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Pathogen recognition receptors in channel catfish: II. Identification, phylogeny and expression of retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs)

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ABSTRACT

Vertebrates including teleost fish have evolved an array of pathogen recognition receptors (PRRs) for detecting and responding to various pathogen-associated molecular patterns (PAMPs), including Toll-like receptors (TLRs), nucleotide-binding domain, leucine-rich repeat containing receptors (NLRs), and the retinoic acid inducible gene I (RIG-I) like receptors (RLRs). As a part of the series of studies targeted to characterize catfish PRRs, we described 22 NLR receptors in the sister contribution. Here in this study, we focused on cytosolic PRRs recognizing nucleotide pathogen-associated molecular patterns (PAMPs) of invading viruses, the retinoic acid-inducible gene I (RIG-I)-like receptors (RLR receptors). Three RLRs with DExD/H domain containing RNA helicases, retinoic acid inducible gene-I (RIG-I), melanoma differentiation-associated gene 5 (MDA5) and laboratory of genetics and physiology 2 (LGP2), were identified from channel catfish, *Ictalurus punctatus*. The catfish RIG-I encodes 937 amino acids that contains two CARDs, a DExDc, a HELICc and a RD domains. MDA5 encodes 1005 amino acids with all the domains identified for RIG-I. LGP2 encodes 677 amino acids that contain other domains but not the CARD domain at the *N*-terminus. Phylogenetic analyses of the three genes of catfish showed close clustering with their counterparts from other teleost fish. All the genes were found to be constitutively expressed in various tissues of catfish with minor variations. Channel catfish ovarian cells when infected with channel catfish virus showed significant increase in the transcript abundance of all the three genes. Further, RLR genes showed significant increases in expression in the liver tissue collected at different time-points after bacterial infection as well. The results indicate that the catfish RLRs may play important roles in antiviral and anti-bacterial immune responses.

1. Introduction

Pathogen recognition receptors (PRRs) play a crucial role in pathogen surveillance in all eukaryotic organisms. PRRs recognize the conserved molecular signatures associated with pathogens termed pathogen-associated molecular pattern (PAMPs), that include proteins, lipids and nucleotides, and result in activation of host innate immune response (Pichlmair and Reise Sousa, 2007). After sensing the PAMPs, host innate immune cells initiate a broad spectrum of defense mechanisms that result in the development of inflammation and host resistance to infection (Akira et al., 2006). PRRs comprise an array of sensors and are found in the extracellular space, membrane-associated variant cell types or in the cytosol. Three major groups of PRRs have been identified: toll-like receptors (TLRs), nucleotide oligomerization domain (NOD) containing protein-like receptors (NLRs) and retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) (Akira et al., 2006; Meylan et al., 2006; Franchi et al., 2010; Chen et al., 2009; Hansen et al., 2011).

RLRs are key cytosolic pattern recognition receptors for detecting nucleotide PAMPs of invading viruses and they are crucial for the RNA virus-triggered interferon response (Takeuchi and Akira, 2008; Zou et al., 2009). Similar to Dicer of the RNAi pathway, RLRs belong to the phylogenetically conserved DExD/H-box family of helicases (Lu et al., 2009). Three genes encode RIG-I-like receptors: retinoic acid-inducible gene I (RIG-I), melanoma differentiation-associated antigen 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2), which share a common functional RNA helicase domain near the *C*-terminus (HELICc) specifically binding to the RNA molecules with viral origin and a *C*-terminal regulatory domain (RD) (Yoneyama et al., 2004, 2005; Holm et al., 2007; Venkataraman et al.,

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2007; Yoneyama and Fujita, 2007, 2008; Zou et al., 2009). The structure of RIG-I and MDA5 are very similar, as the *N*-terminal region of RIG I and MDA5 are characterized by the presence of two tandem arranged caspase activation and recruitment domains (CARDs) involved in protein–protein interactions. This activation triggers the interferon response via activation of interferon regulatory factor 3 and NFkB (Yoneyama et al., 2004; Holm et al., 2007). On the contrary, LGP2 does not possess a CARD domain at the *N*-terminal.

Each RLR plays a different role in recognizing viral PAMPs in the cytoplasm (Takahasi et al., 2009). RIG-I is a key mediator of antiviral immunity by inducing interferon (IFN) production after recognizing both RNA and DNA viruses (Ablasser et al., 2009; Choi et al., 2009; Gack et al., 2010; Pothlichet et al., 2009; Rehwinkel et al., 2010). However, among the PRRs, RIG-I is known to have a key role in recognizing RNA viruses (Baum et al., 2010). Recently it has been reported that RIG-I has broad regulatory functions that include its role in antibacterial responses, apart from its previously described antiviral roles (Kong et al., 2009). RIG-I is capable of discriminating host RNA from viral RNA based on the chemical nature of the 5′ end of RNA (Hornung et al., 2006). Further, RIG-I can recognize short double-stranded RNA in contrast to MDA5 which recognizes long dsRNA (Takahasi et al., 2009). The ability to discriminate and bind viral originated nucleic acids is provided by the specific amino acid differences in the RD domains of the RLRs (Kato et al., 2008; Yoneyama and Fujita, 2008; Takeuchi and Akira, 2008; Pichlmair et al., 2009).

