Cloning and Analysis of N-Acetyltransferase 9 Genes in Yellow Catfish Pelteobagrus fulvidraco

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Abstract

N-acetyltransferase 9 (NAT9) is an important reproduction-related gene in fish. In this study, we cloned the full-length NAT9 cDNA sequence from yellow catfish Pelteobagrus fulvidraco. P. fulvidraco NAT9 cDNA is 1253 bp, which including non-coding region (584 bp) and open reading frame (669 bp). NAT9 gene encodes a 222 amino acids-protein which shares high homology with NAT9 in four species: Ictalurus punctatus (81%), Astyanax mexicanus (83%), Danio rerio (81%) and Lepisosteus osseus (81%). NAT9 protein is 25.789 kD, 4.86 theoretical isoelectric point and C_{14}H_{27}N_{4}O_{8}S_{2}, as revealed by computer-assisted analysis. Tissue transcription profile analysis indicated that the catfish NAT9 gene is generally but differentially expressed in the detected tissues, including gonads, brain, liver, kidney, spleen, intestine, blood, gills, muscle and heart. Identification and tissue distribution of yellow catfish NAT9 genes provided initial step towards understanding their biological roles in yellow catfish.

Keywords: N-acetyltransferase 9; Pelteobagrus fulvidraco; Gene clone; Bioinformatics analysis

Introduction

N-acetyltransferases (NATs) are well characterized xenobiotic-metabolizing enzymes which catalyze the acetyl Coenzyme A (AcCoA)-dependent N-, O- and N,O-acetylation of aromatic amines and hydrazines. They played important endogenous roles, as well as having potential as novel targets for pharmacological intervention. NAT9 is a member of NATS which has N-acetyltransferase, acetyltransferase and transferase activities. NAT9 has important biological function, due to the neutral protein electropositivity [1]. NAT acetylates wide range of drugs and xenobiotics into prominence, as acts in the polymorphic metabolism of the anti-tubercular agent isoniazid [2].

Latest studies reported that NAT9 was highly expressed in human brain, gonad, and specifically expressed in the heart, spleen, gonad of adult mouse. Whole-mount in situ hybridization showed that NAT9 specifically expressed in E7.5 and E8.5 mouse embryo brains and in HH10 stage chicken embryo brain. These results suggest that NAT9 may play important roles in the development of embryonic brain and adult brain and gonad's function [3]. As above reports, NAT9 is an important reproduction related gene. Although NAT9 gene was found has expression in human [4], rat [5], cattle [6] and other animals, this gene expression in yellow catfish has not been reported yet. Whether NAT9 gene is associated with the reproductive traits in yellow catfish is still unknown.

Yellow catfish Pelteobagrus fulvidraco, an omnivorous freshwater fish, is regarded as a good candidate for freshwater culture in China for its delicious meat and high market value [7]. In this study, we obtained the full-length cDNA sequence of NAT9 gene, and analyzed tissue expression and putative proteins through bioinformatics approaches. The results of our study provide the basic information of NAT9, and illuminate the molecular mechanism of this gene in P. fulvidraco.

Materials and Methods

RNA isolation

The P. fulvidraco were obtained from the Chinese Academy of Fishery Sciences P. fulvidraco Breeding Engineering Center. The yellow catfish were 2 years old, 60.6 ± 2.7 g weight and 18.5 ± 3.4 cm length. The extraction of total RNAs from the yellow catfish was conducted using the Trizol (Invitrogen, China) technique following the manufacturer's instructions. The total RNA concentration was determined by measuring the absorbance at OD260. RNA integrity was checked by electrophoresis. The total RNA was reverse-transcribed into cDNA using the aM-MLV RTase cDNA Synthesis Kit (TaKaRa, Japan).

NAT9 cloning

Every tissue total RNA used cDNA reverse treasure article number for RR047A transcription kit and used minus 20 degrees to put on cDNA template. According to Ictalurus punctatus in Genbank madtoms NAT9 (serial number DQ353801.1) cloning NAT9 cDNA partial sequence. After the sequence by Heilongjiang River Fisheries Research Institute of products research, development of genomics and molecular breeding research of yellow catfish are found in ninety-nine percent of similarity in the method of the transcriptome gene sequence and gene sequence designed primers. We designed the PCR primers NAT9-F1, NAT9-R1, NAT9-F2, NAT9-R2, NAT9-F3, NAT9-R3, NAT9-F4, NAT9-R4, NAT9-F5 and NAT9-R5 to clone the NAT9 cDNA partial sequence (Table 1).

