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Abstract The cysteine-rich β-defensin peptides are broad-spectrum bactericidal agents expressed in epithelial and myeloid tissues. The human β-defensin-1 (hBD-1) gene maps adjacent to the human α-defensin cluster and is expressed in the respiratory, gastrointestinal and genitourinary tracts. Here, we characterize a mouse β-defensin gene (mBD-1) which is: (1) closely related to hBD-1 both in sequence and gene organization; (2) expressed at high levels in the mouse kidney and at lower levels in brain, heart, lung, uterus, spleen, skeletal muscle, stomach, and small intestine; and (3) maps to mouse chromosome 8 at or near the location of the mouse α-defensin genes. These data indicate that mBD-1 is a close homolog of hBD-1, and suggest that analysis of its role in mouse host defense may provide significant insights into human epithelial innate immunity.

Key words: Antimicrobial peptide; Defensin; Mouse

1. Introduction

Beta-defensins are cationic, cysteine-rich antimicrobial peptides isolated from both myeloid and epithelial tissues [1]. The bovine β-defensin family includes at least fifteen related peptides with two expressed in epithelial tissues [2,3] and thirteen in neutrophils [4]. In marked contrast, a single human β-defensin, termed hBD-1, was isolated from plasma [5] and subsequently shown to be expressed at highest levels in the kidney and lower levels in epithelial tissues including the lung, pancreas, and genitourinary tract [6,7]. Evidence from studies of both cattle and human β-defensins demonstrates their involvement in epithelial host defense [3,8–10]. Impaired function of hBD-1 may play a prominent role in the pathophysiology of recurrent bacterial lung infections in the disease cystic fibrosis [10,11].

Several mouse cDNA sequences reported recently in the Sequence Tagged Site database encode a putative β-defensin. Using this sequence information, we have begun a molecular characterization of the mouse β-defensin gene family as an animal model for studying the role of β-defensin peptides in host immunity.

2. Materials and methods

2.1. Southern and Northern blots

Mouse genomic DNA was isolated from the liver of an adult FVB/NJ male, digested with restriction endonucleases, subjected to electrophoretic separation on a 1% agarose gel, and transferred to a Gene Screen Plus nylon membrane (NEN, Boston, MA) according to standard protocols [12]. Total RNA was isolated from adult FVB/NJ mouse tissues, subjected to electrophoresis in 1.3% agarose, and transferred to nylon membranes as described [13]. Northern and Southern blot hybridizations were overnight at 42°C in 50% formamide, 5×SSC, 1% SDS; 100 mcg/ml Salmon DNA, and 10% dextran sulfate. Post-hybridization washes were 2×10 min in 2×SSC at room temperature, followed by 2×15 min in 0.2×SSC, 0.5% SDS at 50°C.

2.2. Hybridization probes

A plasmid containing the mBD-1 cDNA was obtained from the ATCC (#974407 corresponding to GenBank #AA065510). A 261-bp PCR fragment encompassing the entire coding sequence was generated using the oligonucleotide primer pair: mBD-F1, 5′-GGCTGCCACCACTATGAAAACTC-3′ and mBD-R1, 5′-CCATCGCTCGTCCTTTATGCTCA-3′. A 140-bp PCR fragment encoding the predicted 3′ mature peptide was generated using the primer pair: mBD-F2: 5′-TGCCTTCAACATGGAGGATTCTG-3′ and mBD-R1. In each case, the PCR product was gel purified and then used as template for generation of radioactive probe using either random primed DNA labeling or PCR amplification in the presence of radioactive nuclides (NEN, Boston, MA). A 1.3-kb genomic Hind III fragment encoding the mBD-1 exon 1 sequence was labeled by random priming and used as a probe to identify exon 1 and 5′ sequences.

2.3. RT-PCR

Total RNA from adult mouse tissues was reverse transcribed using SuperScript according to manufacturer's protocol (Gibco BRL, Gaithersburg, MD). PCR amplification of first strand product used the following profile: 95° for 5 min then 35 cycles of 94° for 0.5 min, 58° for 0.5 min, 72° for 0.5 min. PCR products were analyzed on 2% NuSieve/1% SeaKem LE agarose gels (FMC, Rockland, ME).