MDA5 was initially discovered by Kang et al., 2002 as an interferon-inducible putative RNA helicase with double-stranded RNA-dependent ATPase activity and melanoma growth-suppressive properties in human melanoma cells. Later, it has been reported that MDA5 plays a crucial role in intracellular signal transduction pathway that could lead to the activation of the IFN-β promoter and could mediate type I IFN responses against nucleic acid PAMPs (Andrejeva et al., 2004; Gitlin et al., 2006; Kato et al., 2006). Although MDA5 is known to recognize long dsRNA (Takahasi et al., 2009), it has been reported later that it is not simply long molecules of dsRNA that are required for the activation of MDA5 but that higher-order RNA structures generated during virus infection are also required (Pichlmair et al., 2009).

The third RLR, LGP2, is a regulatory protein (Komuro et al., 2008) and structurally similar to the other two RLRs except for the lack of a CARD domain (Yoneyama et al., 2004, 2005; Rothenfusser et al., 2005; Holm et al., 2007). It has been shown to interfere with the binding process of RIG-I/MDA5 to viral RNAs (Saito et al., 2007) and negatively regulate RLR signaling (Komuro et al., 2008). However, recently, it has been suggested that LGP2 is a positive regulator of RIG-I- and MDA5-mediated antiviral responses and can potentiate IFN production during viral infection (Satoh et al., 2010).

Most of the studies on RLRs have been focused on mammals and only a little is known about such molecules in other organisms. However, a survey of purple sea urchin genome has revealed multiple putative RIG I-like homologues (Hibino et al., 2006). Recently, RIG-like homologues have been identified *in silico* in the nucleotide databases of many invertebrates and vertebrates including teleost fish (Zou et al., 2009). In addition, lately, RLRs have been reported in many teleosts species such as zebrafish, Atlantic salmon, grass carp and Japanese flounder and fathead minnow-derived EPC cell line (Biacchesi et al., 2009; Lauksund et al., 2009; Huang et al., 2010; Ohtani et al., 2010, 2011; Su et al., 2010; Yang et al., 2011).

Channel catfish, *Ictalurus punctatus*, is the major aquaculture species in the United States, and its immune system has been well characterized (Vallejo et al., 1992; Wilson et al.,1990, 1997; Clem et al., 1990, 1996; Miller et al., 1994, 1998; Khayat et al., 2001; Bengtén et al., 2002; Barker et al., 2000, 2002; Zhou et al., 2001, 2003; Shen et al., 2002, 2003; Shen, 2004; Quiniou et al., 2005; Ghaffari and Lobb, 1993; Magor et al., 1994; Hogan et al., 1999; Godwin et al., 2000; Antao et al., 2001; Hawke et al., 2001; Ventura-Holman and Lobb, 2002; Shen, 2004). A number of immune genes/innate PRRs such as chemokines (Peatman et al., 2006; Peatman and Liu, 2007; Bao et al., 2006) anti-microbial peptides (Bao et al., 2005, 2006; Xu et al., 2007), several TLRs (Bilodeau and Waldbieser, 2005; Baoprasertkul et al., 2006, 2007a,b), a number of lectin family of proteins (Takano et al., 2008; Zhang et al., 2011) and a few NLRs have been characterized in catfish (Sha et al., 2009). In a sister contribution, we reported a complete set of NLR receptors from channel catfish (Rajendran et al., 2012), and here we focus on RLRs. The objectives of the present study were to identify RLR genes in catfish through *in silico* analysis of the transcripts generated through RNA-seq, validate of the transcripts through in silico analysis with the catfish genome database (Lu et al., 2011, and unpublished genome sequences), elucidate the phylogenetic relationships of the genes, determine tissue expression patterns, and analyze expression profiles of the RLR genes after infection with channel catfish virus (CCV) or bacterial infection with *Edwardsiella ictaluri*.

2. Materials and Methods

2.1. Database mining and sequence analysis

To identify the RLR genes, RNA-seq and the whole genome database of catfish, *I. punctatus*, were searched using available zebrafish (*Daniorerio*) and human RLRs as queries. The RNA-seq database was generated from the transcriptome assembly of expressed short reads of a doubled haploid channel catfish (Liu et al., 2011, 2012). The quality of three cDNA sequences obtained from RNA-seq database was confirmed by comparison with the preliminary catfish whole genome assembly (unpublished data) which was also originated from sequencing a doubled haploid channel catfish. The retrieved reconstructed transcripts were translated using ORF finder (http://www.ncbi.nlm.nih.gov) and GENSCAN (Burge and Karlin, 1997). The predicted ORFs were verified by BLASTP against NCBI non-redundant protein sequence database. The RLR genes from other organisms were retrieved from the NCBI database for analysis. The cDNA sequences in this work were obtained and submitted to GenBank as we have done for NLRs of catfish (Rajendran et al., 2012). The Simple Modular Architecture Research Tool (SMART) was used to predict the conserved domains based on sequence homology and further confirmed by conserved domain prediction from BLAST. The full-length amino acid sequences as well as the partial sequence coding for the conserved domains were used in the phylogenetic analysis. Multiple protein sequence alignment was done using the ClustalW program. Neighbor-joining and maximum parsimony analyses were conducted using MEGA version 4 (Tamura et al., 2007).

