

CHANNA STRIATUS TNFR-1: MOLECULAR CLONING, CHARACTERIZATION AND GENE EXPRESSION

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ABSTRACT

Tumor necrosis factor receptor (TNFR) superfamily entails in various biological functions including cell proliferation, differentiation, survival and providing co-stimulatory signals for programmed cell death or apoptosis. In this report, we have identified and characterized TNFR-1 from *Channa striatus* (Cs) at molecular level. *In silico* analyses revealed that the CsTNFR-1 cDNA sequence was 1200 bp in length, which includes an open reading frame of 1197 bp that encoded a putative protein of 399 amino acids (45 kDa). It contains three 'Cys' rich domains (CRDs) at CRD1⁴¹⁻⁴⁶, CRD2⁷⁹⁻¹¹⁸ and CRD3¹²⁰⁻¹⁵⁹ in the extracellular region, also it possessed a putative TRAF6-binding site in the cytoplasmic tail. In addition, CsTNFR-1 cytoplasmic region contained a death domain between 297 and 388, which is important for apoptosis induction. It shared a maximum identity with its homologous from *Paralichthys olivaceus* (82%). Real-time PCR findings revealed that CsTNFR-1 was expressed predominantly in head kidney, further it is up-regulated upon fungal (*Aphanomyces invadans*) and bacterial (*Aeromonas hydrophila*) infection. Overall, the gene expression results indicated that CsTNFR-1 involvement in the innate immune response against pathogenic infections. The recombinant CsTNFR-1 protein was over-expressed and purified using an *Escherichia coli* expression vector system. The purified recombinant CsTNFR-1 derivative binds with TNF- α at high affinity and inhibits its cytotoxic activity *in vitro* in a dose dependent manner. CsTNFR-1 was also analyzed for respiratory burst activity upon two different ROS inducers such as opsonized zymosan and phorbol 12-myristate 13-acetate (PMA). The results showed that the recombinant CsTNFR-1 protein alone did not produce any ROS, but upon induction with opsonized zymosan, it considerably increased the ROS production in a concentration dependent manner. Overall, the findings showed the potential involvement of CsTNFR-1 in the pathogen-induced inflammatory process of *C. striatus*.

KEYWORDS

Channa striatus; Tumor necrosis factor receptor 1; TNF- α inhibition activity; Cytotoxic activity; Respiratory burst activity.

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