Research in Veterinary Science 95 (2013) 1021-1025

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc



Molecular cloning and expression analysis of pig CD138

Joonbeom Bae^a, Seonah Jeong^a, Ju Yeon Lee^a, Hyun-Jeong Lee^b, Bong-Hwan Choi^b, Ji-Eun Kim^c, Inho Choi^{c,*}, Taehoon Chun^{a,*}

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^a Division of Biotechnology, School of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Republic of Korea ^b Division of Animal Genomics and Bioinformatics, National Institute of Animal Science, Rural Development Administration, Suwon 143-13, Republic of Korea ^c School of Biotechnology, Yeungnam University, Gyeongsan City 712-749, Republic of Korea

ARTICLE INFO

Article history: Received 6 August 2013 Accepted 20 September 2013

Keywords: cDNA cloning CD138 Expression Pig Syndecan

ABSTRACT

CD138 (syndecan-1) interacts with various components of the extracellular matrix and associates with the actin cytoskeleton. In this study, we cloned pig CD138 cDNA and determined its complete cDNA sequence. Pig CD138 cDNA contained an open reading frame (930 bp) encoding 309 amino acids with five well conserved putative glycosaminoglycan attachment sites, a putative cleavage site for matrix metalloproteinases, and conserved motifs involved in signal transduction among mammalian species. Pig CD138 mRNA was detected in various tissues, including lymphoid and non-lymphoid organs, indicating the multicellular functions of CD138 in pigs. Western blot and flow cytometry analyses detected an approximate 35 kDa pig CD138 expression was mainly observed in submucosa and lamina propria of the pig small intestine. Further study will be necessary to define the functional importance of CD138 during specific infectious diseases in pigs.

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Syndecans are type I transmembrane proteins consisting of a core polypeptide attached with heparin sulfate proteoglycans as an extracellular domain that interacts with various components of the extracellular matrix and associates with the actin cytoskeleton (Couchman, 2010; Teng et al., 2012). Additionally, syndecans interact with many heparin or heparin sulfate binding molecules (Bernfield et al., 1999; Fears and Woods, 2006). Therefore, syndecans are biological significant for their connecting the extracellular matrix to the intracellular cytoskeleton and by regulating many biological activities through specific ligand-receptor interactions. Four isoforms of syndecan proteins have been identified in mammals (Teng et al., 2012). Each protein is encoded by a distinct gene and has a highly conserved transmembrane and cytoplasmic tail with at least three attachment sites for heparin sulfate (Teng et al., 2012). CD138 (syndecan-1) is the first identified protein among mammalian syndecans (Saunders et al., 1989) and is expressed mainly on epithelial cells (Teng et al., 2012). However, CD138 is highly expressed in precursor B cells and plasma cell stages during B cell maturation in secondary lymphoid tissues (Sanderson et al., 1989).

The functional roles of CD138 have been proposed to include cell growth, cell migration, and tissue remodeling because CD138 interacts with many cell surface molecules expressed on particular cells (Teng et al., 2012). Indeed, CD138 negatively regulates leukocyte infiltration by inhibiting the interactions between integrins and their binding partners such as intercellular adhesion molecule 1 and vascular cell adhesion molecule 1 during inflammation (Kharabi Masouleh et al., 2009). Some studies have also shown that CD138 inhibits chemokine secretion by activated leukocytes (Li et al., 2002; Xu et al., 2005; Hayashida et al., 2009). Therefore, CD138 has anti-inflammatory activities during the development of several inflammatory diseases such as allergic lung disease, endotoxic shock, and vascular injury (Xu et al., 2005; Fukai et al., 2009; Hayashida et al., 2009). During early infection by several pathogens, CD138 serves as a receptor that facilitates initial attachment and subsequent entry of pathogens into host cells (Bhanot and Nussenzweig, 2002; Kalia et al., 2009; Bacsa et al., 2011) and subverts host defense mechanisms by inhibiting activation of innate immune cells (Park et al., 2001; Hayashida et al., 2011). Therefore, CD138-deficient mice are more resistant to several viral and bacterial pathogens, compared with those of normal mice (Teng et al., 2012). Many viral and bacterial pathogens share the same niche when reproducing in mammalian species. Therefore, identifying other examples of mammalian CD138 may be important to elucidate the functional role of CD138 during infection by specific pathogens and analyze the structural relationship between the receptor-ligand interaction for host cell entry.

