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Molecular cloning characterization and expression of porcine immunoreceptor SIRPα

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Abstract

SWC3 is a porcine CD that has been the reference marker of myeloid lineage. It is expressed in every myelomonocytic cell from early bone marrow precursors. We have identified the molecule recognized by anti-SWC3 antibodies as a member of the signal-regulatory proteins (SIRPs)α family.

Here, we describe the cloning of a cDNA coding for a porcine SIRPα protein. The sequence is 2470 nucleotides long and contains an open reading frame encoding a 507 amino acid sequence. The predicted polypeptide was composed of a 30 amino acids putative signal peptide, a 342 amino acid extracellular region, a 23 amino acid transmembrane segment and a 112 amino acid cytoplasmic domain. Analysis of the sequence reveals a high degree of homology with known SIRPs in other species, being easily identified the three extracellular Ig type domains and two cytoplasmic ITIM motifs characteristic of this molecule.

The gene coding for porcine SIRPα has been mapped to porcine chromosome 17, in a region syntenic to the human chromosome 20 where *SIRP* genes have been mapped.

During the analysis of SIRP gene expression in tissues by RT-PCR, we noticed the existence of a shorter mRNA, and cloned the corresponding cDNA. This coded for a splicing variant of SIRPα that lacked the two membrane proximal Ig domains.

In transfection experiments, we have been able to show that anti-SWC3 antibodies recognize both forms of the molecule, mapping the SWC3 epitopes to the N-terminal IgV type domain.

Keywords: Signal-regulatory protein; Porcine; Myeloid; Immunoglobulin-super-family

1. Introduction

Signal-regulatory proteins (SIRPs) are a family of transmembrane glycoproteins, involved in signal transduction, which are expressed at high levels in neurons and myeloid cells, including macrophages,

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monocytes, granulocytes and dendritic cells (DCs) [1–3].

Structurally, SIRPs are a family of proteins which have closely related extracellular regions consisting of one to four immunoglobulin (Ig)-like domains, and large differences in their carboxy-terminal segments, with different types of transmembrane and cytoplasmic regions, or even lacking those [4,5]. Based on these differences, SIRP proteins have been classified in four groups named with greek letters [5,6].

SIRPα has cytoplasmic domains containing immunoreceptor tyrosine-based inhibitory motifs (ITIMs). Tyrosine phosphorylation of these motifs leads to the recruitment of src homology 2 (SH2) domain-containing tyrosine phosphatases SHP-2 and SHP-1 that negatively regulate signal transduction cascades. SIRPα has been shown to interact with CD47 through its amino-terminal Ig-like domain [7,8].

The second type of SIRP proteins, called SIPRβ, are highly homologous to SIRPα in the extracellular region, but have a short intracytoplasmic region devoid of ITIMs. Instead, they contain a positively charged lysine residue in the transmembrane domain involved in interaction with small transmembrane adapter proteins such as DAP12 which contain immunoreceptor tyrosine-based activation motifs within their cytoplasmic domains, and transduce stimulatory signals [9]. Several types of SIPRβ proteins have been identified in different species, differing in the number and type of Ig domains [5].

SIRPγ and SIRPδ have not been identified in every species studied to date. SIRPγ are very similar to SIRPβ, except in their transmembrane and cytoplasmic segments that do not allow interaction with the adapter proteins. SIRPδ proteins are soluble proteins [5].

In human, SIPRα and SIRPβ molecules are encoded by different genes clustered at the same locus [10]of human chromosome 20p12.2-13, but in other species *SIRP* genes are located in two chromosomes [5].

SIRPα proteins have been involved in a variety of biological phenomena, including inhibition of signalling through different receptor tyrosine kinases and cytokine receptors and negative regulation of immune cells phenotypic and functional maturation [11–18].

SWC3 was the first cluster of differentiation specific for myeloid cells defined in swine [19] and has been extensively used as a marker for monocytes/macrophages in this species. In a previous report, we have identified the porcine myelomonocytic marker SWC3 as a member of the family of SIRP [20]. Here, we describe the cloning and expression of cDNAs coding for two different forms of swine SIRPα and their reactivity with anti-SWC3 mAbs.

2. Materials and methods

2.1. Tissues and cells

All tissues used for cDNA preparations were obtained fresh from Large White pigs with average weight between 30 and 40 kg. Alveolar macrophages (MØ) were collected by bronchoalveolar lavage as described by Carrascosa et al. [21].

2.2. Screening of pig cDNA libraries

A probe was generated by RT-PCR using mRNA from swine MØ. The oligonucleotides, used as primers, named 460F and 1274R were designed from conserved regions in the sequences of bovine *SIRP* family genes (GenBank accession numbers: Y11045 and Y11046). The 835-bp PCR product was labelled with the Digoxigenin (Dig) system (Roche, Basel, Switzerland) according to the manufacturer's protocol. PCR reactions were performed in a final volume of 50 μl containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 0.005% Tween 20, 0.005% Nonidet P-40, 1 mM $MgCl_2$, 0.05 mM each dATP, dCTP and dGTP, 0.04 mM dTTP and 0.002 mM digoxigenin-11-dUTP, 1 μM oligonucleotide primers and 2 U of DyNAzyme II DNA polymerase (Finnzymes, Espoo, Finland); 5 μl of cDNA from pig MØ was subjected to 35 amplification cycles of 45 s at 94 °C, 45 s at 58 °C and 1 min at 72 °C. The labelled probe was purified and used to screen approximately 5×10^5 plaque colonies of two different libraries. A porcine aortic smooth muscle Uni-ZAP^{TM} XR cDNA library (M) (Stratagene, La Jolla, CA, USA) and a λZAP II cDNA library constructed from a mixture of mRNA from several porcine tissues (hereafter named F) [22]. Each library was plated at 5×10^4 plaque forming units (pfu) per plate and grown on a lawn of XL1-Blue *Escherichia coli* for 8 h. Plaques were transferred onto Nytran-nylon membranes (Schleider & Schuell, Dassel, Germany) denatured in 1.5 M NaCl/0.5 M NaOH, neutralized in 1.5 M NaCl/1 M