2.2. Expression analysis of catfish PRRs

Reverse-transcriptase PCR (RT-PCR) and quantitative real-time PCR (qRT-PCR) were employed to study the mRNA expression of selected RLR genes. To study the normal expression of these genes in healthy fish, blood, skin, muscle, gill, heart, liver, spleen, intestine, head kidney, trunk kidney and brain were collected from five individual fish and pooled. Three such pools were used in the present study. The tissues were snap-frozen in liquid nitrogen and immediately subjected to RNA extraction using RNeasy Minikit (Qiagen, USA) following manufacturer's protocol. The extracted total RNA was quantified using UV-spectrophotometer and an aliquot (1 μg) of RNA was treated with 1 unit of RNase-free DNAse (Qiagen) prior to reverse transcription. Uniform quantity of

DNA-free RNA was reverse transcribed using iScript cDNA Synthesis Kit (Bio-Rad, USA) following manufacturer's protocol.

PCR was carried out using Platinum Taq DNA polymerase, 10X buffer and 50 mM $MgCl_2$ (Invitrogen). The 20 μl PCR reaction mixture contained 2.0 μl 10X buffer 1.0 μl of $MgCl_2$ (25 mM), 1.0 μl of dNTP (10 mM), 0.4 μl of Taq polymerase (1 U), 1 μl (10 pmol/μl) of each pair of primers, 2 μl cDNA and 12.6 μl PCR-grade water. Gene-specific primers and internal reference 18SrRNA-specific primers were used separately in the PCR amplification. Thermal cycling was performed on a Bio-Rad PCR system for 35 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s and extension at 72 °C for 1 min.The PCR products were resolved on a 2% agarose gel.

2.3. Virus infection in cell culture

Channel catfish ovarian cells (ATCC-CRL-2772) were grown in Eagle Minimum Essential Medium with 10% inactivated fetal bovine serum. Cells were maintained at 28 °C in 25 cm^2cell culture flasks until appropriate confluency was attained. For virus infection, the cells were infected with channel catfish virus (CCV) (ATCC VR-665), or *ictalurid herpesvirus* 1, a cytopathic herpesvirus that causes a severe hemorrhagic disease in young channel catfish (Wolf and Darlington, 1971) at a multiplicity of infection (MoI) of 0.25. For studying the time-dependent expression profile of all the RLR genes, the cells were harvested at 0, 6, 12, 18 and 24 h post-infection. For each time-point, cells grown in three separate flasks were used as replicates. RNA was extracted from the harvested cells using Trizol (Qiagen) following the manufacturer's protocol and reverse transcribed as described elsewhere.

2.4. Bacterial challenge

A bacterial challenge study on healthy channel catfish (Marion strain) was carried out as described in a previous study (Peatman et al., 2007). Liver tissue collected at different time-points (4 h, 24 h, 4 day and 6 day post-infection (p.i)) from both experimental and control groups was used in the RNA extraction and subsequent real-time PCR to study the expression pattern of RLR gene.

2.5. Quantitative real-time PCR

Three replicate RNA samples (biological replicates) derived from both normal and infected liver tissue of fish and the virus-challenged CCOcells collected at different time points were tested to investigate the gene expression profile using qRT-PCR with 18S rRNA as the internal reference (Small et al., 2008). For each replicate there were three additional well replicates (statistical replicates). Each qRT-PCR reaction consisted of a total volume of 10 μl containing 5.0 μl SsoFast EvaGreen Supermix (Bio-Rad, USA), 0.5 μl of each primer (5 pmol/μl), 2 μl cDNA (150 ng/μl)and 2 μl PCR-grade water. The thermal cycling was carried out on a C1000 Thermal Cycler (BIO-RAD, USA) using the cycling conditions: denaturation, 95 °C/30 s, 40 cycles of 95 °C/5 s, 57 °C/5 s, and 72 °C/5 s followed by dissociation curve analysis to verify the specificity of amplification. The qRT-PCR data were exported into Microsoft Excel Sheet for analysis. The relative expression ratio of target gene in experimental group and the control group was calculated using the $2^{-\Delta\Delta}$CT method. The data generated were further analyzed statistically (one-way ANOVA) for determining significance.

3. Results

Three RLR genes, RIG-I, MDA5 and LGP2, were identified in channel catfish. The transcript sequences were initially obtained from the assembled full-length cDNA (Liu et al., 2012). To verify the open reading frames, the identified sequences were aligned against partial draft genome sequences (not published data). All these full length cDNAs have been deposited to the NCBI Transcriptome Shotgun Assembly (TSA) database with continuous accession numbers of JQ008940, JQ008941, JQ008942 (Table 1).

RIG-I encodes 937 amino acids and contains two CARDs (caspase recruitment domain), a DEXDc (DExD/H box-containing domain), a HELICc (helicase superfamily *C*-terminal domain), and a RD (regulatory domain) (Table 1).The deduced catfish RIG-I protein sequence was longer than most of the mammalian and bird sequences but shorter than the amphibian RIG-I protein sequence. Among the known RIG-I genes, the catfish RIG-I shared maximum identity with cyprinid fish RIG-I (63%).

The catfish MDA5 gene encodes 1005 amino acids long and possesses two CARD domains at the *N*-terminus in addition to the conserved DExDc and HELICc domains and the *C*-terminal regulatory domain (RD) (Table 1).Catfish MDA5 is longer than all other teleost MDA5, except that of zebrafish and pufferfish (1034 and 1038 amino acids long, respectively). As in the case of RIG-I, catfish MDA5 also showed similarity with other vertebrate MDA5. In comparison, similar to RIG-I, catfish MDA5 showed maximal identity with cyprinid fishes (68%). Human and mouse MDA5 were found to be 49% identical with the catfish gene and the lowest identity was with *Branchiostoma floridae*.