In this study, we cloned and determined the full-length cDNA sequence of pig CD138 and analyzed pig CD138 mRNAs in various pig organs, according to the detailed "Materials and methods" in



Abbreviation: Gapdh, glyceraldehyde-3-phosphate dehydrogenase.

^{*} Corresponding authors. Tel.: +82 2 3290 3069; fax: +82 2 3290 3499. *E-mail addresses:* inhochoi@ynu.ac.kr (I. Choi), tchun@korea.ac.kr (T. Chun).

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(B)

D)						
	1 ← signa	al peptide -		E0	CD	
Pig CD138	MRRAAFWLWL	CALALRLQPA	LLETVATNVP	PEDQDGSGDD	SDNFSGSGAG	ALPDVTLSQQ
Cow CD138	MRRAALWLWL	CALALRLQPA	LLHSVAVNMP	PEDQDGSGDD	SDNFSGSGAG	ALPDIT.SSH
Human CD138	MRRAALWLWL	CALALSLQPA	LPQIVATNLP	PEDQDGSGDD	SDNFSGSGAG	ALQDITLSQQ
Chimpanzee CD138	MRRAALWLWL	CALALSLQPA	LPQIVATNLP	PEDQDGSGDD	SDNFSGSGAG	
Rhesus CD138	MRRAALWLWL	CALALSLQPA	MPQIVATNLP	PEDQDGSGDD	SD N FSGSGAG	ALQDITLSQQ
Aouse CD138	MRRAALWLWL	CALALRLQPA	LPQIVAVNVP	PEDQDGSGDD	SDNFSGSGTG	ALPD.TLSRQ
Rat CD138	MRRAALWLWL	CALALRLQPA	LPQIVTANVP	PEDQDGSGDD	SD N FSGSGTG	ALPDMTLSRQ
Hamster CD138	MRRAALWLWL	CALALRLQPV	LPQIMAVNVP	PEDQDGSGDD	SDNFSGSGTG	ALPDITLSRQ
				•	• •	
	61		EC	CD		120
Pig CD138	TPSTWKDTGL	LTTMPTAPEP	TSPDTVATST	SVLPAGERPG	RGRAVLL.DL	DPGLTAQE
Cow CD138	TPSTWKDLGP	VTTTATAPEP	TSPDAIAAST	TILPTGEQPE	GGRAVLLAEV	EPGLTAQ
Human CD138	TPSTWKDTQL	LTAIPTSPEP	TGLEATAAST	STLPAGEGPK	EGEAVVLPEV	EPGLTARE
Chimpanzee CD138					EVVMG	
Rhesus CD138	TPSTWKDTWL	VRATPMSPEP	TGLEATAAST	STIQAGEGPK	EGEAVVLLEV	EPDLTARE
Aouse CD138	TPSTWKDVWL	LTATPTAPEP	TSSNTETAFT	SVLPAGEKPE	EGEPVLHVEA	EPGFTARDKE
Rat CD138	TPSTWKDVWL	LTATPTAPEP	TSRDTEATLT	SILPAGEKPE	EGEPVAHVEA	EPDFTARDKE
Hamster CD138	TSSTLKDVWL	LTATPTAPEP	TSRDTEATFT	SILPAGEKPG	EGEPVLIAEV	DTSSTTWDKE
	121		EC	CD		180
Pig CD138	KEATHSPSET	TQHPTTHRAS	T.AGATTAQV	PATSHPHRDV	PPDHPETSAP	AGHGQLDPHT
Cow CD138	KEATHPPSET	TLHPTTHSVS	T.ARATMAPG	PATSHPHRDV	QPDHHETSAP	TGRGRMEPHR
Human CD138	QEATPRPRET	TQLPTTHQAS	T.TTATTAQE	PATSHPHRDM	QPGHHETSTP	AGPSQADLHT
Chimpanzee CD138	APRPK	T.LPTTHQAS	T.TTATTAQE	PATSHPHRDM	QPGHHETSTP	AGPSQADLHT
Rhesus CD138	QEATPQPTET	TQLPTTHQAP	T.ARATTAQE	PATSHPHRDM	QPGHHETSAP	AGPGQADLHT