2.4. Screening of genomic library

The 261-bp mBD-1 PCR product was used as a probe for filter hybridization to a mouse 129/SvJ genomic library (Stratagene, La Jolla, CA). Duplicate positives were plaque purified, their phage DNA isolated and analyzed by restriction endonuclease digestion, and subfragments cloned for sequencing as described [14].

2.5. DNA sequencing

Purified plasmid DNA was sequenced from both strands using a thermal cycling method with florescent dye-labelled dideoxynucleotide terminators and Taq DNA polymerase. Sequence data were analyzed using the DNA and protein analysis software MacVector (IBI, New Haven, CT).

2.6. Chromosomal mapping

The mBD-1 locus (referred to as *Defb1*) was mapped by analysis of two sets of multilocus crosses: (NFS/N or C58/J×M. m. *musculus*)×M. m. *musculus* [15] and (NFS/N×M. *spretus*)×M. *spretus* or C58/J [16]. Progeny of these crosses have been typed for over 800 markers distributed over the 19 autosomes and X chromosome including the Chr 8 markers *Col4a* (collagen 4a), *Plat* (plasminogen activator, tissue), *Polb* (DNA polymerase beta), *Ram1* (replication of amphotropic virus 1), and *Plcd1* (phospholipase C, delta 1) as described previously [17,18]. Recombination was determined according to Green [19] and loci were ordered by minimizing the number of recombinants.

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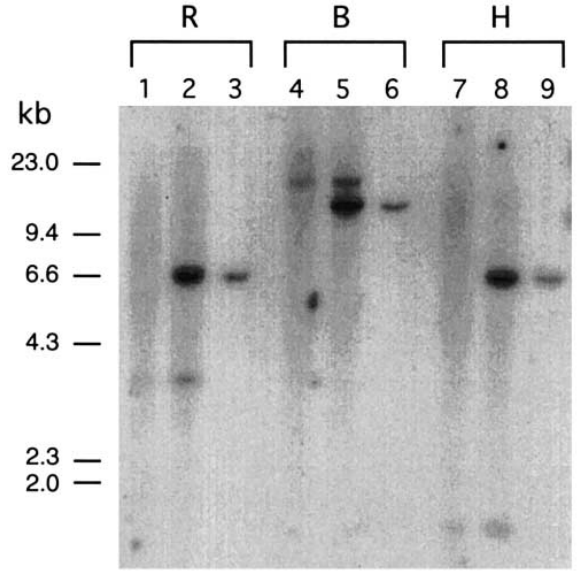

Fig. 1. Compilation of Southern blot hybridization of mouse genomic DNA. R, *Eco*RI; B, *Bam*HI; H, *Hin*dIII. Probes used were mBD-1 entire coding sequence (lanes 2, 5, 8), mBD-1 5′ sequences (lanes 1, 4, 7), and mBD-1 3′ sequences (lanes 3, 6, 9) (see Section 2).

3. Results

3.1. mBD-1 genomic sequence

Nearly identical cDNA sequences encoding a putative β-defensin peptide were found in mouse kidney, heart, and macrophage cDNA libraries (GenBank #AA065510, AA104376 and AA071757, respectively). A 261-bp PCR fragment encompassing the entire open reading frame of this mouse β-defensin (mBD-1) cDNA was used as a probe in Southern hybridization (Fig. 1). Two bands, one intense and one weak, were identified in each of three different restriction enzyme digestions of mouse genomic DNA (lanes 2, 5 and 8). Based on the previously reported two-exon structure of the human and bovine β-defensin genes, we predicted that the 5′ and 3′ regions of the mBD-1 cDNA would be encoded by separate exons, and that the two bands detected represented the two exons of mBD-1. This was supported by rehybridization of the same filter with a probe specific for the 5′ mBD-1 sequences (lanes 1, 4, 7), which identified only the less intense band in each lane, and a probe specific for the 3′ mBD-1 sequences (lanes 3, 6, 9), which identified only the more intense band.

We used the same 261-bp mBD-1 PCR fragment as a probe to identify mBD-1 sequences in a mouse 129/SvJ phage genomic library. From approximately 10^6 pfu screened we identified fifteen duplicate positives, three of which were plaque-purified and their DNA analyzed. Phage 4E-2 contains a genomic insert of approximately 20 kb including a 2.8-kb *Eco*RI fragment encoding the mBD-1 exon 1 sequence (Fig. 2). Phages A7 and 9D-1 contain overlapping genomic inserts of 13–14 kb which encode the mBD-1 exon 2 sequence.

