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Short communication

# Molecular cloning and expression analysis of pig CD79α

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**Abstract**

The CD79α (immunoglobulin α, Igα), a part of B cell receptor (BCR) complex, forms a heterodimer with CD79β (Igβ) and plays an important role in the B cell signaling. In this study, we have cloned pig *Cd79a* cDNA using RT-PCR and determined the complete cDNA sequence of pig *Cd79a*. Pig *Cd79a* cDNA contains an open reading frame (672 bp) encoding 223 amino acids. The putative amino acid identity of pig CD79α with those of human, cattle and mouse are 70.4, 81.4, and 67.7%, respectively. Alignment of the CD79α amino acid sequence with those of mammalian species showed that the extracellular domain is the most divergent, whereas transmembrane region and cytoplasmic tail including immunoreceptor tyrosine-based activation motif (ITAM) are largely conserved. Pig *Cd79a* mRNA was detected mainly in lymphoid tissues by RT-PCR. The highest level of *Cd79a* mRNA expression was observed in mesenteric lymph node and spleen. Relatively low level of *Cd79a* mRNA expression was observed in lung, thymus and small intestine. The lowest level of *Cd79a* mRNA expression was observed in large intestine. Flow cytometry analyses demonstrated that human CD79α antibody recognizes a CD79α in pig B cells. Further, immunohistochemistry analysis using human CD79α antibody on pig spleen was revealed that CD79α is strongly expressed in the follicular mantle zone rather than in the germinal center. Future study will be focused on defining the functional role of CD79α during the course of pig infectious diseases and the formation of neoplasm.


*Keywords:* B cell; B cell receptor (BCR) complex; cDNA cloning; CD79α; Expression; Pig

## 1. Introduction

The B cell receptor (BCR) complex is composed of membrane immunoglobulin (Ig) heavy and light chain with heterodimers of CD79α (Igα) and CD79β (Igβ) (Van Noesel et al., 1991; Reth, 1992). BCR has two important functions. One is capturing antigens and another is transducing a signal determining B cell behavior (DeFranco, 1993). However, the membrane portion of Ig heavy chain has only three amino acid residues, not enough to transduce a signal through

*Abbreviations:* BCR, B cell receptor; CHO, Chinese hamster ovary; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Ig, immunoglobulin; ITAM, immunoreceptor tyrosine-based activation motif; MFI, mean fluorescence intensity; mAb, monoclonal antibody.

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cytoplasm (Reth, 1992). Thus CD79α and CD79β mediate a signal into the downstream of the cell (Ha et al., 1994). To transduce a signal, both the cytoplasmic tails of CD79α and CD79β contain a conserved immunoreceptor tyrosine-based activation motif (ITAM) that is essential for signaling (Reth, 1992).

The expression of CD79α is thought to be mainly restricted to B cell lineage. The expression of CD79α is already observed in the cytoplasm of pro-B cells before the onset of Ig gene rearrangement and its expression is terminated in plasma cell stage (Ha et al., 1994). Thus, mutations in CD79α result in a failure of B cell development and cause of agammaglobulinemia (Minegishi et al., 1999). This finding indicates that CD79α-mediated signal transduction is required to support B cell development (Kraus et al., 2001). In addition, CD79α is necessary for BCR assembly and trafficking to the cell surface (Dylke et al., 2007). The expression of CD79α has been also observed in non-B-cell lineage including thymocytes and T cell lineage neoplasm, although the signaling potential of CD79α in these cells is not fully characterized (Mason et al., 1995; Hashimoto et al., 2002).

Despite increasing evidence that CD79α is important in mediating the downstream signal to develop or activation of B cell, only mouse, human and cattle cDNAs have so far been identified and characterized subsequently (Hombach et al., 1990; Yu and Chang, 1992; Youn et al., 1996). Identification of other examples of mammalian CD79α may be important to elucidate the structural relationships between CD79α and downstream signaling molecules in B cells. In this study, we cloned and sequenced full-length cDNA of pig *Cd79a*, and examined the tissue expression of pig *Cd79a* mRNAs. We also tested whether anti-human CD79α antibody recognizes pig CD79α and examined pig CD79α expression in pig spleen by immunohistochemistry using anti-human CD79α antibody. These results could provide valuable molecular information to be used to study the role of CD79α in a pig immune system.

## 2. Materials and methods

### 2.1. Generation of cDNA sequence and gene expression analyses of pig Cd79a

The pig *Cd79a* cDNA sequence was generated by RT-PCR. In brief, total RNAs from splenocytes of domestic female pig (18 weeks, female, Duroc) were isolated using a guanidium 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 *Cd79a* were designed based on the cDNA sequences of human *Cd79a* (GenBank Accession NM_001783), cow *Cd79a* (GenBank Accession NM_174266) and mouse *Cd79a* (GenBank accession NM_007655). Primer sequences were 5′-AACTCACTGGGAGAAGATGCCTG-3′ (sense), 5′-ACCCACGTCTCACTCGCATCTTC-3′ (anti-sense), resulting in a 754 bp RT-PCR product. To confirm if these RT-PCR products were pig *Cd79a*, we also performed the nested PCR. Primer sequences for nested PCR were 5′-CAGCAACAGCACTAACATCACCTGG-3′ (sense), 5′-CCTCATACATGGAGCAGTCATCAAGG-3′ (anti-sense), resulting in a 425 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). The pig *Cd79a* cDNA sequence was derived from three independent clones of three different animals.

For detecting *Cd79a* 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). After isolation of total RNAs from various tissues, RT-PCRs were performed as described above using the nested PCR primers yielding a 425 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′-ATGACCACAGTCCATGCCATC-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.

### 2.2. Retroviral transduction of pig CD79α to CHO cell

The pig *Cd79a* cDNA was generated via RT-PCR as described above. The purified RT-PCR products were then ligated into pGEM-T vectors and transformed into competent cells. The pig *Cd79a* cDNA was then subcloned into the pLNCX2 retroviral vector (Invitrogen) with NotI as restriction enzyme sites. The