PCR was conducted for 5 min at 94°C followed by 35 cycles at...
Bioinformatics analysis

The physicochemical properties, protein domains, secondary structures, secretory proteins, subcellular localization signals, transmembrane regions, and 3D structure models of the putative proteins of NAT9 were predicted and analyzed using online bioinformatics tools, as listed in Table 2. To strengthen the conclusions, more than one bioinformatics tool was used in the prediction of
secretory proteins, subcellular localization signals, and transmembrane regions for the protein. The sequence alignment of amino acids was performed by using the BLASTX program, which is available on the NCBI website. The amino acid sequences of HsHDR1 and HsHDR2 were aligned, and the phylogenetic tree was constructed with neighbor-joining criteria by the MEGA 6.0 software.

**Statistical analysis**

All data obtained from the qRT-PCR were calculated to the value of 2^{-△△CT} and differences were evaluated statistically using a t-test in SPSS 20.0 software (IBM, Chicago, USA). The relative expression levels of each gene in different tissues were calculated to the 2^{-△△CT} of the mean and the standard error (SE) from the three replications. Statistical significance was set with a P value less than 0.01.

**Results**

**Phylogenetic analysis of the NAT9 gene**

A phylogenetic tree was constructed based on the amino acid sequences of selected NAT9 using the neighbour-joining (NJ) method (Figures 1 and 2). Data were analyzed by Poisson’s correction, and gaps were removed by complete deletion. *Neolamprologus brichardi*, *Astatotilapia burtoni* and *Reochromis* spp. belong to Perciformes; *Fundulus heteroclitus*, *Oryzias latipes*, *Xiphophorus maculatus* and *Pecilia formosa* belong to Cyprinodontiformes; *Fugu rubripes* of Tetraodontiformes and yellow catfish NAT9 amino acids have different branches. The results showed that the molecular NAT9 evolutionary relationships and the corresponding species are known similar evolutionary relationships. The NAT9 gene encodes a protein of 222 amino acids which shares high homology with the NAT9 from four species: *Ictalurus punctatus* (91%), *Astatotilapia burtoni* (83%), *Danio rerio* (81%) and *Lepisosteus oculatus* (81%) (Figure 2).

**Tissue expression of NAT9 gene**

Quantitative real-time PCR was used to determine tissue distribution of NAT9 gene expression in both male and female *P. fulvidraco*. NAT9 genes were widely expressed in all 10 tissues (gonads, brain, liver, kidney, spleen, intestine, blood, gills, muscle and heart). NAT9 gene expression in the male gut and liver were higher than in female. NAT9 gene expression in the female heart, gill, muscle and spleen were higher than males (Figure 3).

**Analysis of the physicochemical properties**

We analyzed the physicochemical properties of the putative NAT9 protein using the ProtParam (http://www.expasy.org/tools/protparam.html). The molecular weight of NAT9 protein was 25,789 kDa and 4.86 theoretical isoelectric point. The negatively charged amino acid residues (Asp + Glu) was 40, and positively charged amino acid residues (Arg + Lys) was 26. The instability coefficient of NAT9 protein was 48.15, indicating that the protein was unstable. Theoretical isoelectric half-life of NAT9 protein was about 30 h. The fat coefficient was 0.669 and grand average of hydrophobic was 71.94. Amino acid content: Glu 12.2%, Leu 9.0%, Thr 7.7%, Arg 7.2%, Gly 6.8%, Ser 6.3%, Asp 5.9%. The hydropathicity were 0.669 (Table 3). The other detailed results of the physicochemical properties for the protein, including the