Sequences from the deduced exons correspond to the reported cDNA sequence, and the predicted splice site is analogous to that of hBD-1 (Fig. 3A). The mouse mBD-1 gene sequence demonstrates significant nucleotide identity to the hBD-1 sequence as shown in Fig. 3B and C. Homology search of the GenBank database using the mBD-1 genomic sequence identified the hBD-1 sequences as well as a partial cDNA clone labeled CDK4 (#89820). Interestingly, CDK4 was isolated from rat kidney and predicts a peptide similar to mBD-1 [20].

3.2. Chromosomal localization

The hybridization probe for mBD-1 identified *Pvu*II fragments of 6.9 kb in M. *spretus* and 6.0 kb in NFS/N and C58/J. Southern blot analysis of the parental DNAs of the M. m. *musculus* cross failed to identify any RFLPs. Inheritance of the variant fragments was compared with inheritance of over 800 markers previously typed in the *M. spretus* crosses and linkage was observed with markers on Chr 8. As indicated in Fig. 4, the mBD-1 locus (*Defb1*) mapped near *Polb*, *Plat*, and

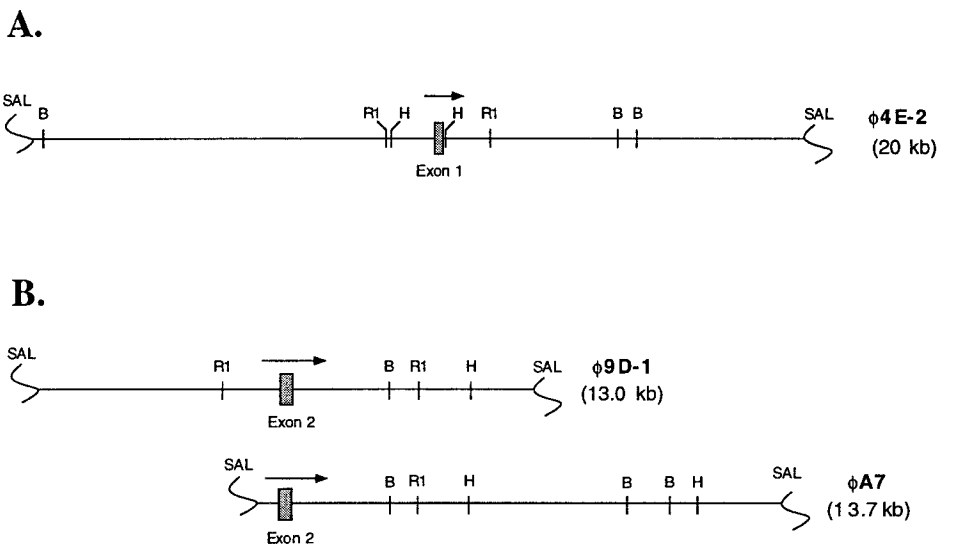

Fig. 2. Restriction enzyme maps of lambda phage clone genomic inserts encoding mBD-1 exon 1 (ϕ4E-2) and exon 2 (ϕA7 and ϕ9D-1). SAL, *Sal*I; R1, *Eco*RI; B, *Bam*HI; H, *Hin*dIII. Location of additional *Eco*RI and *Hin*dIII sites present in ϕ4E-2 downstream from the exon 1 coding sequence have not been determined.