4ouse CD138	KEVTTRPRET	VQLPITQRAS	T.VRVTTAQA	AVTSHPHGGM	QPGLHETSAP	TAPGQPDHQP
Rat CD138	KEATTRPRET	TQLPVTQQAS	TAARATTAQA	SVTSHPHGDV	QPGLHETLAP	TAPGQPDHQP
Hamster CD138	LEVTTRPRET	TQLLVTHRVS	T.ARATTAQA	PVTSHPHRDV	QPGLHETLAP	TAPGQPDHQP
			_	_		
	181		E0	CD		240
Pig CD138	PGVGDGGPAT	TEKAAEGEAS	TQLPVGEGSG	EPDFTFDVSG	ENTAGDALDP	DQRNEPP
Cow CD138	PHVEEGGPPA	TEKAAEEDPS	TQIPVGEGSG	EQDFTFDLSG	ENAAGAAGEP	GSRNGAPEDP
Human CD138	PHTEDGGPSA	TERAAEDGAS	SQLPAAEGSG	EQDFTFETSG	ENTAVVAVEP	DRRNQSP
Chimpanzee CD138	PHTEDGGPSA	TERAAEDGAS	SQLPAAEGSG	EQDFTFETSG	ENTAVVAVEP	DHR N QSP
Rhesus CD138	PRTEDGGPSA	TERAAEDGAS	SQLPAAEGSG	EQDFTFETSG	ENTAIVAVEP	DHR N QSP
Mouse CD138	PRVEGGGTSV	IKEVVEDGTA	NQLPAGEGSG	EQDFTFETSG	ENTAVAAVEP	GLRNQPP
Rat CD138	PSVEDGGTSV	IKEVVEDETT	NQLPAGEGSG	EQDFTFETSG	ENTAVAGVEP	DLR N QSP
Hamster CD138	PSGGTSV	IKEVAEDGAT	NQLPTGEGSG	EQDFTFETSG	ENTAVAAIEP	DQRNQPP
			•		~	
	241 ← E	CD	- 1	M		Y 300
Pig CDI38	VDQGTTGASQ	SLLDRKEVLG	GVIAGGLVGL	IFAVCLVGFM	LYRMKKKDEG	S <u>Y</u> SLEEPKQA
COW CD138	EATGATGASQ	GLLDRKEVLG	GVIAGGLVGL	IFAVCLVGFM	LYRMKKKDEG	S <u>Y</u> SLEEPKQA
Human CD138	VDQGATGASQ	GLLDRKEVLG	GVIAGGLVGL	IFAVCLVGFM	LYRMKKKDEG	S YSLEEPKQA
Chimpanzee CD138	VDQGATGASQ	GLLDRKEVLG	GVIAGGLVGL	TRAVCLVGFM	LYRMKKKDEG	S <u>Y</u> SLEEPKQA
Anesus CDIS8	VDPGATGASQ	GLLDRKEVLG	GIIAGGLVGL	TFAVCLVGFM	LIRMAKADEG	S ISLEEPKQA
Mouse CD138	VDEGATGASQ	SLLDRKEVLG	GVIAGGLVGL	IFAVCLVAFM	LYRMKKKDEG	S <u>Y</u> SLEEPKQA
Rat CDIS8	VDEGATGASQ	GLLDRKEVLG	GVIAGGLVGL	TRAVCLVARM	LIRMAKADEG	S I SLEEPKQA
Hamster CD138	VDEGATGASQ	GLEDRKEVEG	GVIAGGLVGL 00000	IFAVCLVGFM	LIRMARADEG	S I SLEEPKQA
	301 — CV					
Dia (D120	NCCAVORDED	OFFEVA				
CDIDE	NGGA <u>IQ</u> KFSK	QEEF IA				
JUW CDIDO	NGGAIQKFIK	QEEF IA				
Human CD138	NGGA <u>IQ</u> KPTK	QEEF <u>I</u> A				
Inimpanzee CD138	NGGA <u>IQ</u> KPIK	QEEF <u>I</u> A				
Anesus CD138	NGGA <u>IQ</u> KPTK	QEEF <u>I</u> A				
Mouse CDISS	NGGA <u>IQ</u> KPIK	QEEF IA				
Rat CDIS8	NGGA <u>IQ</u> KPIK	QEEF <u>I</u> A				
Hamster CD138	NGGA <u>Y</u> QKPTK	QEEF <u>Y</u> A				
()						
() Mw	MLN M	к н	Lv Lu S	ip T Si	Li	
500 bp						
400 bp	Marrow and				→ →	Cd138
300 bp -		_				Sandh
200 bp -	1				- (ларан

