Molecular and immune response characterizations of IL-6 in large yellow croaker (*Larimichthys crocea*)

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ABSTRACT

Interleukin-6 (IL-6) is a multifunctional inflammatory cytokine which exists in multiple tissues and cell lines. In the present study, the full-length cDNA and the genomic sequence of IL-6 (*LcIL-6*) were cloned from large yellow croaker, *Larimichthys crocea*. The full-length cDNA of *LcIL-6* was 1066 base pairs (bp), containing an open reading frame (ORF) of 678 bp encoding for 225 amino acids, a 5' untranslated region (UTR) of 71 bp and a 3' UTR of 317 bp. The predicted *LcIL-6* protein included a 24 amino acids (aa) signal peptide and a conserved IL-6 domain. However, the polypeptide sequence identities between *LcIL-6* and its counterparts in mammals and other fish are from 12% to 45%. The genome sequence of *LcIL-6* gene was composed of 2126 bp, including five exons and four introns. Phylogenetic analysis revealed that *LcIL-6* showed a close relationship with the IL-6 from other bony fish. Quantitative real-time PCR (qRT-PCR) analysis revealed that *LcIL-6* mRNA was expressed in most examined tissues, with the most predominant expression in stomach,

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followed by blood and very weak expression in other tissues. The expression levels of *LcIL-6* after challenged with LPS, poly I:C and *Vibrio parahaemolyticus* were investigated in spleen, head-kidney and liver. *LcIL-6* transcripts were induced significantly after immune challenge, with the peak-value of 33.5 times as much as the control in the head-kidney at 3 h after LPS injection ($p < 0.05$). Overexpression of *LcIL-6* enhanced tumor necrosis factor (TNF)- α transcripts significantly ($p < 0.05$) in *L. crocea* kidney (LCK) cells. Additionally, recombinant *LcIL-6* mature peptide was obtained in the supernatant of *Escherichia coli* BL21 (DE3). The purified recombinant *LcIL-6* fusion protein was also demonstrated to improve the transcriptional expression levels of TNF- α significantly in LCK cells ($p < 0.05$). However, no significant changes of Mx (myxovirus resistant protein), IL-1 β , janus kinase (JAK)2, signal transducers and activators of transcription (STAT)3 and STAT5 in LCK cells was detected after *LcIL-6* overexpression or recombinant *LcIL-6* protein stimulation. Our results indicated that *LcIL-6* might be important in large yellow croaker immune response and improve the inflammatory response by through activation TNF- α expression.

Key words: Interleukin-6; gene structure; large yellow croaker; immune challenge; overexpression; recombinant expression; inflammatory response

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