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Molecular cloning and expression analysis of interferon-γ-inducible-lysosomal thiol reductase gene in orange-spotted grouper, *Epinephelus coioides*

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Abstract

In mammals, interferon-γ-inducible-lysosomal thiol reductase (GILT) has been demonstrated to play a key role in the processing and presentation of MHC class II-restricted antigen (Ag) by catalyzing disulfide bond reduction, thus unfolding native protein Ag and facilitating subsequent cleavage by proteases. In this study a cDNA containing the orange-spotted grouper GILT (OsgGILT) coding sequence has been cloned and its complete sequence determined. The full-length cDNA of OsgGILT gene is 1066 bp nucleotides (nt) encoding a protein of 260 amino acids (aa), with a putative molecular weight of 28.7 kDa. The deduced OsgGILT 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 result of real-time PCR showed that OsgGILT mRNA was expressed in heart, liver, brain, gill, kidney and muscle and more highly expressed in spleen. The OsgGILT expression is obviously up-regulated in spleen and kidney after induction with LPS, these results suggest that OsgGILT may be involved in the immune response to LPS challenge in orange-spotted grouper.

Keywords: Orange-spotted grouper (*Epinephelus coioides*); GILT; cDNA cloning; mRNA expression; Lipopolysaccharide (LPS)

1. Introduction

Exogenous antigens are internalized by antigen-presenting cells and delivered to MHC class II-containing compartments. During this process, they are unfolded and degraded, generating peptides that bind to MHC class II molecules. Following transport to the cell surface, MHC class II-peptide complexes stimulate CD4-T cells [1,2]. Within the MHC class II antigen processing pathway, the reduction of disulfide bonds in exogenous Ags is a critical step [3–5]. Gamma-interferon (IFN-γ)-inducible lysosomal thiol reductase (GILT) has been shown to be involved in this processing, which functions to catalyze disulfide bond reduction, thus unfolding native protein antigen and facilitating further cleavage via cellular proteases [5–8]. Recent studies show that GILT may have additional roles including

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negative regulation of T cell activation and neutralization of extracellular pathogen and/or clearance of cell debris resulting from infection [9,10].

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 [5,7,11–13]. GILT is synthesized as a 35-kDa precursor, and following delivery to major histocompatibility complex (MHC) class II-containing compartments (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 [5,6]. Absence of GILT or relatively lower level of 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 [7]. These studies suggest a major role for GILT in Ag processing in mammals.

Orange-spotted grouper (*Epinephelus coioides*) is a coral fish of high commercial value and farming of this economic fish has been the focus of finfish mariculture in Southeast Asia, especially in China. However, a low level of larval survival has been the major problem encountered by fish farms for mariculture of groupers. In this study, we first reported the cloning of a GILT gene homologue from orange-spotted grouper (*Epinephelus coioides*), a marine fish (OsgGILT), and demonstrated the tissue specific up-regulation of its expression in the spleen and kidney after induction with LPS.

2. Materials and methods

2.1. Fish, stimulation and RNA isolation

Orange-spotted grouper were purchased from Huimingqiao marine fish market (Nanjing, China). The heart, liver, brain, gill, spleen, muscle and kidney were aseptically removed from 3 individual 500 g fish. The tissues were washed with PBS (with penicillin/streptomycin) and each tissue was minced into pieces with dissecting scissors under sterile conditions. After washed with PBS three times, the pieces of tissues were cultured in RPMI 1640 (Cambrex Bioscience, MD, USA) medium supplemented with 5% Foetal Bovine Serum (Cambrex Bioscience, MD, USA), 1% streptomycin/penicillin (Invitrogen, USA) at 22 °C. After cultured at 22 °C 2 h, the tissues were treated with 10 μg ml^{-1} lipopolysaccharide (purified LPS; SIGMA, USA) for 0, 4, 8, 12 h. Total RNA was extracted from the above tissues using ISOGEN (NipponGene, Japan) according to the manufacturer's instruction.

2.2. RT-PCR and Rapid amplification of cDNA ends (RACE)

A first-strand cDNA was synthesized from 1 μg of RNA isolated from spleen using 5 U Reverse Transcriptase XL (Takara, Japan) according to the manufacturer's protocol. A pair of degenerate primers F1 and F2 (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. PCR conditions (32 cycles) were as

Table 1
Sequences of the primers used in this study

Primer	Nucleotide sequence (5′-3′)
F1	GAGACNCTNTGNCCNGNATGCAGA (N = A, T, G, C)
F2	TAANGGTNANCCANGGCACATATT (N = A, T, G, C)
F3	GCCCTGACATTCTGGTCCATGCTGG
F4	GATGTCCTCGGTTCAGCCGAGAGCTG
F5	ACGCGGGGGGGGCAGTGCTGTGTC
F6	AAGAAAGTGAATTGTATTTATAC
R1	GAGCACACAGAGGAACTGCAGGAC
R2	GTGGCAGTAGCTTCTGAAGTACTC
AC-F	AGATCATTGCACCCACCAGAGTCG
AC-R	GATCTCATCGTACGTCCTGCTTGC