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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 |

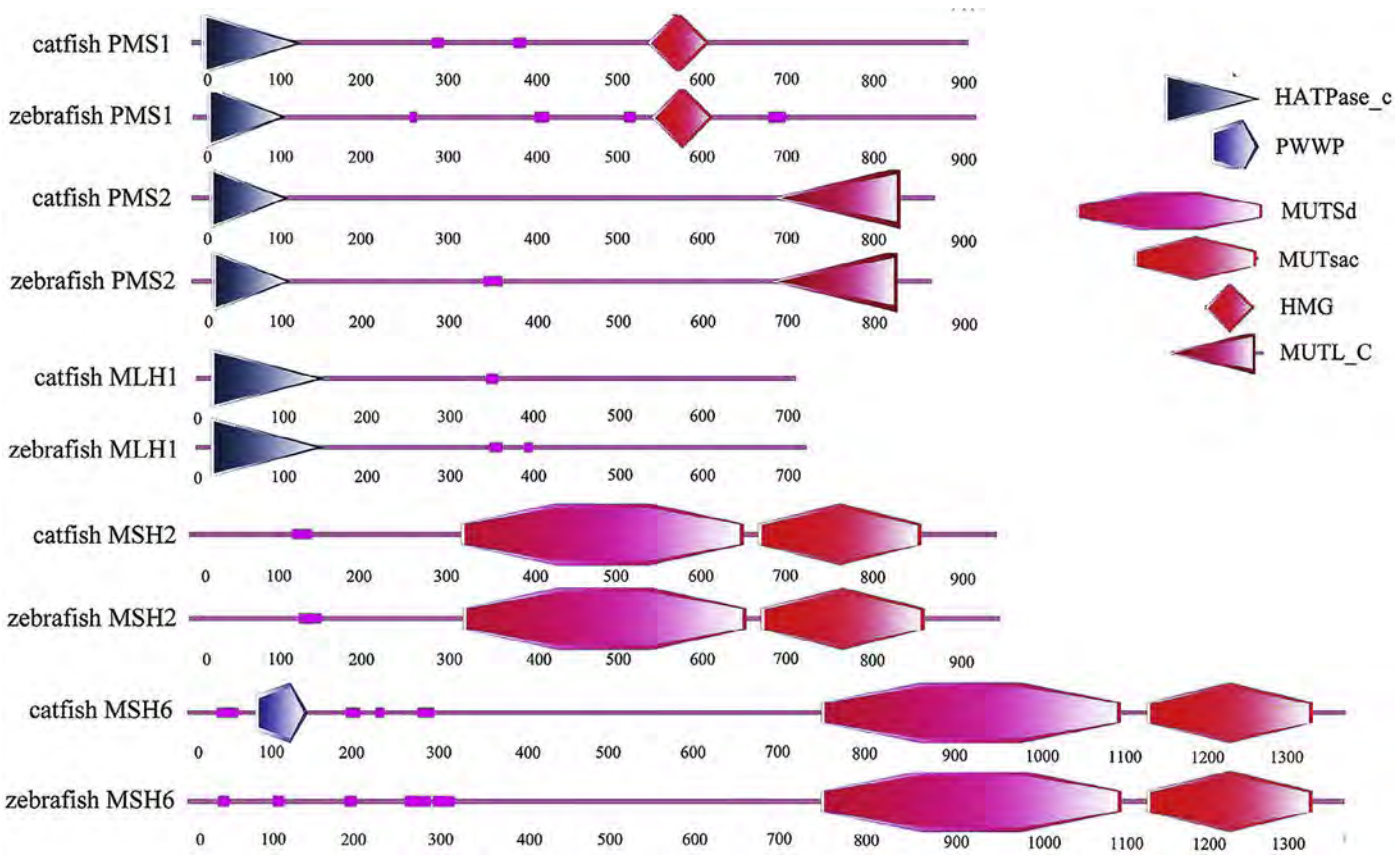


**Fig. 1.** Schematic presentation of the domain architecture of tumor suppressor genes (PMS1, PMS2, MLH1, MSH2, and MSH6) between channel catfish and zebrafish.