A. Exon 1

```
4E-2    tataaaaaaacaagctcattcaagcctcatctgtcagcccaactacctgggagtttcacatcctctctgcactctggaccctggca
cDNA                                                                ..............................

Codon                              M  K  T  H  Y  F  L  L  V  M  L  C  F  L  F  S  Q  M  E  P  G
4E-2    ctctggaccctggctgccaccactATGAAAACTCATTACTTTCTCCTGGTGATGATATGTTTTCTTTTCTCCCAGATGGAGCCAG
cDNA    ...............................................................................
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Exon 2

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Codon    V  G  I  L  T  S  L  G  R  R  T  D  Q  Y  K  C  L  Q  H  G  G  F  C  L  R  S  S  C
A7      GTGTTGGCATTCTCACAAGTCTTGGACGAAGAACAGATCAATACAAATGCCTTCAACATGGAGGATTCTGTCTCCGCTCCAGCTGC
cDNA    ...............................................................................

Codon    P  S  N  T  K  L  Q  G  T  C  K  P  D  K  P  N  C  C  K  S
A7      CCATCTAATACCAAACTACAGGGAACCTGTAAACCAGATAAGCCCAACTGTTGTAAGAGCTGACAGTAGTTTGAAGAATGGACATA
cDNA    ...............................................................................AG....

A7      AAGGACGAGCGATGGATTGTAAAATTAGTGTTTTAATAAATGAAATGTTTTTGAAGTTTATTTACATCATATCAAGATAAATTTTA
cDNA    ...............................................................................

A7      TTTCTCAGTTTAGAAGAGCAAATTTTTTT-AAAAAGTATTGGGCTTAGAACAAGAGGTGGGAAAATCCAGAACATCTGCCTGG
cDNA    ......T.............T.......T.............................A.....................
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B.

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        (--------Exon 1-----)(-----------------Exon 2----------------------)
codon   1        10        20        30        40        50        60
mBD-1   MKTHYFLLVMLCFLFSQMEPGVGILTSLGRRTDQYKCLQHGGFCLRSSCPSNTKLQGTCKPDKPNCCKS
        | | | ||  || | | |  |   || || | | | |   || || | ||  || |||| | |||
hBD-1   MRTSYLLLFTLCLLLSEMASGGNFLTGLGHRSDHYNCVSSGGQCLYSACPIFTKIQGTCYRGKEKCCK
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C

% nucleotide identity	5' UTS (109 bp)	Exon 1 Coding (61 bp)	Intron 5' (57 bp)	Intron 3' (33 bp)	Exon 2 Coding (146 bp)	3' UTS (92 bp)
mBD-1 vs hBD-1	50.5	67.2	56.1	57.6	68.5	63.0

Fig. 3. (A) Nucleotide and predicted amino acid sequence for mBD-1 cDNA versus genomic sequences. (.) nucleotide identity; (—) spacing characters to optimize alignment. Predicted stop codon underlined. (B) Amino acid alignment of predicted prepropeptides for mouse (mBD-1) and human (hBD-1) [7] β-defensins. Active peptide underlined. (C) Nucleotide identity between hBD-1 and mBD-1 gene sequences. GenBank accession numbers: mBD-1 cDNA (AA065510), genomic exon 1 (AF003524) and genomic exon 2 (AF003525); hBD-1 genomic exon 1 (U50930) and genomic exon 2 (U50931).

Ram1 with no recombination in 93 mice indicating that these genes are within 3.2 cM at the upper limit of the 95% confidence level. This is the same regional localization described for the mouse α-defensins (*Defcr*) [21]. Comparative analysis of the mouse and human genomes indicates that this gene falls within a region of conserved synteny to human chromosome 8, consistent with the human map location of the Def locus containing both the hBD-1 gene and the family of human α-defensin genes [22,23].

3.3. Expression pattern