Searches of catfish RNA-seq results and the genome database identified a 677 amino acids long, full-length sequence of LGP2. Unlike RIG-I and MDA5, it lacked a CARD domain at the *N*-terminus. However, the gene possessed a DExDc and a HELICc domain and *C*-terminal regulatory domain (RD) homologous to their corresponding motifs in the other two RLR proteins (Table 1). The identified catfish gene also showed comparable length with the non-mammalian LGP2 which is reported to be in the range of 671–730 amino acids long. The deduced LGP2 amino acid sequence of catfish was found to be similar to the LGP2 sequences from all the teleosts and showed 60–66% identity except for the *Gadus mohrua* LGP2 for which only partial sequence is available in the GenBank. Human LGP2 showed 49% identity with the catfish gene.

To compare the sequence homologs and construct the phylogenetic tree we collected all the vertebrate RLR sequences available in the GenBank. The analysis showed all the teleost RIG-Is, MDA5s and LGP2s converged into their corresponding clade separately (Fig. 1A–C). The RIG-I and LGP2 homologous proteins were found to be separated into three distinct groups, consisting of mammals, birds and teleostei (Fig. 1A and C) whilst MDA5 had an additional cephalochordate branch (Fig. 1B). Analyses using several algorithms, such as neighbor joining and maximum parsimony methods, produced almost identical phylogenetic trees.

Tissue distribution of the three RLR genes was studied in healthy catfish using RT-PCR with 18S rRNA as the internal reference gene. For this, eleven tissues (blood, skin, muscle, gill, heart, liver, spleen, intestine, head kidney, trunk kidney, and brain) of pooled samples with five fishes in each pool were examined. As shown in Fig. 2, all three RLR genes, RIG-I, MDA5 and LGP2 were ubiquitously expressed in all the tissues tested.

To determine the antiviral response of all the RLR genes, channel catfish ovarian cells were infected with channel catfish virus, and cells collected at 0, 6, 12, 18 and 24 h post-infection were subjected to quantitative real-time PCR with 18SrRNA as the internal reference. RIG-I did not show any change in its expression at 6 h post-infection. However, the gene showed up-regulated expression from 18 to 24 h with the highest level being detected at 24 h (2.7-fold, $P < 0.05$). MDA5 showed a significant up-regulation trend even at 6 h post-infection with the highest level (3.4-fold, $P < 0.05$) attained at 24 h p.i. Unlike RIG-I and MDA5, LGP2 showed no significant change in its expression until 18 h post-infection. However, at

Table 1
A list of RLR-like receptor genes characterized from channel catfish (*Ictalurus punctatus*).

Gene	Accession No.	Length (nucleotide)	Length (amino acids)	Domains identified and amino acid positions
RIG-I	JQ008940	3048	937	CARD-DEXDc-HelicC-RD (1–187)-(249–457)-(644–743)-(813–935)
MDA5	JQ008941	4417	1005	CARD-DEXDc-HelicC-RD (7–193)-(306–521)-(713–801)-(877–997)
LGP2	JQ008942	3083	677	DEXDc-HelicC-RD (1–201)-(318–388)-(550–670)

