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Expression of tumor suppressor genes in channel catfish after bacterial infections

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ABSTRACT

Tumor suppressor genes are negative regulators of tumor formation. While their anti-tumor functions have been well studied, they have been found to be also involved in immune responses and innate immunity. In this study, 21 tumor suppressor genes in channel catfish (*Ictalurus punctatus*) were characterized. Phylogenetic and syntenic analyses allowed annotation of all 21 catfish tumor suppressor genes. The expression profiles of the 21 catfish tumor suppressor genes were determined using the RNA-Seq datasets. After *Edwardsiella ictaluri* infection, expression of five of the 21 tumor suppressor genes was up-regulated at 3 days in the intestine, and four of the 21 genes were up-regulated in the liver 14 days post-infection. With *Flavobacterium columnare* infection, seven genes were up-regulated in the gill at 48 h post-infection. These results expanded our knowledge on the tumor suppressor genes in teleosts, setting a foundation for future studies to unravel functions of tumor suppressor genes in response to stresses, particularly after bacterial disease infections.

1. Introduction

Tumor suppressor genes are negative regulators of tumor formation (Knudson, 1993). In general, they deliver anti-tumor functions through repression of genes necessary for the continuing of cell cycle, mismatch repair, or apoptosis (Banno et al., 2014; Eskander et al., 2014; Shoemaker et al., 1998). For instance, Rb1, WT1, P53, BRCA1 and BRCA2 function as transcriptional and cell-cycle regulators in mammals (Chinnam and Goodrich, 2011; Dehbi and Pelletier, 1996; Hickman et al., 2002; Miki et al., 1994); APC, DPC4, PTEN and NF1 function in intracellular signaling pathways (Cantley and Neel, 1996; Cichowski et al., 2003; Hata et al., 1998; Kikuchi, 2003); and MSH2, MSH6, PMS1, PMS2 and MLH1 function in mismatch repair pathways (Baker et al., 1996; Edelmann et al., 2000; Harfe and Jinks-Robertson, 2000; Prolla et al., 1994; Wind et al., 1995). While these traditional anti-tumor functions are well studied, recent studies seem to link expression of many tumor suppressor genes with immune responses and innate immunity. For instance, depletion of RB in hepatoma cells resulted in a compromised immunological response to multiple stimuli and reduced the potential of these cells to recruit myeloid cells (Hutcheson et al., 2014). Down-regulation of tumor suppressor genes has been observed under stresses that are often accompanied with inhibition of immune functions (Sarkar and Zhang, 2013). The interactions of P53 and immune responses have been well studied (Dharel et al., 2008; Komarova et al., 2005; Menendez et al., 2013). For instance, P53 can modulate the expression of many TLR genes (Menendez et al., 2013). The absence of p53 resulted in delayed cytokine and antiviral gene responses in lung and bone marrow, decreased dendritic cell activation, and reduced IAV-specific CD8 (+) T cell immunity (Muñoz-Fontela et al., 2011).

Many tumor suppressor genes have been identified in mammals, including, but not limited to, Rb1, WT1, P53, NKX3.1, PTC, BRCA1, BRCA2, APC, DPC4, P19, LKB1, PTEN, NF1, TSC2, MSH2, MSH6, PMS1, PMS2, MLH1 and VHL (Macleod, 2000). However, characterization of tumor suppressor genes from teleost fish is limited. Previous studies have characterized tumor suppressor genes P53 and VHL in fish (Kraus et al., 1997; Luft et al., 1998; van Rooijen et al., 2009). Interestingly, activation of the tumor suppressor gene VHL in zebrafish displayed a general systemic hypoxia response (van Rooijen et al., 2009), suggesting involvement of tumor suppressor genes in hypoxia responses as well as in disease responses.

Enteric septicemia of catfish (ESC) is caused by a bacterial pathogen, *Edwardsiella ictaluri* (Hawke et al., 1981). It is one of the most common diseases in channel catfish and caused major economic losses to the catfish industry (Li et al., 1993). Another bacterial

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disease, Columnaris disease caused by the bacterium *Flavobacterium columnare*, can also cause huge economic losses. It is characteristic of pronounced erosion or necrosis of external tissues, with the gills often being the major site of damage (Davis, 1922; Farkas and Oláh, 1986).

With fish species, stress responses are often linked with reduced immunity and increased disease incidence and severity. While many of the innate immune genes have been characterized from catfish, analysis of the involvement of tumor suppressor genes in disease responses or under stress conditions have not been conducted. Thus, analyses of the gene expression of tumor suppressor genes under disease situations are of interest. In this study, we characterized a set of 21 tumor suppressor genes from channel catfish, and analyzed their expression patterns after bacterial infections using existing RNA-Seq datasets (Li et al., 2012; Sun et al., 2012; Wang et al., 2013).

2. Materials and methods

2.1. Database mining and sequence analysis

To identify the tumor suppressor genes, the transcriptome databases (Liu et al., 2011, 2012) and whole genome database of channel catfish were searched using available tumor suppressor protein sequences of human, chicken, frog and teleosts as queries. TBLASTN was used to obtain the initial pool of the transcriptome sequences. ORF finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) and FGENESH (Solovyev et al., 2006) were used to predict amino acid sequences. BLASTN was used to verify the cDNA sequences by aligning the cDNA sequences with the whole genome sequences. The predicted amino acid sequences were verified by searching against NCBI non-redundant protein sequence database using BLASTP. Conserved domains of these proteins were identified by SMART (http://smart.embl.de/).