transduction and transcription regulation, APC, LKB1, NKX3.1, PTCH, SMAD4 and WT1 (Fig. 2). Six N-terminal ARM domains ($S^{324}$-$H^{376}$, $D^{443}$-$F^{494}$, $D^{496}$-$W^{537}$, $D^{540}$-$A^{581}$, $C^{583}$-$S^{628}$, $N^{633}$-$A^{673}$, Fig. 2) were found in catfish APC, which were also present in the APC gene of zebrafish. The LKB1 gene, possessing the S_Tkc domain ($Y^{47}$-$R^{307}$), was well conserved between catfish and zebrafish. Within NKX3.1, a HOX domain ($Q^{99}$-$D^{161}$) was observed in both the catfish and zebrafish genes. The catfish PTCH was composed of 12 transmembrane domains ($V^{441}$-$T^{460}$, $A^{472}$-$L^{494}$, $T^{504}$-$F^{526}$, $G^{547}$-$I^{569}$, $A^{579}$-$L^{600}$, $S^{766}$-$V^{785}$, $I^{805}$-$M^{822}$, $H^{1040}$-$L^{1062}$, $A^{1066}$-$I^{1088}$, $I^{1095}$-$G^{1117}$, $F^{1137}$-$V^{1151}$, $F^{1166}$-$L^{1188}$), while zebrafish only possessed 11 transmembrane domains. The catfish Wt1 possessed four Znf C2H2 domains ($F^{292}$-$H^{316}$, $Y^{322}$-$H^{346}$, $F^{352}$-$H^{374}$, $F^{383}$-$H^{407}$), which was in consistence with Wt1a and Wt1b of zebrafish that also contained these conserved domains.

**Table 2**
Comparison of gene copy numbers in various selected species.

| Name | Human | Mouse | Chicken | Catfish | Zebrafish | Medaka | Fugu | Tilapia |
|---|---|---|---|---|---|---|---|---|
| APC | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| BRCA1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 |
| BRCA2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| FHIT | 1 | 1 | 1 | 1 | 2 | 0 | 1 | 1 |
| P53 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 |
| PMS1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| PMS2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| PTCH | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 |
| PTEN | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 |
| P19 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 |
| LKB1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| MLH1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| MSH2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| MSH6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NF1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 |
| NKX3.1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 |
| Rb1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| DPC4 | 1 | 1 | 0 | 1 | 2 | 2 | 2 | 2 |
| TSC2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| VHL | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Wt1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 |

### 3.2. Phylogenetic analyses

Phylogenetic analysis was conducted to determine the identities of the catfish tumor suppressor genes. While annotations for 19 of the 21 tumor suppressor genes were relatively straightforward with phylogenetic analysis (Supplementary Figs. S1–19), the catfish P53 genes cannot be annotated by phylogenetic analysis alone. Two P53 genes were identified in catfish genome but only one P53 gene was found in human, chicken, mouse and several fish species. Although high levels of sequence similarities existed and the functional domains were well conserved between the two genes (Fig. 3), Phylogenetic analysis (Fig. 4) resulted in a phylogenetic tree, where catfish P53a and P53b fell into two distinct clades, each of which contained P53 genes from different teleost species, suggesting that one of the P53 genes may have been lost in various teleost species.

### 3.3. Syntenic analysis of P53 gene

As shown in Fig. 5, syntenic analysis of catfish P53 genes showed that they shared similar neighboring genes as compared with human, zebrafish, fugu and tilapia. The *gps2* gene was closely linked with P53a, which was similar with human and zebrafish (Fig. 5). Catfish P53a was located next to *capga*, which was found in fugu, tilapia and zebrafish as well. Catfish P53b was located next to *yb2*,

