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Short communication

cDNA cloning, genomic structure, expression analysis and biological activity of porcine A Proliferation-Inducing Ligand (APRIL)

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Abstract

A Proliferation-Inducing Ligand (APRIL) is a novel member of the tumor necrosis factor (TNF) superfamily. In this study, a novel cDNA has been isolated from pig spleen by homology cloning and 3′- and 5′-rapid amplification of cDNA ends (RACE) strategies and designates porcine APRIL (pAPRIL). The open reading frame (ORF) of this cDNA covers 756 bases, encoding 251 amino acids. The soluble part of pAPRIL shows 89% identity with its human counterpart at the level of the primary protein structure. The *pAPRIL* gene is approximately 2.1 kb in size and comprises six exons and five introns. Southern blotting analysis indicated that the *pAPRIL* gene is a single copy gene. Real-time PCR analysis revealed that pAPRIL is constitutively expressed in various tissues. Recombinant His_6-tagged psAPRIL protein was efficiently expressed in *Escherichia coli* BL21 (DE3) and its expression was confirmed by SDS-PAGE and Western blotting analysis. In vitro, purified recombinant psAPRIL protein co-stimulated the proliferation of porcine splenic B-cells in response to formalin-fixed *Staphylococcus aureus* Cowan 1 (SAC).

Keywords: The TNF family; RACE; Gene; Real-time PCR; Bioinformatics analysis; Recombinant protein

1. Introduction

APRIL (A Proliferation-Inducing Ligand, also named TALL-2, TRDL-1 and TNFSF-13a) is a type α membrane protein which belongs to the TNF family (Hahne et al., 1998). It does not exist as a membrane-bound form, but is processed intracellularly within the Golgi apparatus by a furin pro-protein convertase prior to secretion of the biologically active form (Lopez-Fraga et al., 2001). Two TNF receptor family members, B-cell maturation antigen (BCMA) and transmembrane activator and cyclophilin ligand interactor (TACI), bind to APRIL with high affinity (Rennert et al., 2000; Yu et al., 2000; Wu et al., 2000; Marsters et al., 2000). Both receptors are shared with BAFF (B-cell activating factor of the TNF family, also named BLyS, THANK, TALL-1, zTNF-4), another important ligand of the TNF family. APRIL and BAFF and their receptors, also termed ‘‘the BAFF/APRIL system’’, play important immunological

Abbreviations: PBLs, peripheral blood leukocytes; RNA, ribonucleic acid; cDNA, DNA complementary to RNA; UTR, untranslated region; IPTG, isopropy-β-D-thiogalactoside; RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcription-PCR; GAPDH, glyceraldehyde phosphate dehydrogenase; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electropheresis.

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roles, especially in the B-cell arm of the immune system (Mackay et al., 2003, 2007). Recent studies have localized APRIL expression to immune cell subsets that also produce BAFF: monocytes, dendritic cells, macrophages and T cells. In addition, APRIL is expressed in cells outside the immune system, including osteoclasts and tumor tissues. APRIL binds to proteoglycan structures on the cell surface (Hendriks et al., 2005; Ingold et al., 2005). However, the relevance of this binding is unclear, but may serve to accumulate and/or multimerize APRIL in the extracellular matrix or at the surface of syndecan-positive cells. It thus may facilitate access to its receptor TACI, which also interacts with syndecans, or to intracellular BCMA upon syndecan internalization (Dillon et al., 2006; Bossen and Schneider, 2006). The latest research advancements revealed that neutrophil-derived APRIL accumulated on tumor cells via proteoglycan binding correlates with human B-cell lymphoma aggressiveness (Schwaller et al., 2007) and simultaneous binding of TACI and HSPG on B-cells with APRIL is crucial for IgA production (Sakurai et al., 2006).

APRIL serves an important role in immunological responses, such as the contribution to B-cell survival, Ig secretion, isotype switching and T-independent antibody responses (Dillon et al., 2006; Stein et al., 2002; Litinskiy et al., 2002; Castigli et al., 2004; Mackay and Leung, 2006). These activities indicate that APRIL might have an adjuvant-like effect on the immune system to enhancing antigen-specific humoral immunity. In this study, we report for the first time the molecular characterization of porcine APRIL (pAPRIL) and raise the possibility that pAPRIL could be used as an immunoadjuvant in raising the immunity of pigs against infectious diseases.

2. Materials and methods

2.1. RNA isolation, RT-PCR and rapid amplification of cDNA ends (RACE)

Pigs used in this study were named Large White Pigs with average weight between 30–40 kg from Red-sun breed cultivation farm, Jiangsu, China. Tissue from pig heart, spleen, liver, lung, kidney, thymus, PBLs and intestine was removed and processed for RNA isolation using TRIzol reagent (Gibco-BRL, USA).

cDNA was synthesized from 5 μg of total RNA of spleen by murine leukaemia virus reverse transcriptase (MLV RT) (Takara, Japan) primed with Oligo$(dT)_{18}$ following the manufacturer's instructions, and used as a template for PCR. A pair of degenerate primers, forward primer A1 (5′-CAGAGNNCNGATGNCCTG-GAAGCCTG-3′) and reverse primer A2 (5′-TCA-CAAACCCCAGGAANGTTCCATG-3′), was designed based on multiple alignments of the highly conserved TNF domains of human and mouse APRIL cDNA sequences. PCR product of expected size was cloned into pMD18-T vector (Takara, Japan) and sequenced. The degenerate primers yielded a 490 bp fragment homologous to known mammalian APRILs.

RACE-PCR technique was used to obtain the full-length cDNA of pAPRIL including the 3′- and 5′-untranslated regions (UTRs). Briefly, two specific primers A3 (5′-GTCTCCTGCCTTCCCTGGCCCTC-CCGAG-3′) and A4 (5′-CAAGCGTGGGAGAGGT-CTGGAGGCCCAAG-3′) were synthesized based on the cDNA sequence obtained by the internal amplification above. For each 5′- and 3′-RACE, the cDNA was synthesized according to the manufacturer's protocols (SMART™ RACE cDNA Amplification Kit, Clontech, Japan). PCR conditions (32 cycles) were as follows: denature for 30 s at 94 °C, annealing for 30 s at 65 °C, and extension for 2 min at 72 °C. PCR products of 5′- and 3′-RACE were both cloned into pMD18-T vector and sequenced.

The full-length cDNA was generated by long distance PCR using Advantage 2 polymerase (Clontech, Japan) with primers A5 (5′-GCTTCCTAGAGAAACT-GACACTAAATTCTC-3′) and A6 (5′-CTGTCAAAC-CTGGGGTTCCAGTCAAAC-3′) designed from the distal ends of both 5′- and 3′-RACE products. The full-length cDNA sequence of pAPRIL was deduced from six independent clones.

2.2. Bioinformatics analyses

The searches for nucleotide and protein sequence similarities were conducted with BLAST algorithm at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast). For the exact localization of the exon/intron boundaries the mRNA-to-genomic alignment program Spidey (http://www.ncbi.nlm.nih.gov/IEB/Research/Ostell/Spidey/index.html) was used. The putative promoter sequence was analyzed "in silicon" with the program PROSCAN Version 1.7 from Web Promoter Scan Service (http://www-bimas.cit.nih.gov/molbio/proscan/). The deduced amino acid sequence was analyzed with the Expert Protein Analysis System (http://www.expasy.org/) and the protein domain features of pAPRIL were determined by using Simple Modular Architecture Research Tool (http://smart.embl-heidelberg.de/). Isoelectric point and molecular weight prediction was carried