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Short communication

Molecular cloning and expression analysis of porcine γ-interferon-inducible lysosomal thiol reductase (GILT)

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Abstract

A porcine interferon-γ-inducible lysosomal thiol reductase (GILT) cDNA, designated pGILT, was cloned by RT-PCR and rapid amplification of cDNA ends (RACE) strategies. The full-length cDNA of pGILT consists of 1062 bp with a 741 bp open reading frame, encoding 246 amino acids, with a putative molecular weight of 29.5 kDa. The deduced pGILT possesses the typical structural feature of mammalian GILT, including an active-site CXXC motif, a GILT signature sequence $CQHGX_2ECX_2NX_4C$, and 10 conserved cysteines. The genomic DNA sequence of pGILT contains seven exons and six introns, which is similar to vertebrate GILT exon–intron organization. The result of real-time PCR showed that GILT is expressed in many tissues in the pig, including spleen, liver, lung, heart, intestine, blood and kidney. And the pGILT expression is obviously up-regulated in spleen and blood after induction with LPS. These results suggesting that pGILT is highly likely to play a role in the innate immune responses in porcine. It also provided the basis for investigations on the role of GILT in this important domestic species and an animal model for human diseases.

Keywords: Porcine; GILT; RACE; mRNA expression; Lipopolysaccharide (LPS)

Antigen-presenting cells such as dendritic cells, macrophages and activated B cells stimulate CD4+ T cells by presentation of major histocompatibility complex (MHC) class II-peptide complexes. Generation of class II epitopes requires proteolysis of endocytosed antigens in MHC class II-containing compartments (Watts, 1997). Several studies have shown that reduction of disulfide bonds in proteins is a critical step for antigen processing, mainly by facilitating unfolding of proteins and their subsequent cleavage by proteases (Collins et al., 1991). In mammals, an IFN-γ-inducible lysosomal thiol reductase (GILT) has been identified as the only thiol reductase involved in MHC class II-restricted Ag processing, which functions to catalyze thiol bond reduction, thus facilitating unfolding of protein Ag and their subsequent cleavage by cellular proteases (Arunachalam et al., 2000; Haque et al., 2002; Li et al., 2002).

GILT is constitutively expressed in antigen-presenting cells, and is inducible by IFN-γ in other cell types such as fibroblasts, endothelial cells and keratinocytes (Arunachalam et al., 2000; Maric et al., 2001; Phan et al., 2001; Zheng and Chen, 2006; Luster et al., 1988). GILT is synthesized as a 35-kDa precursor, and following delivery to MIICs, is processed to the mature 30-kDa form via cleavage of N- and C-terminal propeptides. Both precursor and mature GILT reduce disulfide bonds with an acidic pH optimum (Arunachalam et al., 2000; Maric et al., 2001). Absence of GILT or relatively lower level of

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Table 1
Sequences of the primers used in this study

Primer	Nucleotide sequence (5′–3′)
P1	GAGACNCTNTGNCCNGNATGCAGA (N = A, T, G, C)
P2	TAANGGTNANCCANGGCACATATT (N = A, T, G, C)
P3	CTTGTTCATCTTGCACTCCTGCTCGCCG
P4	GCAAGATGAACAAGGTGGAGGCCTGCC
P5	ACGCGGGGCGGCTTAAAGGCGGCT
P6	ATCAAATGAATCACAACATCTCAA
P7	ATCGGCCTCGTCGCCTCTCCTGCCGTT
P8	TCACTTGAAGCAGACTTCCTTGTGG
R1	GCCCAACTCACAGATGCTCTGAAGC
R2	GGCAGACGAGGCGTAAGAGCTGGT
GF	TGCTGGGGCTGGCATTGCCCTCAACGAC
GR	GCCATGTGGACCATGAGGTCCACCACCC

GILT expression in human melanomas affected the display of immunodominant epitopes by MHC class II molecules. In GILT-free mice, the Ag processing in professional APCs is shown to be defective (Maric et al., 2001). These studies suggest a major role for GILT in Ag processing in mammals.