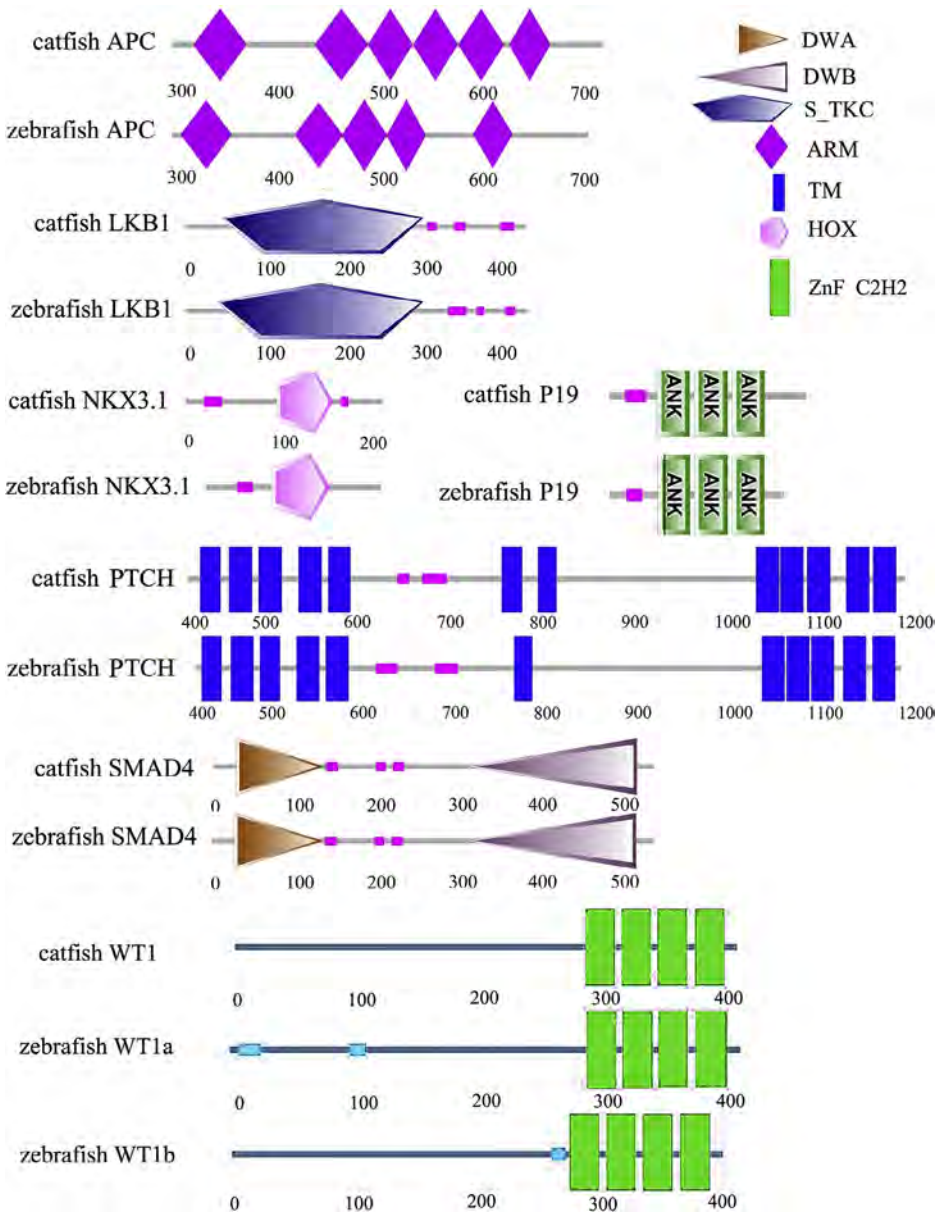


**Fig. 2.** Schematic presentation of the domain architecture of tumor suppressor genes (APC, LKB1, NKX3.1, P19, PTCH, SMAD4 and WT1) between channel catfish and zebrafish.

which was similar as in fugu and tilapia. Thus, syntenic analysis provided further evidence supporting the two P53 genes from catfish being derived whole genome duplication, and hence should be named P53a and P53b, according to the Zebrafish Nomenclature Guidelines (http://zfin.org).

