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SCIENCE DIRECT

Genomics 85 (2005) 352–359

GENOMICS

www.elsevier.com/locate/ygeno

Molecular cloning, characterization, and chromosomal assignment of porcine cationic amino acid transporter-1 ☆

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Received 26 August 2004; accepted 10 November 2004
Available online 18 December 2004

Abstract

We have cloned and characterized the gene encoding the porcine cationic amino acid transporter, member 1 (CAT-1) (HGMW-approved gene symbol *SLC7A1*) from porcine pulmonary artery endothelial cells. The porcine *SLC7A1* encodes 629 deduced amino acid residues showing a higher degree of sequence similarity with the human counterpart (91.1%) than with the rat (87.3%) and mouse (87.6%) counterparts. Confocal microscopic examination of porcine CAT-1–GFP-expressing HEK293 cells revealed that porcine CAT-1 localizes on the plasma membrane. Amino acid uptake studies in *Xenopus oocytes* injected with cRNA encoding this protein demonstrated transport properties consistent with system y^+. Radiation hybrid mapping data indicate that the porcine *SLC7A1* maps to the distal end of the short arm of pig chromosome 11 (SSC11). This map location is consistent with the known conservation of genome organization between human and pig and provides further confirmation that we have characterized the porcine orthologue of the human *SLC7A1*.

Keywords: L-Arginine; Cationic amino acid transporter; CAT-1; *SLC7A1*; System y^+; Nitric oxide; Endothelial nitric oxide synthase; Endothelium

Pulmonary artery endothelial cells (PAEC) are a rich source of nitric oxide (NO), a nitrogen-centered free radical with multiple and unique physiologic bioregulatory activities [1,2]; e.g., NO functions as a signaling molecule and plays a major role in the regulation of pulmonary vascular tone, leukocyte and platelet adhesion to endothelium, and pulmonary vascular smooth muscle proliferation. A number of lung diseases associated with primary or secondary pulmonary hypertension are characterized by impaired vascular production of NO [3–5]. As such, modulation of NO generation has the potential to become a novel strategy in the prevention and treatment of a number of lung diseases.

PAEC generate NO from L-arginine (L-Arg) via the catalytic action of an NADPH-requiring, Ca^{2+}/calmodulin-dependent constitutive NO synthase [2]. Synthesis requires O_2 as a cosubstrate and results in NO and the coproduct L-citrulline. Cofactors required include NADPH, BH4, flavin adenine dinucleotide, and flavin mononucleotide. L-Arg is the exclusive precursor of NO, and NOS-mediated formation of NO is critically dependent upon an adequate and continuing supply of L-Arg [6–8]. The L-Arg content of endothelial cells is derived primarily from plasma membrane-dependent transport of extracellular L-Arg, although L-Arg can also be synthesized from L-citrulline [9–12]. The importance of transport to the maintenance of intracellular L-Arg content is clear from work demonstrating that removal of L-Arg from the medium results in rapid depletion of intracellular L-Arg in cultured endothelial cells [13,14].

Transport of L-Arg is mediated by several independent transport activities in mammalian cells [15]. The distribution

☆ Sequence data from this article have been deposited with the GenBank Data Library under Accession No. AY371320.

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doi:10.1016/j.ygeno.2004.11.006

and relative contribution of each of these transport activities to the total L-Arg uptake by a particular cell type vary widely due to cell-specific expression of the corresponding genes. Under physiologic conditions L-Arg transport in PAEC is mediated by two transport agencies [9,16–18]: system y^+, a Na^+-independent system encoded by the *SLC7A* gene family, and system $B^{O,+}$, a Na^+-dependent cotransport system encoded by the $ATB^{O,+}$ gene [19,20]. Previous studies from our laboratory using plasma membranes from porcine PAEC indicate that 94% of saturable L-Arg transport in these cells is mediated by system y^+ [18]. System y^+ has been characterized in porcine PAEC by our lab [18,21] and its properties in this cell type are similar to those described for many others. These properties include Na^+-independent transport that is responsive to membrane potential, nonobligatory exchange, and, at neutral pH and physiological substrate concentrations, specificity restricted to amino acids with net positive charge.