In this study, we reported the characterization of a GILT homologue from porcine (pGILT), examined its expression profile in adult animals, and tested the effects of lipopolysaccharide (LPS) on pGILT expression. Additionally, its genomic DNA sequence were identified and analyzed.

A first-strand cDNA was synthesized from 1 μg of RNA isolated from spleen using Reverse Transcriptase XL (Takara, Japan) according to the manufacturer's protocol. A pair of degenerate primers P1 and P2 (Table 1), whose design was based on regions of high homology among the sequences of human, mouse, large yellow croaker, zebrafish and amphioxus GILT analyzed by ClustalW program, was used in the PCR. A 392 bp cDNA fragment which has high sequence similarity to mammalian GILT was obtained. RACE technique was used to obtain the full-length cDNA of pGILT including the 3′ and 5′ untranslated regions (UTRs). Briefly, two specific primers P3 and P4 (Table 1) were synthesized based on the cDNA sequence obtained by the internal amplification above. For each 5′ (primer P3) and 3′ RACE (primer P4), the cDNA was synthesized according to the manufacturer's protocol (SMARTTM RACE cDNA Amplification Kit, Clontech, Japan). The full-length cDNA was generated by long distance PCR using Advantage 2 polymerase (Clontech, Japan) with primers P5 and P6 (Table 1) designed from the distal ends of both 5′ and 3′ RACE products. The obtained full-length GILT cDNA of 1062 bp contains an open reading frame (ORF) of 741 bp flanked by 74 and 247 bp of 5′- and 3′-UTR, respectively (GenBank accession no. EF644197). The741 nt ORF encodes a 246 aa protein, with a putative molecular weight of 29.5 kDa. The nucleotide and predicted amino acid sequences of the full-length cDNA were shown in Fig. 1.

The deduced protein possesses all the main features characteristic of known GILT proteins including the signature sequence $CQHGX_2ECX_2NX_4C$ spanning residues 113–128, CXXC motif at residues 68–71, two potential sites for Asn-linked glycosylation at residual positions 91 and 104 (Net-NGLYc 1.0 sever, http://www.cbs.dtu.dk/services/NetNGlyc). It also has a putative signal peptide consisted of the N-terminal sequence of 26 aa (SignalP 3.0 server, Nielsen et al., 1997). The presence of Asn-linked glycosylation sites suggest that they could theoretically be derivatized with mannose-6-phosphate (M6P), which has been considered necessary for transportation of GILT to lysosomal system (Fig. 1).

To determine the genomic structure of pGILT, a LdPCR was used to amplify the genomic DNA of pGILT. Genomic DNA of porcine was prepared from spleen by standard methods (Sambrook et al., 2001). Two primers P7 and P8 (Table 1) were designed against the 5′ and 3′ coding sequences of the full-length genomic DNA of pGILT. Sequence analysis showed that the genomic DNA sequence of pGILT gene obtained here is 4018 nt and composed of seven exons and six introns (GenBank accession no. EU154970). The seven exons of 149, 154, 75, 93, 153, 54 and 63 bp, respectively, are interspaced by six introns of 1025, 93, 783, 801, 185 and 86 bp, which all begin with GT and end with AG dinucleotide. Analysis of the genomic structure showed that pGILT gene exhibits a similar exon–intron organization to that of human and mouse except for a slightly more compact intron arrangement, whereas the GILT gene of fruit fly, a representative invertebrate model organism, has only four exons and three introns (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene&cmd=Retrieve&dopt=full report&listuids=41650).

Indication about the physiological function of a protein might be obtained from its tissue distribution studies. The pGILT mRNA expression in different tissues was analyzed by real-time PCR. Real-time quantitative RT-PCR (qRT-PCR) was performed with the SYBR Green 2× Supermix (Applied Biosystems, USA) on an ABI 7300 Real-Time Detection System (Applied Biosystems, USA) to investigate the expression of pGILT. Two pGILT-specific primers, forward primer R1 and reverse primer R2 (Table 1) were used to amplify a PCR product of 106 bp. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was chosen as reference gene