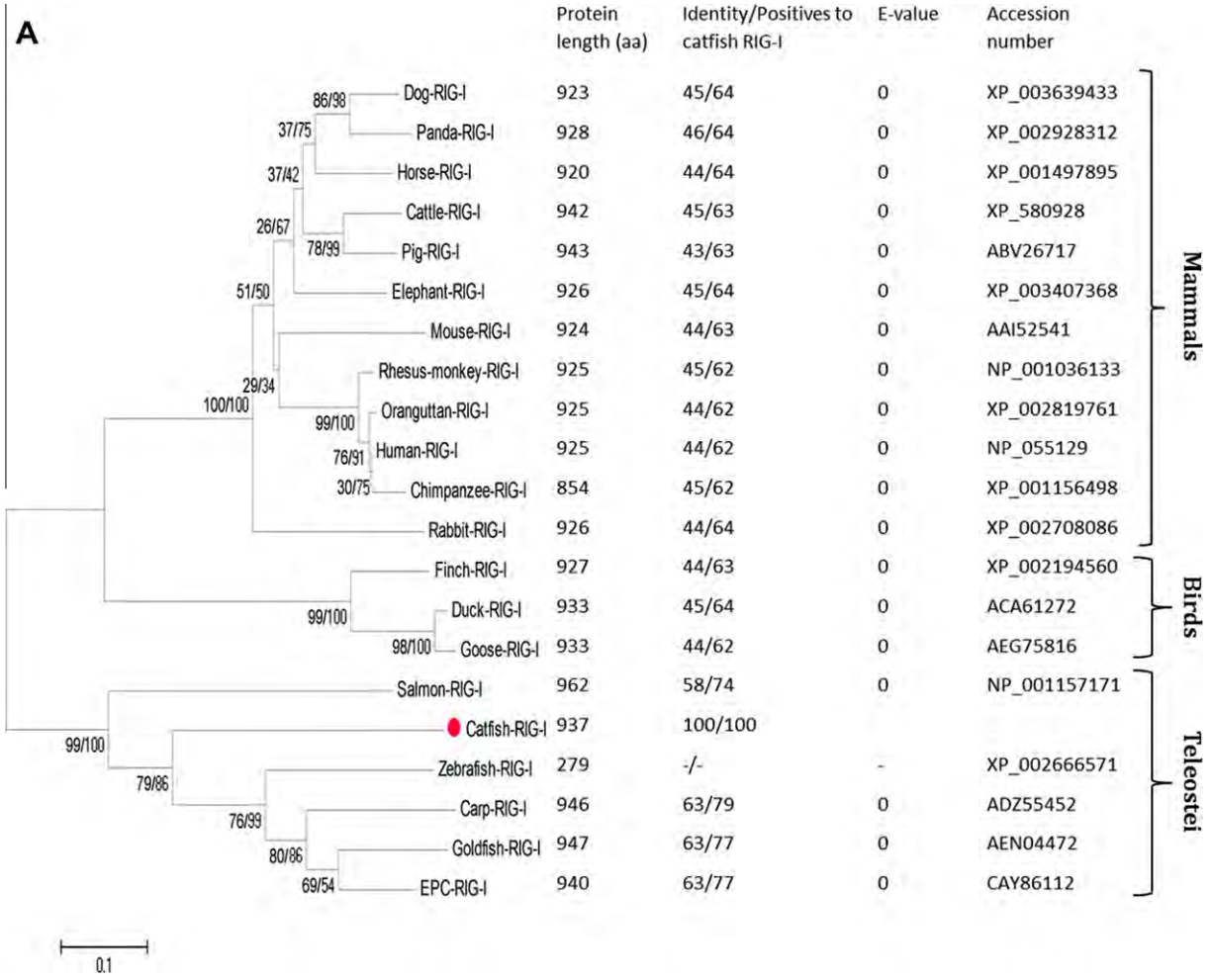


Fig. 1. Phylogenetic analysis of RLR receptors. (A): Phylogenetic relationship of catfish and other vertebrate RIG-I. ClustalW alignments of all the amino acid sequences were used to generate a phylogenetic tree using Neighbor joining and Maximum parsimony methods. The tree is shown with 10,000 replicates from the bootstrap test and the percentage of bootstrap values were given next to the branches (Maximum parsimony bootstrap value/Neighbor joining bootstrap value). (B): Phylogenetic relationship of catfish and other vertebrate MDA5. ClustalW alignments of all the amino acid sequences were used to generate a phylogenetic tree using Neighbor joining and Maximum parsimony methods. The tree is shown with 10,000 replicates from the bootstrap test and the percentage of bootstrap values were given next to the branches (Maximum parsimony bootstrap value/Neighbor joining bootstrap value). (C): Phylogenetic relationship of catfish and other vertebrate LGP2. ClustalW alignments of all the amino acid sequences were used to generate a phylogenetic tree using Neighbor joining and Maximum parsimony methods. The tree is shown with 10,000 replicates from the bootstrap test and the percentage of bootstrap values were given next to the branches (Maximum parsimony bootstrap value/Neighbor joining bootstrap value).

24 h p.i. the gene showed a significant increase in its expression with a 3.1fold change ($P < 0.05$). The expression pattern of all the three genes is depicted in Fig. 3.

To examine whether RLR genes are involved in intracellular bacterial infection, liver tissues collected 0 h, 4 h, 24 h, 4 days and 6 days after infection with *E. ictaluri* were subjected to real-time PCR. Strikingly, all the RLR genes, RIG-I, MDA5 and LGP2 showed significant up-regulation as early as 4 h p.i. ($P < 0.05$) (Fig. 4). While RIG-I and LGP2 showed more than 3-fold increase in expression at 4 h, MDA5 showed more than 10-fold upregulation at the same time-point. MDA5 was observed to maintain a similar level of expression until 6 day p.i. RIG-I and LGP2 showed a similar pattern of expression with the highest level observed at 6 day p.i. with 7- and 6-fold increases, respectively.

4. Discussion

Several studies have revealed RIG-I, MDA5 and LGP2 gene orthologs in avian, amphibian and teleost species (Biacchesi et al., 2009; Zou et al., 2009; Barber et al., 2010; Huang et al., 2010; Ohtani et al., 2010, 2011; Su et al., 2010; Yang et al., 2011). Among the three RLRs, RIG-I is apparently not present in all teleost genomes potentially due to gene loss in particular fish genomes or divergence into a non-recognizable form (Zou et al., 2009). Substantiating this, it appears that MDA5 and LGP2 are common to all teleost genomes whilst RIG-I has been identified only in cyprinids and salmonids (Biacchesi et al., 2009; Zou et al., 2009; Barber et al., 2010; Huang et al., 2010; Ohtani et al., 2010, 2011; Su et al., 2010; Yang et al., 2011). Interestingly, both the RNA-seq and genome database of