**Table 3: Physicochemical properties of the putative NAT9 proteins.**

| Protein characteristic Website | NAT9 |
|-------------------------------|------|
| The number of amino acids     | 222  |
| Formula                       | C_{112}H_{175}N_{26}O_{36}S_{1} |
| Molecular weight              | 25.7899 kDa |
| Isoelectric point (pI)        | SWISS-MODEL |
| Secretary protein Positively charged residues (Arg+Lys) | 40 |
| Negatively charged residues (Asp+Glu) | 26 |
| Instability index             | 48.15 |
| Aliphatic index               | 0.669 |
| Grand average of hydropathicity | 71.94 |
Prediction of signal peptide and transmembrane domain

We analyzed the signal peptides in NAT9 by using the SignalP 3.0 Server (http://www.cbs.dtu.dk/services/SignalP/). Significant signal peptides were predicted based on the putative protein sequence, which indicated that NAT9 was secretory proteins and might play an important role (Table 4). The TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/) were also used to analyze transmembrane domains. No transmembrane domains were found in this protein, which indicated that NAT9 may locate in the cellular cytoplasm.

Prediction of secondary structure and construction of 3D structure model

The secondary structure of the putative protein was predicted by Predictica protein server software (http://www.predictprotein.org/). The percentage of α-helix, β-sheet, and random coil was 45.05%, 4.95%, and 50.00%, respectively. The 3D structures of the putative NAT9 protein were predicted by the SWISS-MODEL, using the GANT (Gcn5-related N-acetyltransferase) as the template. Disorder prediction and fold recognition showed that P. fulvidraco NAT9 protein was similar with c3tcvB, genetic code template structure (Figure 4). Compared the template and P. fulvidraco NAT9 PDF files by Rasmol view fish; we found two monomer structure were very similar (Figure 4). Gcn5-related N-acetyltransferase super family is a comprehensive super family. They have a huge number of homologous region and acetylated related motif similar kind of material [8], including general control nonderepressible-5', Hat1, Elp3, Hpa2, other eukaryotic and prokaryotic acetyltransferase of different substrates. The Gcn5-related super family is a comprehensive super family. They have a huge number of amino acids, formula and isoelectric point were summarized in Table 3.

HATs maintain cell dynamic equilibrium that many diseases incidence relate to histone acetylation/deacetylation balance disorder. Tissue expression analysis shown yellow catfish NAT9 gene has huge differentially expressed in detected tissues. The suitable explanation for this under current conditions is that at the same time those biological activities related to the mRNA expression of yellow catfish NAT9 gene were presented diversely in different tissues. This indicates that NAT9 gene has diverse roles in different tissues because of the expression difference. To further understand the function of this novel gene, more research based on these primary results is needed.

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In summary, based on the sequence from full-length cDNA of the NAT9 belong to NAT family in P. fulvidraco, we analyzed and detected tissue transcription profile and also predicted the putative protein using bioinformatics tools. These results of our study will pave the way to furthers researches the reproduction and disease resistance of NAT9 in P. fulvidraco.

Discussion

In the current study, we firstly cloned the full-length cDNA of P. fulvidraco NAT9 gene. Through sequence analysis, it can be seen that the encoding protein of P. fulvidraco NAT9 gene is highly homologous with NAT9 in other fishes. This implied that the NAT9 genes were highly conserved in some fishes, and NAT9 of yellow catfish might have similar functions as in other fishes. NAT9 protein of yellow catfish has new feature compared with other fishes’ NAT9, hints that P. fulvidraco NAT9 gene has some unique functions than other fishes.

Number of amino acids, formula and isoelectric point were summarized in Table 3.

Table 4: Results of signal peptide sequence prediction.

| Measure | Position | Value | Cutoff | Signal peptide | Secretory protein |
|---------|----------|-------|--------|----------------|------------------|
| Max.C   | 30       | 0.565 | 0.32   | YES            | YES              |
| Max.A   | 30       | 0.690 | 0.33   | YES            | YES              |
| Max.S   | 12       | 0.989 | 0.87   | YES            | YES              |
| Mean S  | 1-29     | 0.852 | 0.48   | YES            | YES              |
| D       | 1-29     | 0.771 | 0.43   | YES            | YES              |

Figure 4: 3D model for NAT9 and c3tcvB. A - NAT9 Monomer 3D structure B - c3tcvB Monomer 3D structure
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