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Molecular cloning, characterization and expression analysis of QM gene from grass carp (*Ctenopharyngodon idellus*) homologous to Wilms' tumor suppressor

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Abstract

QM, a novel gene that was originally identified as a tumor suppressor, has been cloned from species encompassing members of higher vertebrate, plant and fungal kingdoms, but it is not well documented in fish. In present study, a gene homologous to QM was obtained from grass carp (*Ctenopharyngodon idellus*) head kidney and spleen cDNA library. The full-length grass carp QM (GcQM) cDNA of 759 bp contains a short 5′UTR of 22 bp, a 3′UTR of 89 bp and an open reading frame of 648 nucleotides that translates into a 215-amino acid peptide with a molecular weight of 24.5 kDa. The predicted GcQM contains a series of functional motifs that belong to the QM family signature conserved among different species. Multiple alignment analysis reveals that GcQM shares an overall identity of 62.4%~97.7% with other members of QM family. The fish QM has a closest genetic relationship to chicken homologue Jif-1. The GcQM expresses constitutively in spleen, heart and brain, and significantly up-regulated by *Aeromonas hydrophila* and grass carp haemorrhagic virus (GCHV) in head kidney, spleen and liver. The results suggest that grass carp QM homolog is an inflammatory stress inducible gene associated with anti-bacterial and viral defense, and it plays an important role in immune defense.

Keywords: Characterization; Cloning; *Ctenopharyngodon idellus*; Grass carp; Immune response; QM gene; Tissue distribution; Wilms' tumor suppressor

1. Introduction

The QM gene was originally identified from human by subtractive hybridization as a cDNA transcript elevated in a non-tumorigenic Wilm's tumor microcell hybrid relative to the tumorigenic parental cell line, and as such was proposed to be a tumor suppressor (Dowdy et al., 1991). Since then, much attention has been paid to this gene in different species due to its putative important function. So far, QM gene has been cloned from species encompassing high vertebrate, plant and fungal kingdoms, and sequence comparison indicates that the homologs of QM are highly conserved throughout eukaryotic evolution. More and more studies demonstrated that QM is an essential gene and plays an important role in cell growth, differentiation and apoptosis (Marty et al., 1993; Green et al., 2000; Lillico et al., 2002). In chicken, it has been reported that Jif-1, a homolog of QM, is a negative transcription regulator of c-Jun. It binds specifically to the leucine zipper region of c-Jun and represses its binding to a promotor containing the AP-1 binding sequence, leading to the inhibition of gene expression transactivated by c-Jun and suppression of AP-1 function (Monteclaro and Vogt, 1993). Since c-Jun and AP-1 have shown to be important transcriptional factors involved in cell proliferation, apoptosis and response to oxidative stress, it is possible to deduce that Jif/QM plays an important role in these cellular processes through association to c-Jun and AP-1 (Inada et al., 1997; Karin et al., 1997). In yeast, two groups reported that deletion of QM homolog GRC5/QSR1 was lethal and mutation of GRC5/QSR1 resulted in a defect in protein synthesis, growth and

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cell division arrest, abnormalities of actin cytoskeleton and mitochondrial respiration (Tron et al., 1995). In mammalian, QM is a member of multigene family in both mouse and man, appears to be expressed in a broad range of adult and embryonic tissues, and the level varies at different stages of development and differentiation, suggesting that QM might be involved in posttranslational protein processing which are essential for differentiation of specific tissues during embryogenesis and development (Mills et al., 1999). Furthermore, several recent studies revealed that QM protein was associated peripherally with 60S ribosomal subunit in the rough endoplasmic reticulum, but not with free ribosome in cytoplasm, which suggests convincingly that QM protein is involved in membrane-associated protein synthesis (Dick et al., 1997; Eisinger et al., 1997; Loftus et al., 1997; Nguyen et al., 1998). In addition, QM protein showed interactions with c-Yes and other Src family members in various tumor cell lines in vivo. Two different regions of QM protein were associated with the SH3 domain of c-Yes, resulting in the reduction of c-Yes kinase activity. The results suggest that the QM protein might be a regulator or mediator for various signal transduction pathways involving SH3 domain-containing membrane proteins and other cytoplasmic molecules, even in translation and transcription processes (Anderson et al., 1990; Kay et al., 2000; Shima et al., 2001).