By Northern blot hybridization, we detected mBD-1 mRNA only in kidney RNA (Fig. 5). Two hybridizing bands were present, one at approximately 550 nucleotides and one at approximately 450 nucleotides. Using the more sensitive technique of RT-PCR, mBD-1 expression was detected in multiple tissues including those of the mouse respiratory, gastrointestinal, and genitourinary tracts but not in bone marrow (Fig. 6).

4. Discussion

This report characterizes the single mouse β-defensin family member mBD-1. Similar to previously described β-defensin cDNAs [2,3,6,10], a basic peptide with a characteristic array of 6 cysteines is encoded at the 3′-end of a 63–68 codon open reading frame. The mBD-1 gene structure, chromosomal localization, and pattern of tissue expression indicates that it is a close homolog of hBD-1, and notably different from the bovine β-defensins as discussed below.

The mouse β-defensin gene, like hBD-1 [22], has a single long intron which is positioned at codon twenty-one. Analysis of the genomic inserts of phages 4E-2 and 9D-1 places a minimum size on the intron of 9.5 kb. The chromosomal

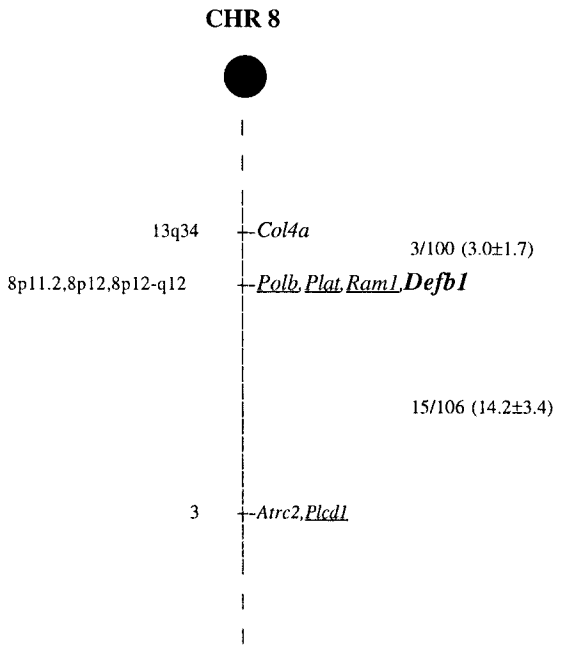

Fig. 4. Genetic map location of *Defb1* on mouse Chr 8. To the right of the map are given recombination fractions for adjacent loci. In parentheses the recombinational distances ± S.D. are given. Map locations for the human homologs are given to the left of the map.

map location of the mouse and human β-defensins lies close to the α-defensin clusters on chromosome 8 of each species [21–23]. By Northern blot analysis, the highest level of β-defensin mRNA is found in the kidney for both the mouse and human genes. Taken together, these results suggest that unlike the α-defensin families of mice and humans, which have evolved in markedly differing patterns [1], the nearby β-defensin genes have maintained significant similarity in structure and perhaps function.

By contrast, the single intron of each of the three characterized bovine genes is short (~2 kb) and positioned at codon twenty (GenBank #L13373, U60447 and AF000362). The highest levels of bovine β-defensin expression were found in the bone marrow and epithelial tissues, including the trachea, tongue, and colon, while only trace β-defensin signal was found in kidney [3,4]. Although it is most probable that these bovine β-defensin genes were derived from the same ancestral precursor as both mBD-1 and hBD-1, their gene structure and

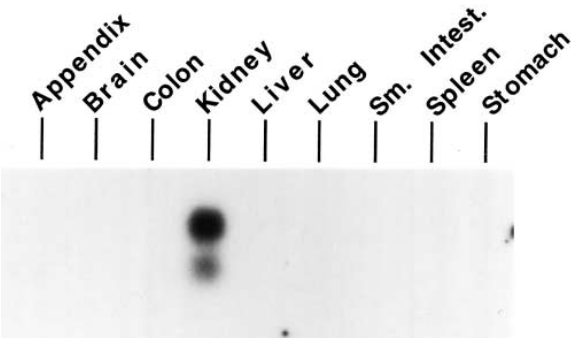

Fig. 5. Northern blot hybridization of mouse RNA using a 261-bp mBD-1 coding sequence probe. 15 μg of total RNA per lane.

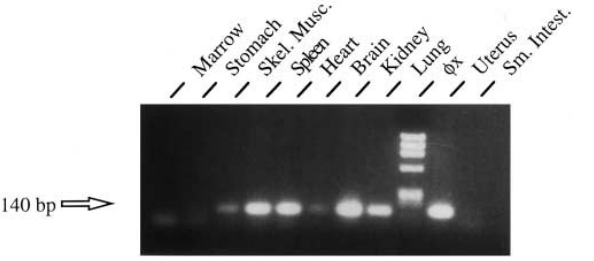

Fig. 6. RT-PCR of mouse β-defensin 1 sequences using adult mouse RNA as template. Oligonucleotide primers were mBD-F2 (forward) and mBD-R1 (reverse) (see Section 2).

expression patterns are significantly different from that of the only known mouse and human β-defensins. This suggests to us that additional mouse and human β-defensin sequences may be present which do not cross-hybridize to mBD-1 and hBD-1 sequences and which demonstrate alternative expression patterns.

In many antimicrobial peptide gene families examined to date there have been significant and unexpected inter-species differences which limit the generalization of biological findings [1,24]. While our data confirm that a close genetic relationship exists between the mouse and human β-defensin families, further characterization of mBD-1 and hBD-1, focusing on gene regulatory mechanisms and biological activity, will be both valuable and necessary in validating the mouse system as a model of hBD-1 function in vivo.

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