MOLECULAR CLONING, EXPRESSION ANALYSIS AND CHARACTERIZATION OF GRASS CARP IKK-BETA

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ABSTRACT:

IKK-beta is a member of the IKK complex and plays an important role in the innate immune response. IKK-beta can interact with PKR to activate the NF-κB pathway in response to dsRNA in mammals. In this study, a grass carp (Ctenopharyngodon idellus) IKK-beta gene (CiIKKβ) was obtained by homology cloning and RACE technique. The full length of CiIKKβ cDNA is 3428 bp, including 185 bp 5'UTR, 906 bp 3'UTR and 2337 bp open reading frame encoding 778 aa. SMART predicts that there is a serine/threonine kinase region at CiIKKβ protein N-terminal, followed by a UBQ structural domain and a leucine zipper structure (LZ), then a NEMO binding domain (NBD) at C-terminal. Phylogenetic tree showed that CiIKKβ is highly homologous to zebrafish IKKβ (DriKKβ). The real-time PCR result showed that the expression of CiIKKβ was detected in brain, intestine, liver, spleen, kidney, gill and heart tissues. For the purpose of searching for the mechanism of NF-κB activation mediated by the interaction between CiIKKβ and grass carp PKR (CiPKR), a glutathione S-transferase(GST) pull-down assay was performed. By GST pull down, CiIKKβ was shown to be physically associated with the N-terminal of CiPKR.

KEYWORDS: IKKβ, Tissue expression, PKR, Interaction, GST pull down

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