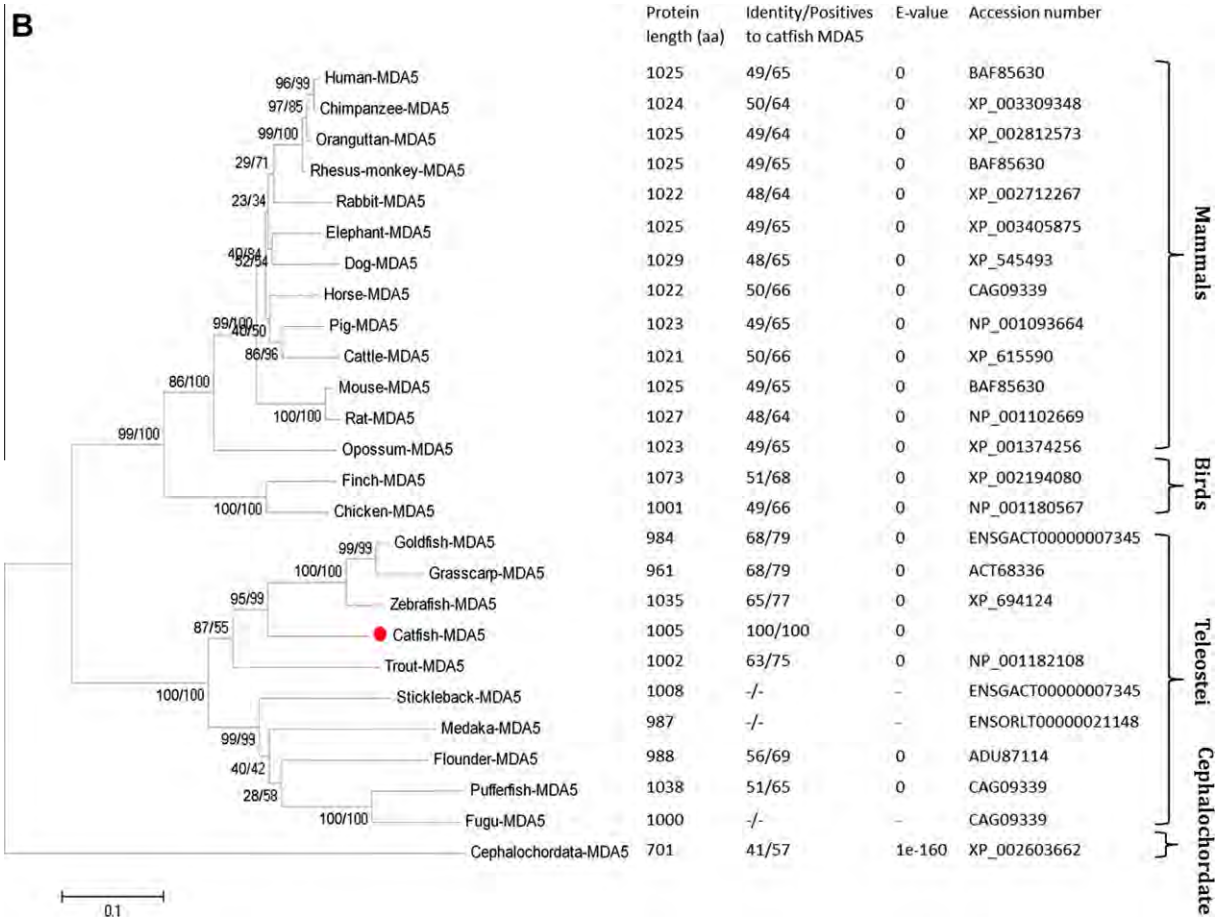


Fig. 1 (*continued*)

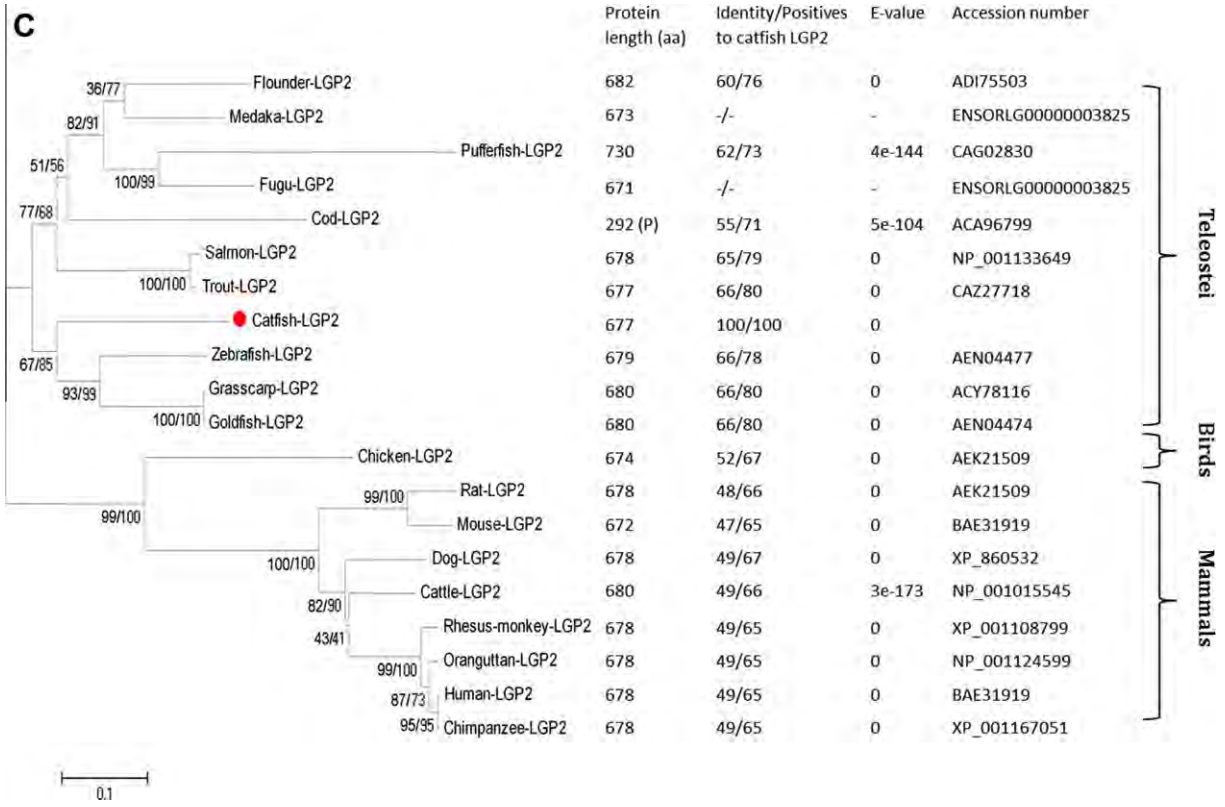
catfish revealed the presence of all the three RLRs. As observed in carps and salmon, the catfish RIG-I is characterized by the presence of two CARD domains at the *N*-terminus, a DExD/H domain, a HELICc domain, and a regulatory domain (RD) at the *C*-terminus. However, the RIG-I gene in zebrafish has only one CARD domain and no such domain has been determined in lamprey.