Although QM gene has been found in a wide variety of eukaryotes, fish QM gene are still not well documented. In catfish, a 60S ribosomal protein L10 was found to be homologous to QM family, and it was reported as a mark for phylogenetic studies and comparative genomics (Patterson et al., 2003). In this paper, a gene homologous to QM is isolated from a lambda Uni-ZAP XR grass carp (*Ctenopharyngodon idellus*) head kidney and spleen cDNA library by anchored PCR protocol. Analyses of molecular characterization, expression pattern in various tissues and potential immune function of grass carp QM (GcQM) were undertaken. The study of QM gene in grass carp will provide significant evolutionary information of this gene and help identify the function of QM protein in fish.

2. Materials and methods

2.1. Experimental fish

Grass carp, *C. idellus*, 2 years old of both sexes, weighing approximately 600~1000 g, was obtained from the Institute of Freshwater Fisheries of Zhejiang, China. The fish were kept in the recirculating water at 20 °C and fed with commercial pellets at a daily ration of 0.7% of their body mass. All fish were held in laboratory for at least 2 weeks prior to use in experiments to allow for acclimatization and evaluation of overall fish health. Only healthy fish, as determined by general appearance and level of activity, were used for studies.

2.2. Induction of QM expression and RNA isolation

In an attempt to upregulate QM gene expression, an in vivo stimulation of fish with a strain of *Aeromonas hydrophila* BSK-100 and grass carp haemorrhagic virus (GCHV) was performed. The *A. hydrophila* BSK-100 and GCHV used in this experiment were pathogens of bacterial haemorrhagic septicemia of goldfish (*Carassius auratus*) and viral haemorrhagic disease of grass carp in China, and they were isolated from diseased fish in our laboratory (Shao et al., 2004). A group of grass carps were injected intraperitoneally with 2×10^8 bacteria and $10^{5.8}$ $TCID_{50}$ of GCHV per animal. Twenty-four hours after injection, the fish were anaesthetized in benzocaine (25 μg/mL, Sigma-Aldrich), sacrificed, and the head kidney and spleen were collected. Tissue samples were kept frozen in liquid nitrogen until RNA extraction. Total RNA was prepared for library construction using a TRIzol reagent (Gibco BRL, USA), following the manufacturer's instructions. Poly $(A)^+$ mRNA was purified from total RNA with PolyATtract™ mRNA Isolation System III (Promega, USA) according to the protocols provided by the manufacturer.

2.3. Construction of cDNA library

The cDNA library from head kidney and spleen of grass carp challenged with bacteria and virus as described above was constructed using Stratagene's Lambda Uni-ZAP XR cDNA cloning system based on the manufacturer's instruction manual. Briefly, first strand cDNA was synthesized from mRNA templates using StrataScript™ RNase H reverse transcriptase and a hybrid oligo(dT) linker–primer containing an XhoI restriction site, in the presence of dATP, dGTP, dTTP and 5-methyl dCTP. Second strand was generated with DNA polymerase I, following RNase H digestion, in the presence of normal dNTPs. The double-stranded cDNA was treated with T_4 DNA polymerase to blunt the ends before ligation with *Eco*RI adaptors. Subsequent digestion with XhoI resulted in double-strand template which was size fractionated directionally inserted into the Lambda Uni-ZAP XR vector. Finally, the library was packaged with the Gigapack III Gold Packaging Extract, and titer was estimated on *Escherichia coli* XL1-Blue MRF' strain. Amplification of the library was performed to obtain a more stable secondary library, and this library was subsequently used in PCR based approach to search for grass carp QM gene.

2.4. Prediction of fish QM homolog by in silico cloning in zebrafish EST database

In order to obtain the sequential information of fish QM gene and design specific primers for GcQM cloning, the potential QM sequence of zebrafish, *Danio rerio*, was predicted by in silico cloning. The human Wilms' tumor-related protein (QM) mRNA sequence (accession No.