Cloning of Canine cDNA Encoding Tektin

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ABSTRACT. Tektins are a group of proteins that form filamentous polymers in the walls of ciliary microtubules. The cloning of canine cDNA encoding tektin, was carried out and identified from the testis of beagle dog. Canine tektin cDNA is 1,523 bp in length, has an open reading frame of 1281 bp nucleotides encoding a protein of 426 deduced amino acids. The predicted amino acid sequence has 77% and 33–50% of homology with the murine tektin and the sea urchin tektins. The amino acid sequence RPNVELCRD and four cysteine residues were conserved in the dog, mouse and sea urchin, suggesting the functional significance of this protein domain and the amino acid residues in the tektin proteins.

KEY WORDS: canine, tektin, testis.

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Cilia are tiny hairlike appendages on the surface of many kinds of cells such as neurons, sensory cells, supporting cells and germ cells, in most animal species. Their primary function is to move fluid over the surface of the cell or to propel single cell through a fluid. The movement of a cilium is produced by the bending of the core, a complex structure composed entirely of the doublet microtubules and their associated proteins [1, 3, 12]. There is a set of microtubule-associated proteins named tekin [4] which form filament running longitudinally along the wall of the doublet microtubules, forming the shared wall of the doublet microtubules [5, 7, 15]. In addition, tektins are also present in flagella, centrioles, and basal bodies [10, 13, 14]. The tektins were originally identified from sea urchins as a set of proteins (A, ~53 kDa; B, ~51 kDa; C, ~47 kDa) [5, 6]. The tektin A and B are thought to form the core protofilaments of tektin filaments, and tektin C to form homodimers assembled onto the periphery of these core protofilaments or to form a second separate tektin filament [11]. The function of tektins is thought to provide stability and structural complexity of microtubules. In addition, tektins are thought to play a role in the ciliary movement [7, 10, 11]. Several cDNA of tektins from the sea urchin [2, 9, 10] and mouse [8] have already been identified, though the canine tektin cDNA remains unknown. So the present work was taken up to find the nucleotide and amino acid sequence of canine tektin.

Oligonucleotide primers for amplifying a part of cDNA encoding canine tektin were designed on the basis of the sequence of murine tektin mRNA [8]. The 5' primer sequence was 5'-TGCTTGGAATACAGGGAGAA-3' corresponding to position number 343–362, and the 3' primer sequence was 5'-CTTCTCCACATTGGCATTGG-3' corresponding to position number 662–681 of the coding region sequence of murine tektin mRNA. cDNA was synthesized from total RNA obtained from the testis of a beagle dog by using a RNeasy Mini Kit (QIAGEN, Hilden, Germany)

according to the instructions of manufacturer. The testis has been used as a basic material for the cloning and sequencing of tektin in case of mouse. Also it has been shown that the testis express tektin at higher levels than that of other tissues [8]. AmpliTaq Gold polymerase (Perkin Elmer, U.S.A.) was used in PCR for amplification of the canine tektin cDNA. The conditions of the PCR were set as 9 min at 94°C for an initial hot start followed by 30 cycles of 1 min denaturation at 94°C, 1 min annealing at 60°C and 1 min extension at 72°C with a 10 min final extension at 72°C and soak at 4°C. The product was cloned in pCR2.1 vector (Invitrogen Corporation, CA, U.S.A.) and subjected to sequence analysis by using Thermo Sequenase Pre-mixed Cycle Sequencing Kit (Amersham Pharmacia Biotech, Uppsala, Sweden).

To amplify the 3' end of cDNA encoding canine tektin, the rapid amplification of the 3' cDNA ends (3' RACE) was performed by using a 3' RACE System Kit (GIBCOBRL, U.S.A.). The 5' primer 5'-CAGTGTGTGAGAGTTGAGC-3' was designed on the basis of the canine tektin cDNA sequence obtained at position 817–836. The adaptor primer provided with the kit was used as the 3' primer. The amplified PCR products were cloned in pCR2.1 vector and subjected to sequence analysis.

To amplify the 5' end of cDNA of canine tektin, 5'-Full RACE Core Set Kit (TaKaRa, Japan) was used. The primer for the synthesis of the first cDNA strand was 5'-CAGGGC-CATGA-3' (position number 648–658 of the canine tektin cDNA). For the amplification, 5'-TGAAGGAGGCTGAG-GTCAT-3' as sense primer (position number 621–639 of canine tektin cDNA) and 5'-CTGTTCCACCTCATCAT-GCA-3' as anti-sense primer (position number 594–613 of canine tektin cDNA) were used. The amplified PCR products were cloned in pCR2.1 vector and subjected to sequence analysis.