At present, four members of a family of cationic amino acid transporters (CAT-1, CAT-2A, CAT-2B, CAT-3) have exhibited system y^+ activity [15]. The function of a more distantly related isoform, CAT-4, has remained elusive [22]. Although the deduced amino acid sequences of these proteins are similar and they share common substrate specificity, the tissue-specific expression of these proteins differs. The distribution of CAT-1 expression appears to be nearly ubiquitous, with the notable exception of quiescent liver, in keeping with its identification as the gene encoding constitutive system y^+ activity. Under physiological conditions, CAT-3 exhibits a brain-specific expression [23]. Using immunohistochemistry, we documented the expression of CAT-1, but not CAT-2 or -2a, within the plasma membrane of porcine PAEC [24]. Because of the major role of CAT-1 in L-Arg transport, regulation of CAT-1 expression or activity represents a potential target for modulating cellular NO generation. During the past decade, our lab has defined the functional characteristics and localization of porcine CAT-1 in porcine PAEC [12,16–18,21,24–33]. Such information is not available in other species. So, knowing the sequence of the porcine *SLC7A1* gene would allow for correlation of function of the product and structure of the gene that cannot be offered in other species. Therefore, the goal of the present study was to clone and characterize further the porcine *SLC7A1* gene.

Results

Molecular cloning of porcine SLC7A1

As described under Materials and methods, a 959-bp porcine *SLC7A1* cDNA was obtained from pig PAEC RNA using RT-PCR amplification. The PCR primers were designed based on the conserved regions of the following known mammalian *SLC7A1* cDNAs: human, rat, and mouse. Analysis of the sequence of the 959-bp fragment demonstrated that it shared a high degree of sequence identity with the human, rat, and mouse sequences, indicating it was the porcine homologue of *SLC7A1*. Based on this sequence, two gene-specific primers were synthesized and 3′/5′ rapid amplifications of cDNA ends (RACE) were performed. 3′ RACE (~4.8 kb) and 5′ RACE (~2.7 kb) products were cloned into the pCR4-TOPO vector and sequenced. The sequence of the 3′ RACE cDNA fragment (4783 bp) overlaps the 5′ RACE cDNA fragment by 452 bp. Despite trying several different methods we were unable to identify about 600 bp of the most distal portion of the 5′ end of the cDNA. Finally, 6416 bp of the cDNA was assembled from the overlapping 3′ (4783 bp) and 5′ RACE (2085 bp) fragments (GenBank Accession No. AY371320).

Identification and characterization of porcine SLC7A1

Sequence analysis of the porcine *SLC7A1* cDNA revealed (1) an ORF of 1890 bp that would encode a protein of 629 residues with a calculated molecular mass of 67.8 kDa, (2) 366 bp of 5′ untranslated region (UTR), and (3) 4160 bp of 3′ UTR with a consensus AATAAA polyadenylation signal at 21–26 nt upstream of a poly(A) stretch. BLASTn or BLASTp analysis demonstrated that the porcine sequence shares a high degree of sequence identity, both in the nucleotide sequences, especially in coding sequence (CDS) regions (86, 83, and 82%), and in the deduced amino acid sequences (91, 87, and 87%), with the human (Accession No. AF078107), rat (Accession No. AF245000), and mouse (Accession No. NM_007513) *SLC7A1,* respectively. Furthermore, unlike rat and mouse CAT-1, which are shorter than human CAT-1 by 5 and 7 aa, respectively, the porcine CAT-1 is the same length as human CAT-1. Hydrophobicity prediction suggests 14 putative membrane-spanning domains within porcine CAT-1, similar to other mammalian CATs. Consistent with the results of homologous comparison, phylogenetic analysis shows that the divergence of porcine *SLC7A1* and human *SLC7A1* in evolution seems later than that of rat and mouse *SLC7A1*.

To determine whether there is more than one transcript encoded by the porcine *SLC7A1* gene, total RNA from PAEC was subjected to Northern blot analysis and probed with a 1.376-kb porcine *SLC7A1* cDNA. Unlike other known mammalian *SLC7A1,* only one transcript (7.0 kb) was identified in porcine PAEC. Furthermore, this transcript can be upregulated by lipopolysaccharide (LPS)/interferon-γ (IFN-γ) activation (Fig. 1). This is consistent with our previous functional studies demonstrating that LPS/interferon-γ activated L-Arg uptake in porcine PAEC (unpublished data).

Sequencing shows that porcine *SLC7A1* possesses a lengthy 3′ UTR (4.0 kb) within which a few copies of ATTTA pentamer motifs exist. Since this consensus is characteristic of unstable mRNA [34,35], we next studied the porcine *SLC7A1* mRNA half-life using real-time QRT-PCR and the total RNA from porcine PAEC treated for 0–6