### *3.4. Differentially expressed tumor suppressor genes in catfish after bacterial infection*

With publicly available RNA-Seq datasets (intestine samples after *E. ictaluri* challenge, liver samples after *E. ictaluri* challenge and gill

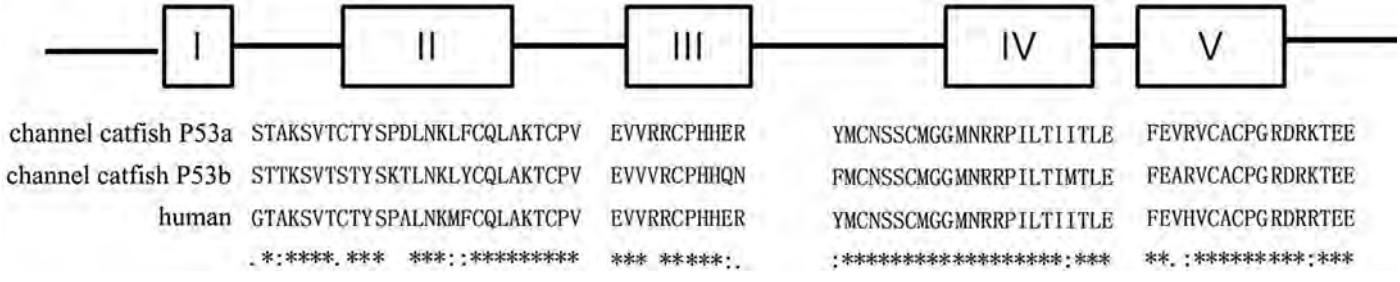


**Fig. 3.** Comparison of P53 conserved domains between channel catfish and human. The five blocks represented I–V domains of P53.

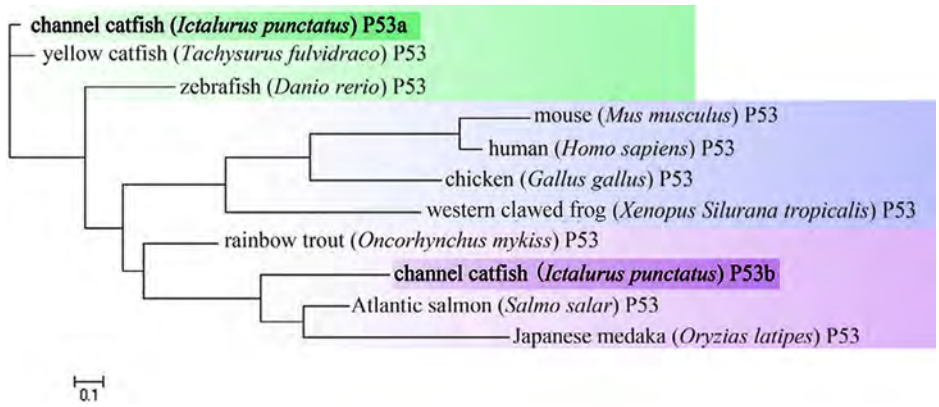


**Fig. 4.** Phylogenetic analysis of P53 in selected fish species and mammals. Gaps were removed by complete deletion and the phylogenetic tree was evaluated with 1000 bootstrap replications. The accession numbers of the protein sequences are: yellow catfish, AEB72290; zebrafish, AAH95597; rainbow trout, NP_001118164; Atlantic salmon, ACN10490; Japanese medaka, AA001196; mouse, AAA39883; human, BAC16799; chicken, NP_990595; western clawed frog, NM_001001903.

samples after *F. columnare* challenge), we determined the expression of tumor suppressor genes after bacterial infection.

### 3.4.1. *Expression of tumor suppressor genes in catfish intestine after ESC challenge*

Of the 21 catfish tumor suppressor genes, seven were found to exhibit significant differential expression at 3 days post-ESC challenge in the intestine (Table 3). Among them, the expression of *APC*, *DPC4*, *NF1*, *PTCH* and *PTEN* was always up-regulated throughout the 3-day infection treatment. The expression of *MLH1* was up-regulated at 24 h, then down-regulated at 3 days post-infection. In addition, the expression of *MSH6* was down-regulated during the 3-day ESC challenge, but only significant down-regulation was observed at 3 h post-infection (Table 3).