By using 5'-TGCTCGGGACTTTGGAAAGCA-3' as sense primer (position number 188–208 of canine tektin cDNA) and 5'-ACGGTGACCAGGGTTTAAT-3' as anti-sense primer (position number 1488–1506 of canine tektin cDNA), the coding region of tektin cDNA was amplified and

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acaccaatgtggaggggagacgcgcgggtttacccgcgggtctgcgggggcccgcagctgcaggtgggtgccaacctgtg	80
ttcagcggttcccgaagcgcacggctagtgcaggcggcccactctctgaccccctgcctggaaccccgccagcccccg	160
ccggcctcctctccacctccctgggctgctcgggactttggaagcagaaatggccaaactcctccaaccttcacccaa	240
M A K L L Q P S P K	10
gttcttgcctgaagagtggcacatcgctaacaagaaccagtaccacagagcagaggcccaaagggtcccggtccgaacgcc	320
F L P E E W H I A N K N Q Y H R A E A Q R S R S E R	36
tggtggcagaaagccagaggcttgtggatgagattgaaaagaccacaagaaagtctcagagcgatgtaacaagaaacta	400
L V A E S Q R L V D E I E K T T R K S Q S D V N K K L	63
gaacagagactcgaggaagtgcaggttctggaagaaggagctggatgacaagctggagcagctagtgtgcaccacagagga	480
E Q R L E E V R F W K K E L D D K L E Q L V C T T E D	90
cctgctcacttatcagactaggctgcagaacgccttgagagcctgaaggagcccctgcacatcacccagatgtgcctgg	560
L L T Y Q T R L O N A L E S L K E P L H I T Q M C L	116
aatacagggacaagcgcattggcatcgacctgggtgcattgaggtggaacaggagctgctgaaggaggctgaggtcatc	640
E Y R D K R I G I D L V H D E V E Q E L L K E A E V I	143
cacggggtcatggccctgctgacccgcacccatggatgaggtgactgagcagatcaggctgaaccgctccgccaagtacaa	720
H G V M A L L T R T M D E V T E Q I R L N R S A K Y N	170
cctagagaaggatttgaaggacaagtttggccttgaccatcgacgatgtttgcttctccctcaacaacaactccccag	800
L E K D L K D K F V A L T I D D V C F S L N N N S P	196
gcattactactcggacagtggtgtgagagttgagccacactccgtgagctcggaggactggctggacttctccaacacc	880
G I Y Y S D S V V R V E P H S V S L E D W L D F S N T	223
aattgtggagaaggcgaacaggcagagaaacaactccctggcgctgaaggccctgggtggaccggatcctgtcccagacagc	960
N V E K A N R Q R N N S L A L K A L V D R I L S Q T A	250
tgacgacctgcgcaggcagtgatgacatgggtggacacggcattccagatggggctgaaggagaccaaggccgagggacc	1040
D D L R R Q C D M V D T A F Q M G L K E T K A A R D	276
agctggctgccacactggccaaggtcatggaagagatcgcatgccaggagaagaacatgacggttctggagaaggccatc	1120
Q L A A H L A K V M E E I A C Q E K N M T V L E K A I	303
cttgatcaagaggggctgccaaggtggctcacacgcgcctggagacccgaacacgcaggcccaacgtagagctgtgccg	1200
L D Q E G P A K V A H T R L E T R T R R P N V E L C R	330
cgatgtcgcacagtaccggctggtcaaggaagtcggagagattgccagaatgtcggaaggctgaaggaggccctggcac	1280
D V A Q Y R L V K E V G E I A Q N V G R L K E A L A	356
aggcgcaggtggagctgaaggccctgaaccgcccggcaactggcgctgcaggaggaagtccaggtcaaggagaacaccatc	1360
Q A Q V E L K G L N R R Q L A L Q E E V Q V K E N T I	383
tacatcgaccgagtgctctgcacccacatgaggaagtccaatcccctgcgggatgggggcgaccaaggccagtgggccag	1440
Y I D R Y L C T H M R K S N P L R D G G D Q G Q W A R	410
ggcctgcgccccgacaccatctgctgaggatggcacttcacacactgattaaaccctggtcaccgtcaaaaaaaaaa	1520
A C A P T P S A E D G T S H T D *	426

Fig. 1. Nucleotide and deduced amino acid sequence of canine tektin (GenBank accession number: AF191546). Asterisk is positioned to show the stop codon. Underlined sequences are predicted to form helices. The conserved sequence RPNVELCRD of tektins is boxed. Four conserved cysteine residues are shadowed.

sequenced. Canine tektin cDNA is 1,523 bp (GenBank accession number: AF191546) in length, and has an open reading frame of 1,281 bp nucleotides encoding a protein of