Zou et al. (2009) have reported that MDA5 is encoded by a single copy gene and identified throughout vertebrate species including fish, amphibians, birds and mammals. The putative protein has a length of 961amino acids (grass carp) to 1285 amino acids (chicken). The catfish gene is found to be relatively longer but falls within the range (1005 amino acids). MDA5 and RIG-I are structurally similar proteins, and the catfish gene showed 25% identity in the full length amino acid sequence. Structural identities of human MDA5 and RIG-I were reported to be about 23% and 35% in their *N*-terminal tandem CARD and *C*-terminal HELICc domains, respectively (Yoneyama and Fujita, 2007). According to Zou et al., 2009, two tandem CARD domains at the *N*-terminal regions are predicted for MDA5 and are well conserved among vertebrate except for the zebrafish MDA5 that lacks a clear CARD domain. Further, according to them, the first CARD motifs at the *N*-terminus are reported be more diverse than the second CARD motif. Similar to the MDA5 genes of other teleosts (Su et al., 2010; Ohtani et al., 2011), two CARD domains could be identified in catfish MDA5.

LGP2 is reported to be an adaptor protein which lacks a CARD domain (Zou et al., 2009). As expected and reported for non-mammalians, the putative catfish LGP2 was shorter than RIG-I and MDA5 proteins with 677 amino acids long and it lacked a CARD domain at the *N*-terminus. However, the length of catfish LGP2 is in the range of available teleost full-length sequences (671–730 amino acids).

Phylogenetic analysis of the three RLR genes showed that all the identified catfish RLRs (RIG-I, MDA5 and LGP2) form distinct groups with their respective genes from other teleosts. Further, as reported by Zou et al., 2009, phylogenetic analysis also showed that vertebrate MDA5 and LGP2 genes form two distinct clusters closely neighboring each other, suggesting the possibility of their divergence from a common ancestor that originated from RIG-I-like protein in invertebrates or early vertebrates (Zou et al., 2009).

Constitutive expression was observed for all the three RLR genes identified in catfish. The expression of RIG-I mRNA in various tissues of catfish showed similar patterns as those reported in carp and grass carp RIG-I (Feng et al., 2011; Yang et al., 2011). Although the expression was found to be relatively low in head kidney in carp and catfish, unlike carp, the relatively high expression was noticed in trunk kidney of catfish. However, Yang et al. (2011) reported RIG-I expression in fifteen tissues tested and observed similar expression pattern in spleen, head kidney and intestine. The tissue expression of MDA5 in catfish showed similarity with that of flounder (*Paralichthys olivaceus*) in which high expression was recorded in head kidney and trunk kidney, spleen and heart (Ohtani et al., 2011). However, the gene was reported to be highly expressed in spleen, skin and gill in the case of grass carp (Su et al., 2010), whilst the catfish showed a comparatively low level of expression in its skin, muscle and brain. Similarly, LGP2 expression in catfish tissues is similar to that in other fish species, as it was ubiquitously expressed in all the tissues tested. In Japanese flounder LGP2 was reported to be highly expressed in head kidney



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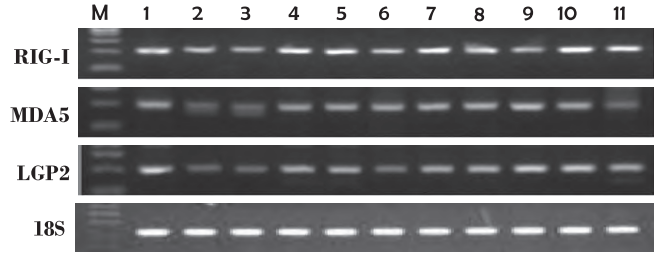

Fig. 2. RT-PCR-based expression analysis of different RLR genes in different tissues of healthy channel catfish. Tissues from five animals were pooled for RNA extraction. M, marker; 1, blood; 2, skin; 3, muscle; 4, gill; 5, heart; 6, liver; 7, spleen; 8, intestine; 9, headkidney; 10, trunkkidney; 11, brain. 18S rRNA was used as an internal control and gene names are indicated on the left of the panel.

