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Short communication

Molecular cloning and expression analysis of pig CD81

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Abstract

CD81, also known as TAPA-1 (target of antiproliferative antibody 1), is a member of the tetraspanin family of proteins and a component of the B cell co-receptor complex. Several studies have shown that CD81 plays significant roles in a variety of immune responses, including activation of B cells and T cells. In this study, we cloned pig *Cd81* cDNA using RT-PCR coupled with rapid amplification of cDNA ends (RACE)-PCR and determined the complete cDNA sequence of pig *Cd81*. Pig *Cd81* cDNA contains an open reading frame (711 bp) encoding 236 amino acids. The identity of pig CD81 with those of human, cattle, rat, and mouse are 90.30%, 92.26%, 86.22%, and 86.22%, respectively. Alignment of the CD81 amino acid sequence with those of mammalian species showed that the large extracellular loop (LEL) is the most divergent, whereas other domains are largely conserved. Pig *Cd81* mRNA was detected by RT-PCR in a broad range of tissues, including lymphoid tissues as well as nonlymphoid tissues, indicated variety of cellular functions of CD81 in most pig tissues. Flow cytometry analyses demonstrated that human CD81 antibody recognizes a pig CD81 on the cell surface. Further, immunohistochemistry analysis using human CD81 antibody on pig spleen was revealed that CD81 expression is widely diffused in spleen tissue. Future study will be focused on defining the functional role of CD81 during the course of pig infectious diseases.

Keywords: B cell; CD81; Co-receptor complex; Pig; Tetraspanin

1. Introduction

CD81 was originally identified as the target of an antiproliferative antibody (TAPA-1) and defined a new transmembrane protein family (Oren et al., 1990). Structurally, CD81 is a member of a tetraspanin family that forms four transmembrane regions and plays significant roles in various immune responses. For example, CD81 forms a B cell co-receptor complex with CD19, CD21, Igα, and Igβ, and enhances B cell signaling during the process of B cell activation (Pierce,

Abbreviations: CHO, Chinese hamster ovary; Con A, concanavalin A; HCV, hepatitis C virus; ID, intracellular domain; LEL, large extracellular loop; LPS, lipopolysaccharide; PHA, phytohaemagglutinin; RACE, rapid amplification of cDNA ends; SEL, small extracellular loop; TAPA-1, target of antiproliferative antibody 1; TM, transmembrane

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2002). In addition to its co-stimulatory effects on B cells, CD81 has been shown to be closely related to T cell activation. The association of CD81 with CD3 increases T cell proliferation and expression of activation markers, such as CD25 and CD69 (Witherden et al., 2000). The intensity of the T cell response is similar to those induced by co-engagement of CD3 and CD28 (Witherden et al., 2000). Furthermore, impaired Th2 responses of CD81-deficient mice revealed the relevance of CD81 in the Th2 response (Maecker et al., 1998). In regards to the T cell response, CD81 plays a critical role in T–B cell interactions that induce Th2 responses (Deng et al., 2002). Its other important function is found in the immunological synapse between T cell and APCs. CD81 is redistributed and accumulated in the contact area of both T cells and APCs (Mittelbrunn et al., 2002). This result may indicate that CD81 is important for the interaction between T cells and APCs for T cell activation. Moreover, CD81 is known to be a receptor for two important human pathogens. The malaria-causing *Plasmodium falciparum* sporozoites fail to infect hepatocytes from CD81-deficient mouse, although the precise receptor for the entry of *P. falciparum* into hepatocytes is not defined (Silvie et al., 2003). CD81 also serves as a receptor entry on hepatocytes for the hepatitis C virus (HCV) by interacting with the envelope glycoprotein E2 of HCV (Pileri et al., 1998). Therefore, CD81 can be used as a modulating agent in clinical strategies.

In this study, we cloned and sequenced full-length cDNA of pig CD81, and examined the tissue expression of pig CD81 mRNAs. We also tested whether anti-human CD81 antibody recognizes pig CD81 and examined pig CD81 expression in pig spleen by immunohistochemistry using anti-human CD81 antibody. These results could provide valuable molecular information to be used to study the role of CD81 in a pig immune system.

2. Materials and methods

2.1. Generation of cDNA sequence and gene expression analyses of pig Cd81

The pig *Cd81* cDNA sequence was generated by RT-PCR coupled with rapid amplification of cDNA ends (RACE)-PCR. In brief, total RNAs from splenocytes of domestic female pig (18 weeks, female, Duroc) were isolated using a guanidinium extraction method (Chomczynski and Sacchi, 1987) and reverse transcribed using M-MLV reverse transcriptase (Promega, USA) and random hexamers (Invitrogen, USA). After cDNA synthesis, PCR was performed with a PTC-100 Thermal Cycler instrument (MJ Research, USA) for 30 cycles of 1 min at 94 °C, 1 min at 55 °C, and 2 min at 72 °C, followed by a 10 min final extension step at 72 °C. Primers for pig *Cd81* were designed based on the cDNA sequences of human *Cd81* (GenBank Accession NM004356) and mouse *Cd81* (GenBank accession AK166521). Primer sequences were 5′-TTCGTCTT-CTGGCTGGCTGGAG-3′ (sense), 5′-GGCCTCAG-TACACGGAGCTGTTC-3′ (anti-sense), resulting in a 661 bp RT-PCR product. To confirm that these RT-PCR products were pig CD81, we also performed the nested PCR. Primer sequences for nested PCR were 5′-CCAGACTACCAGCCTCCTCTACCT-3′ (sense), 5′-TCAATCTTGCTGTGGCAGTCCTCC-3′ (anti-sense), resulting in a 467 bp RT-PCR product. PCR products were then isolated and subcloned into the pGEM-T vector (Promega) by the TA cloning method (Clark, 1988) and sequenced by the dideoxy-mediated chain termination method (Sanger et al., 1973) using a 310 automatic sequencer (ABI, USA). To obtain a 5′ cDNA end of the pig *Cd81* coding region, we performed 5′ RACE-PCR using a 5′-Full RACE Core set (TaKaRa, Japan) according to the instructions of the manufacturer. After 5′ RACE-PCR, the product was cloned using pGEM-T vector and sequenced as described above. The pig *Cd81* cDNA sequence was derived from three independent clones of three different animals.

For detecting *Cd81* mRNA transcripts from various pig tissues, we isolated total RNAs from various tissues of age, sex, and strain matched three individual pigs (18 weeks, female, Duroc). In addition, splenocytes were stimulated with 1 μg/ml of LPS (*E. coli* 0127:B8; Sigma, USA), 1 μg/ml of Con A (Sigma), or 10 μg/ml of PHA (Sigma), respectively, to measure the level of *Cd81* mRNA expression in both unstimulated and stimulated splenocytes. After isolation of total RNAs from various tissues and splenocytes, RT-PCRs were performed as described above using the nested PCR primers yielding a 467 bp RT-PCR product, except performing PCR reactions for 25 cycles instead of 30 cycles. Glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) primers were used as an internal control in equal amount to that of mRNA used. Primer sequences for pig *Gapdh* were 5′-ATGACCACAGTCCATGC-CATC-3′ (sense), 5′-CCTGCTTCACCACCTTCTTG-3′ (anti-sense), resulting in a 271 bp RT-PCR product. Resulting PCR products were loaded on a 1.5% agarose gel containing ethidium bromide and visualized by ultraviolet light.