426 deduced amino acids. Tektins are predicted to form extended rods composed of two helical segments capable of forming coiled coils, which are interrupted by short nonheli-

canine tektin	1	-----MAKLLQ	6
murine tektin	1	-----*****	6
tektin A	1	MDAGATLLSRSYAPTIPVYPTQTTVGTKTDQALSQDLAKMSGLGETGVYGVPTGAP*AQGF	61
tektin B	0	-----	0
tektin C	1	-----S**IK	6
canine tektin	7	PSPKFLPEEWHIANKNQYHRAEAQRSRSERLVAESQRLVDEIEKTRKSQSDVNKKLEQRL	67
murine tektin	7	SP*R*****Y*****S*****Q*****SV*E*****	67
tektin A	62	R*G*HTTQ**ES*Y*K*FQSFTD*DNA**CH**KQ*SN*THAL*MRT*A**T**GD*M	122
tektin B	1	-----MLRNSVITPTPPAK--NLASFVTKPTTDI-MGYH-*NTTR*SN*I	41
tektin C	7	APQR*THG**NYS*HAN*NS**K*ASA**ID**D*I**TDEA*K*T*R*****F****	67
canine tektin	68	EEVRFWKKELDKLEQLVCTEDLLTYQTRLQNALESLEKPLHITQMCLEYPDKRIGIDL	128
murine tektin	68	*****N*****Q*D*****K*****ERS***Y*****EK*****E**V*****	128
tektin A	123	NDIN**F**NREI*EMIEE*DL*CAQKK**E***DATEV**K*ARDN*TC*SR*QD****	183
tektin B	42	DDIET*RET*EKT*ADVDEERK*EEDKDLAER**AKAL*DVASE*KTLL**G*RDN*V*	102
tektin C	68	DD*TY**D**R**KDSKDEI*M**A*K*****E*****ACR***A*VNQ**ND*EG*****	128
canine tektin	129	HDEVEQELLKEAEVIHGVMAALLTRTMDEVTEQIRLNRS AKYNLEKDLKDKFVALTIDDVCF	189
murine tektin	129	**V*****Q***DI*****N**I**LE*****R***T*I*****	189
tektin A	184	G*R**MA*N**VDI*TK*QD**K**LEQSD**K***GS*HK*TM*WS**LS*FK**EK*T	244
tektin B	104	D*LANS*VG**ID**E*IKDA*QAKVSSAF**LC*LQE*RQQ*HA**R**TE*KK**TY*H	163
tektin C	129	**D**KN***R***M*****QK*L*Q*I*****IM**RR*****T***G**D**QD*R	189
canine tektin	190	SLNNNSPGIYSDSVVRVEPHSVSLEDWLD FSNTNVEKANRQRNNSLALKALVDRILSQTA	250
murine tektin	190	*****N*NF*EK***I**N*****A*****DK*L***T***T***Q*****	250
tektin A	245	G*****TE*Q*KEGSAKF*AVQTN PQS*AE**HD**VR*EHE*LA*QQ*RN*I*Q**TD*S	305
tektin B	164	D*TIS**D*C*QPNST*TPKG*TTPT*E***RY*KDR*DAEMRS*QR*REAIHSTVA**D	224
tektin C	190	N*RDDNSTLQFKGG*AKI*TN**TP***QS***E*IL**E*D*K**AD*R*V**SL*KT**	250
canine tektin	251	DDLRRQCDMVDTA FQMG LKETKAARDQLAAHLAKVMEEIACQEKNTVLEKAILDQEGPAK	311
murine tektin	251	N*****EV**E**IN*****D**NK**D*****S*****IMA**N**TQ*****	311
tektin A	306	N*M*E**NT*N*E*ARRIE*MND*KTKMEN**L*TV*D**GM**IKD*TQ*VK*K*A*M*	366
tektin B	225	N**EA*RQATEF*LRKRIH*M*R*K*EDEWQKKNTE***K*R*IRE**Q**K*N*L*	285
tektin C	251	**MQQ*V*DTNL**SKRIR**DMTKGK*ET*****EGQMREM*E*IQK*Q*GVD*KM**K*	311
canine tektin	312	VAHTRLETRTRRPNVELCRDVAQYRLVKEVGEIAQNVGRLKEALQAQVELKGLNRRQL-A	371
murine tektin	312	*****H*****I*****I**I*Q**NH**A*****T*****TQ**A*Y*****	371
tektin A	367	**Q***DH**E*****P*****M*Q*****QDSIDK*QK**ESKAS**D*MDTRM--	426
tektin B	286	L*M*****N**Y*****N*****G**N**H**QDSIKA*EKK*QD*HNA-RDACEK**YR	345
tektin C	312	LSE***DA**N*****PV*****IG**T**NTSIA**QAT*****S*****I*N**N	371
canine tektin	372	LQEEVQVKENTIIYIDRVLC THMRKSNFLRDGGDQGGQWARACAPTPSAEDGTSHTD-	426
murine tektin	372	***I*****T*****Q**ME*****I**P***D**H*A*EG*S*QR**DRSH*EPY-	426
tektin A	427	*EK*I*AL*K*S*FV**DK*LKF*TRY---P-S-T-SKLVGYQ-----	462
tektin B	346	INKDLEL*N*SLDL*-NKMQV*EKLTTGPVTQTMNNLSTFKTDRELYTAQRSLA	400
tektin C	372	***DIDI*SQSLNV*DTQ*MTL*R*INIKR-Y-----	402