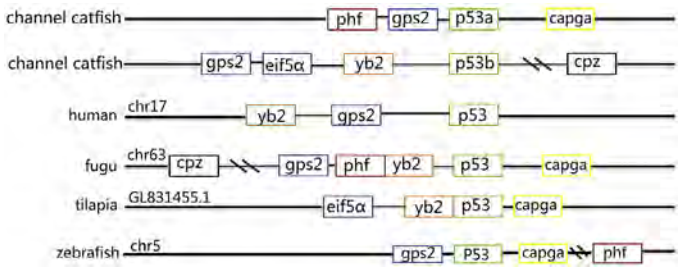


**Fig. 5.** Syntenic analysis of P53 gene. Abbreviations: Phf, PHD finger protein 23; gps2, G protein pathway suppressor 2; yb2, Y box binding protein 2; cpz, carboxypeptidase Z; eif5α, eukaryotic translation initiation factor 5a-1.

**Table 3**
Differentially expressed tumor suppressor genes in the intestine of catfish after ESC challenge. Asterisks indicated the time points where the expressions of the genes were significantly up- or down-regulated compared to the control ($P$-value < 0.05).

| Gene name | 3 hours | 24 hours | 3 days |
|---|---|---|---|
| APC | 1.68* | 1.93* | 1.93* |
| DPC4 | 1.95* | 1.79* | 2.41* |
| MLH1 | −1.07 | 1.59* | −1.32 |
| MSH6 | −1.53* | −1.14 | −1.25 |
| NF1 | 2.25* | 3.16* | 2.30* |
| PTCH | 2.17* | 2.64* | 1.71* |
| PTEN | 2.05* | 2.09* | 1.68* |

### 3.4.2. *Expression of tumor suppressor genes in catfish liver after ESC challenge*

Of the 21 catfish tumor suppressor genes, eight were found to be differentially expressed after ESC challenge during 14-day ESC-challenge in the catfish liver (Table 4). Three of these eight genes were up-regulated, including MLH1, P53b and PMS1. The expression of MSH2, Rb1, VHL and WT1 were down-regulated throughout the 14-day bacterial infection. The expression of *PTEN* was up-regulated at 3 days, then down-regulated at 14 days post-infection.

### 3.4.3. *Expression of tumor suppressor genes following* F. columnare *challenge*

As summarized in Table 5, nine of the 21 catfish tumor suppressor genes were differentially expressed at 48 h after *F. columnare* challenge in the gill. Among these genes, seven were up-regulated within the first 48 h after infection, including APC, BRCA2, MLH1, MSH2, NKX3.1, PMS1 and Rb1. Only one gene, MSH6, was down-regulated. The catfish P53b was down-regulated at 4 h, then was up-regulated at 24 h and 48 h post-infection.

## 4. Discussion

It is well known that there are almost 50,000 living vertebrate species in the world, more than half of which are teleost fish (Harshbarger and Slatick, 2001). All vertebrate species have the potential to develop cancer, and understanding the tumor suppressor genes in teleosts is of great importance (Goessling et al., 2007). Even though a number of studies have been conducted with tumor suppressor genes involved in immune function in vertebrates (Barker

**Table 4**
Differentially expressed tumor suppressor genes in the liver after ESC challenge. Asterisks indicated the time points where the expressions of the genes were significantly up- or down-regulated comparing to the control ($P$-value < 0.05).

| Gene name | 3 days | 14 days |
|---|---|---|
| MLH1 | 2.69* | 1.79* |
| MSH2 | −1.80* | −1.36 |
| P53b | 1.83* | 1.25 |
| PMS1 | 3.22* | 2.15* |
| PTEN | 1.70* | −1.05 |
| Rb1 | −1.41 | −1.97* |
| VHL | −2.50* | −1.99* |
| WT1 | −1.80* | −1.36 |

**Table 5**
Differentially expressed tumor suppressor genes in the gill after *F. columnare* infection. Asterisks indicated the time points where the expressions of the genes were significantly up- or down-regulated compared to the control (*P*-value < 0.05).

| Gene name | 4 hours | 24 hours | 48 hours |
|---|---|---|---|
| APC | 1.44 | 1.33 | 1.57* |
| BRCA2 | 1.29 | 1.72* | 1.37 |
| MLH1 | 1.27 | 1.94* | 1.66* |
| MSH2 | 1.02 | 1.55* | 1.43 |
| MSH6 | −1.29 | −1.64* | −1.14 |
| NKX3.1 | 1.04 | 1.63* | 1.13 |
| P53b | −1.14 | 1.80* | 1.62* |
| PMS1 | 1.58* | 2.06* | 1.32 |
| Rb1 | 1.02 | 1.55* | 1.43 |

et al., 2002; Hutcheson et al., 2014), expression analysis of these genes in teleosts after bacterial infection was lacking. In this work, for the first time, 21 tumor suppressor genes were identified in channel catfish genome, and their expression profiling was determined to provide insights into their involvement in combating bacterial infection.