2.2. Phylogenetic analysis

To conduct phylogenetic analysis, we selected available coding sequences of tumor suppressor genes in NCBI database from various species, including human, mouse, chicken, frog, and several teleost fish species. The full-length amino acid sequences as well as the partial coding sequences (in the absence of full-length sequences) for the conserved domains were used in the phylogenetic analysis. Multiple protein sequence alignments were conducted using the ClustalW (Thompson et al., 2002). Phylogenetic analysis was conducted using MEGA 5 with the maximum likelihood method (Tamura et al., 2011). The bootstrapping with 1000 replications was conducted to evaluate the phylogenetic tree.

2.3. Syntenic analysis of P53 tumor suppressor gene

In cases where phylogenetic analyses provided inconclusive results of the gene identity (P53), syntenic analyses were also conducted to provide evidence for orthologies. The deduced catfish P53 amino acids were used as query to blast the draft catfish genome sequences, and obtained the genomic scaffolds containing the neighboring genes. The neighboring genes of the catfish P53 were identified from the channel catfish scaffolds by FGENESH program (Solovyev et al., 2006). Ensembl database and Genomicus (Louis et al., 2013) were used get the conserved syntenic pattern of P53 genes for zebrafish, fugu, tilapia, and human.

2.4. Expression of tumor suppressor genes after bacterial infections

To determine the expression profiles of tumor suppressor genes in response to bacterial infections, expression analyses were conducted using existing RNA-Seq datasets with bacterial challenges (Li et al., 2012; Sun et al., 2012; Wang et al., 2013), with CLC Genomics Workbench software package. Briefly, the short reads of RNA-Seq were initially mapped onto the channel catfish tumor suppressor genes. Alignment parameters were set as ≥95% of reads in alignment to the reference and maximum mismatches of ≤2. After the number of total mapped reads for each transcript was determined, it was normalized to obtain the Reads Per Kilobase of exon model per Million mapped reads (RPKM). The differentially expressed genes between control and treatment groups were determined by the proportions-based Kal's test with P-value < 0.05, and fold changes were calculated. Transcripts with absolute fold change values of greater than 1.5 and total read number of ≥5 were included in analysis as differentially expressed genes.

3. Results

3.1. Identification and phylogenetic analysis of catfish tumor suppressor genes

A total of 21 tumor suppressor genes were identified in channel catfish, including APC, BRCA2, DPC4, FHIT, P19, LKB1, MLH1, MSH2, MSH6, NF1, NKX3.1, P53a, P53b, PTCH, PTEN, PSM1, PMS2, TSC2, Rb1, VHL and Wt1 (Table 1). All of these sequences have been deposited to the NCBI transcriptome shotgun assembly (TSA) database with accession numbers listed in Table 1.

Main functional domains of 12 genes in catfish were compared with those of zebrafish (Fig. 1 and Fig. 2). Among these genes, five genes possessing the function in mismatch repair were identified, which included PMS1, PMS2, MLH1, MSH2, and MSH6 (Fig. 1). Orthologs of these five genes were found in human, mouse and chicken, as well as zebrafish, medaka, fugu, and tilapia (Table 2). The catfish MSH2 protein possessed MUTsd (T^{323}-Q^{647}) and MUTsac (E^{664}-L^{851}) domains, which was similar to that of zebrafish. Besides MUTsd and MUTsac domains, catfish MSH6 contained a PWWP domain (F^{89}-K^{151}) which was not found in zebrafish. The HATPase_c domain (E^{20}-N^{155}) could be found in both catfish and zebrafish MLH1. Apart from HATPase_c, PMS1 of catfish and zebrafish possessed a HMG domain (S^{527}-A^{597}). The catfish PMS2 owned HATPase_c and MutL_c domain (V^{673}-L^{817}), which was consistent with that of zebrafish.

We also analyzed the functional domains for the tumor suppressor genes of catfish involved in nuclear transport, signal

Table 1
Tumor suppressor genes identified from channel catfish genome. Asterisk indicates the duplication of P53.

Gene	cDNA (bp)	5′-UTR	3′-UTR	Amino acids	Accession
APC	9225	220	722	2760	JT412315
BRCA2	1968	0	0	615	JT444301
DPC4	4519	401	2477	546	JT405846
FHIT	4905	187	4265	150	JT407722
P19	1059	209	337	170	JT467461
LKB1	3162	985	857	439	JT406774
MLH1	2546	184	199	720	JT417010
MSH2	3136	15	304	938	JT407546
MSH6	7758	56	3583	1372	JT457467
NF1	9963	170	1576	2738	JT413455
NKX3.1	949	119	182	215	JT244737
P53a	2163	191	841	376	JT408741
P53b	2341	251	905	394*	JT341182
PTCH	4862	9	497	1451	JT407623
PTEN	4916	933	2774	402	JT409867
PMS1	2948	69	194	894	JT407180
PMS2	3195	368	253	857	JT405684
TSC2	1380	358	389	1788	JT419436
Rb1	2942	170	63	902	JT406432
VHL	1877	259	1099	172	JT408172
Wt1	3487	437	1793	418	JT411207