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Molecular cloning and expression analysis of pig CD138

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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 protein expressed on the cell surface. Further immunohistochemistry analysis revealed that 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.

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.

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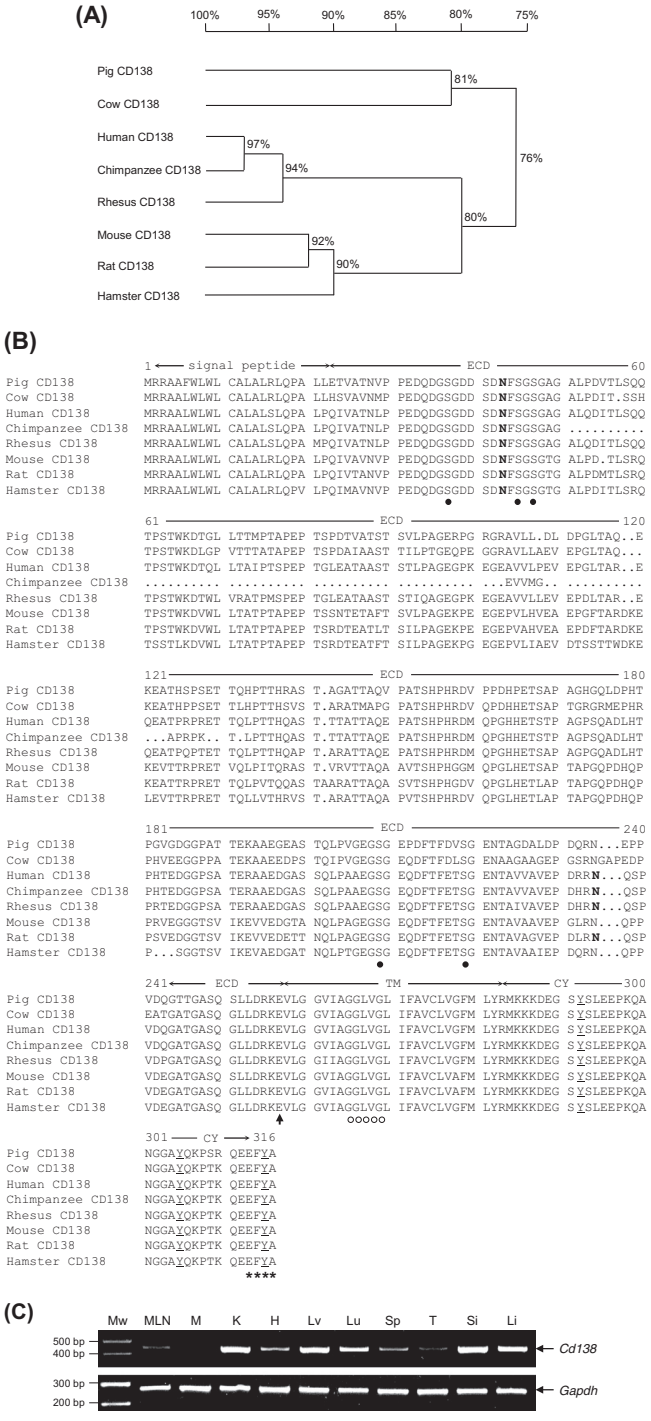

Fig. 1. cDNA cloning and mRNA expression analyses of pig *Cd138*. (A) Phylogenetic analysis of pig CD138 with other mammalian species. The phylogenetic tree was constructed with the DNAMAN software package using each CD138 deduced amino acid sequence with 1000 trials of bootstrap analyses. Very high homology was generally observed among mammalian species. (B) Alignment of putative amino acid sequences of pig CD138 with other mammalian species. Alignment of putative CD138 amino acid sequences among mammalian species. Amino acid sequences for pig CD138 (EMBL accession HF677512), human CD138 (GenBank accession NM_001006946), chimpanzee CD138 (GenBank accession XM_001140545), 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 ($GGLVG^{265-269}$ sequence) within TM is indicated as a white circle. The putative cleavage site for matrix metalloproteinases within the ECD (Arg^{255} and Lys^{256}) 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. *Gapdh* 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