Two copies of P53 were found in channel catfish genome, while only one P53 gene was found in other species. Phylogenetic analysis suggested that both of the catfish P53a and P53b genes were clustered in clades containing teleost P53 genes, suggesting that the two catfish P53 genes may have been derived from whole genome duplication. If that is true, this would also suggest that one copy of the P53 genes have been lost in the teleost species under study. To provide stronger evidence for this, we also conducted syntenic analysis. As shown in Fig. 5, the genomic neighborhood of P53a and P53b contained similar gene contents, suggesting that they indeed were evolved from whole genome duplication. However, due to extensive gene loss in the genomic neighborhood, the level of syntenic conservation was low.

In the present study, we also analyzed the expression of tumor suppressor genes in the intestine and liver after ESC challenge. Five genes including *APC*, *DPC4*, *NF1*, *PTCH* and *PTEN* were induced after infection of *E. ictaluri*. Although the functions of the tumor suppressor genes during bacterial infection remain unknown at the present, existing literatures suggest their involvement in immune responses. For instance, PTEN was reported to be responsible for the elevated production of cytokines in response to TLR agonists (Sahin et al., 2014). Studies of mouse cholangiocellular carcinoma revealed that *DPC4* and *PTEN* had a similar pattern of expression after infection (Xu et al., 2006). A similar expression pattern of the two genes was also observed in this work, suggesting that the two genes may have similar functions after infection. Expression of *NF1*, *PTCH* and *PTEN* genes was induced at 24 h, and then repressed at 3 days post-infection, which verified that acute stress enhanced the innate immunity, while chronic stress damaged it (Demers and Bayne, 1997; Dhabhar and Mcewen, 1997). Reports suggested that the *NF1* tumor suppressor gene was involved in cellular remodeling in response to injury (Giordano et al., 1996). In contrast with genes up-regulated at 3 days post-infection in the intestine, three different genes were up-regulated in the liver at 14 days after ESC treatment, including *P53b*, *MLH1* and *PMS1*. These results indicated that the innate immunity of catfish was affected by different tumor suppressor genes between acute and chronic treatments. Among these up-regulated genes, P53 was well known in inducing cell growth arrest, cell apoptosis, cell differentiation and DNA repair (Gijssel et al., 1997). In the study of rats, P53 had a high expression level under nicotine treatment, but had low expression level with supplementation of Vitamin C (Ahmed et al., 2014). The p53 activity can also be increased by inhibitors of BH4 activity or synthesis (Spurlock et al., 2014). These studies all indicated that P53 are inducible in response to stresses, but its regulation pathways may be quite complex.

Expression of tumor suppressor genes in the gill after *F. columnare* infection was also analyzed. Reports suggested that the adhesion of *F. columnare* to the gill tissue constituted an important step in pathogenesis (Decostere et al., 1998); therefore, the gill was chosen as a critical tissue for *F. columnare* research. After *F. columnare* infection, two genes of *MLH1* and *PMS1* were induced at 24 h, which were also expressed intensely after ESC infection in the liver (Table 4). It is known that MLH1 and PMS1 are two members of DNA mismatch repair system, which are involved in the acquisition of genetic damage in somatic cells and their immune system (Neri et al., 2008). Interestingly, some but not all of DNA mismatch repair genes play important roles in bacterial disease response, further studies are needed to understand the functions of this family in immune responses.

In summary, 21 tumor suppressor genes of catfish were identified and characterized in this study. We analyzed the expression pattern in the intestine and liver after *E. ictaluri* challenge and expression profiles in the gill after *F. columnare* challenge using available RNA-Seq datasets. This study revealed novel expression patterns of teleost tumor suppressor genes and highlighted the roles involved in immune responses.

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## Appendix: Supplementary Material

Supplementary data to this article can be found online at doi:10.1016/j.dci.2014.10.004.

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