Fig. 2. Comparison of amino acid sequence of canine tektin with murine tektin (GenBank accession number AF081947) and sea urchin tektins A (GenBank accession number M97188), B (GenBank accession number L21838), and C (GenBank accession number AAB02680). Asterisk indicates residues similar to those of canine tektin. Box denotes the common sequence RPNVELCRD conserved in three species. Four conserved cysteine residues are shadowed.

cal linkers [10]. Computer analysis showed that 61% of amino acid residues of canine tektin form the helical segments, whereas four highly conserved cysteine residues are present in nonhelical linkers and C terminus (at positions 115, 180, 329 and 390). The sequence RPNVELCRD, likely to form a functionally important protein domain in tektins [8, 10] was observed at position number 323–331 of amino acid sequence (Fig. 1).

Comparison of the amino acid sequence of the predicted protein with sea urchin tektin A, B, C, and mouse tektin revealed that the canine tektin has 77%, 41%, 33% and 50% of homology with mouse tektin and sea urchin tektin A, B, and C, respectively. The homology between canine tektin

and sea urchin tektin C (50% identity) is greater than the similarity (33–42% identity) between any pair of sea urchin tektins, suggesting that the canine tektin may correspond to sea urchin tektin C (Fig. 2).

It is concluded that 1,523 bp of a canine tektin cDNA was identified, which has an open reading frame of 1,281 bp nucleotides encoding a protein of 426 amino acids. The predicted amino acid sequence has 77% and 33–50% of homology with the murine tektin and the sea urchin tektins, respectively. The amino acid sequence RPNVELCRD and four cysteine residues were conserved in the dog, mouse and sea urchin, thus may play an important role in the structure and the function of tektin proteins.

REFERENCES

1. Bost-Usinger, L., Chen, R. J., Hillman, D., Park, H. and Burnside, B. 1997. *Exp. Eye. Res.* 64: 781–794.
2. Chen, R., Perrone, C. A., Amos, L. A. and Linck, R. W. 1993. *J. Cell. Sci.* 106: 909–918.
3. Gibbons, I. R. 1995. *Cell. Motil. Cytoskeleton.* 32: 136–144.
4. Linck, R. W., Albertini, D. F., Kenney, D. M. and Langevin, G. L. 1982. *Prog. Clin. Biol. Res.* 80: 127–132.
5. Linck, R. W., Amos, L. A. and Amos, W. B. 1985. *J. Cell. Biol.* 100: 126–135.
6. Linck, R. W. and Langevin, G. L. 1982. *J. Cell. Sci.* 58: 1–22.
7. Nojima, D., Linck, R. W. and Egelman, E. H. 1995. *Curr. Biol.* 5: 158–167.
8. Norrander, J., Larsson, M., Stahl, S., Hoog, C. and Linck, R. 1998. *J. Neurosci.* 18: 8912–8918.
9. Norrander, J. M., Amos, L. A. and Linck, R. W. 1992. *Proc. Natl. Acad. Sci. U. S. A.* 89: 8567–8571.
10. Norrander, J. M., Perrone, C. A., Amos, L. A. and Linck, R. W. 1996. *J. Mol. Biol.* 257: 385–397.
11. Pirner, M. A. and Linck, R. W. 1994. *J. Biol. Chem.* 269: 31800–31806.
12. Porter, M. E. 1996. *Curr. Opin. Cell. Biol.* 8: 10–17.
13. Steffen, W. and Linck, R. W. 1988. *Proc. Natl. Acad. Sci. U.S.A.* 85: 2643–2647.
14. Stephens, R. E. and Lemieux, N. A. 1998. *Cell. Motil. Cytoskeleton.* 40: 379–392.
15. Stephens, R. E., Oleszko-Szuts, S. and Linck, R. W. 1989. *J. Cell. Sci.* 92: 391–402.