Fig. 1. cDNA cloning and mRNA expression analyses of pig *Cd138*. (A) Phylogenetic analysis of pig CD138 with other mammalian species. The phylogenetic tree was constructed with the DNAMAN software package using each CD138 deduced amino acid sequence with 1000 trials of bootstrap analyses. Very high homology was generally observed among mammalian species. (B) Alignment of putative amino acid sequences of pig CD138 with other mammalian species. Alignment of putative amino acid sequences of pig CD138 with other mammalian species. Alignment of putative cD138 (GenBank accession NM_001006946), chimpanzee CD138 (GenBank accession XM_001140545), rhesus monkey CD138 (GenBank accession HF677512), human CD138 (GenBank accession NM_001006946), chimpanzee CD138 (GenBank accession NM_01140545), rhesus monkey CD138 (GenBank accession XM_001095194), mouse CD138 (GenBank accession NM_011519), rat CD138 (GenBank accession NM_013026), cow CD138 (GenBank accession NM_001075924), and hamster CD138 (GenBank accession L38991) are shown below. The putative glycosaminoglycan attachment sites within the ECD are indicated as black circles and the putative dimerization site (GGLVG²⁶⁵⁻²⁶⁹ sequence) within TM is indicated as a white circle. The putative cleavage site for matrix metalloproteinases within the ECD (Arg²⁵⁵ and Lys²⁵⁶) is indicated by an arrow and putative *N*-glycosylation sites are indicated in bold. Three tyrosine residues possibly phosphorylated within CY are underlined, and the binding motif of the PDZ domain within CY is indicated with an asterisk. ECD, extracellular domain; TM, transmembrane; CY, cytoplasmic tail. (C) Expression analyses of pig CD138 mRNA transcripts from various tissues. Total RNA was isolated from each tissue and used as template for the reverse transcription-polymerase chain reaction. *Gaph* was used as the internal control. Mw, molecular weight; MLN, mesenteric lymph node; M, muscle; K, kidney; H, heart; Lv, liver; Lu, lung; Sp, spleen; T, thymus; Si, small intestine; Li, large intestine.

the Supplementary file. Then, a phylogenetic tree was constructed using the deduced amino acid sequences of the CD138 molecules thus far identified among mammalian species. The amino acid sequence identity of pig CD138 deduced with that of cow was 81%, which shared the highest degree of homology (Fig. 1A). The identity of the pig CD138 deduced amino acid sequence with those

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