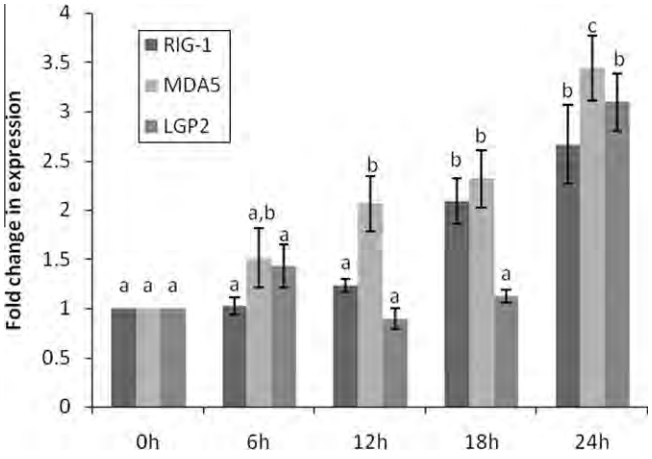

Fig. 3. qRT-PCR-based expression analysis of different RLR genes in channel catfish ovarian cells (CCO). The cells infected with channel catfish virus (CCV) and collected at different time-points (0, 6, 12, 18 and 24 h) after infection were used in RNA extraction andqRT-PCR. 18SrRNA was used as internal reference. The results were expressed as mean ± standard error (bars) from three replicates. Significant difference ($p < 0.05$) between the treated and control group was indicated with alphabets (a–c).

and trunk kidney, spleen, and heart (Ohtani et al., 2011), while in grass carp, beside other tissues, LGP2 was found in abundance in the skin as well (Huang et al., 2010). In catfish, on the contrary, skin tissue showed a relatively low level of LGP2 mRNA. The constitutive expression of the RLRs observed in various tissues of catfish and other teleosts indicate the role of these proteins in the innate immune system and it also suggests that many organs have the potential to be involved in the RLR-mediated response to viral infection.

There is increasing evidence of induction/up-regulation of RLR genes during viral infection in fish. Biacchesi et al. (2009) has reported up-regulation of expression of RIG-I in viral hemorrhagic septicemia virus (VHSV) infection. Similarly, induction of RIG-I has been observed in different tissues of SVCV (a negative single-stranded RNA virus)-infected grass carp (Yang et al., 2011). Expression of another RLR gene, MDA5, was also found to be up-regulated in grass carp infected with Grass Carp Reovirus (GCRV) (Su et al., 2010). Further, significant increases in the MDA5 transcript abundance has been reported in Japanese flounder kidney tissue infected with viral hemorrhagic septicemia (VHSV) as well as kidney and peripheral blood leukocytes stimulated with poly I:C *in vitro* (Ohtani et al., 2011). Similar to the other two RLR genes, fish LGP2 has also been reported to be induced by virus infection both *in vitro* and *in vivo* (Ohtani et al., 2010; Huang et al., 2010). In accordance with the previous reports, channel catfish ovarian cells when infected with channel catfish virus showed significant increases in the expression of RIG-I, MDA5 and LGP2. All the genes showed the maximum up-regulation at 24 h post-infection. The

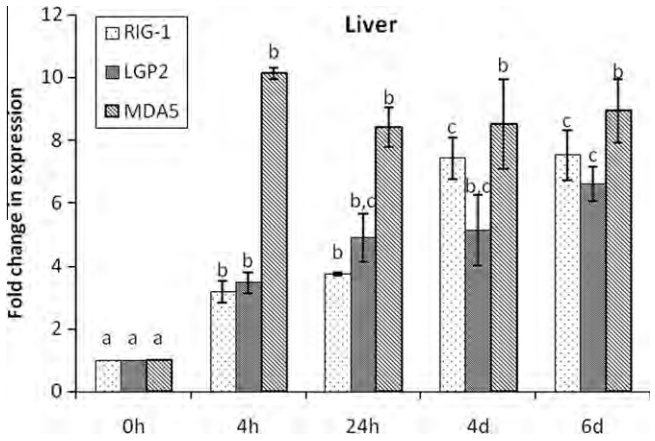

Fig. 4. qRT-PCR-based expression analysis of RLR genes in the liver tissue of channel catfish following bacterial infection. Healthy catfish were exposed to *E. ictaluri* through immersion. Tissues were collected at four different time points after bacterial challenge and RNA extracted from fifteen fishes (3 pools of 5 each) were used in the qRT-PCR. RLR genes were tested with 18SrRNA as internal reference. The results were expressed as mean ± standard error (bars) from three replicates. Significant difference ($p < 0.05$) between the treated and untreated group was indicated with alphabets (a–c).

present study did not examine the *in vivo* effect of the virus on the expression profile of the catfish RLRs, but further studies are warranted in the future.

In our previous study on NLR genes in catfish (Rajendran et al., 2012), the most striking modulation of expression was observed in the liver tissue after bacterial infection. Based on this, we have evaluated the expression patterns of RLR genes in catfish liver infected with the Gram negative bacterium *Edwardsiella ictaluri*. A consistent and significant increase in the levels of RIG-I, MDA5 and LGP2 expression were noticed till 4 days post-infection and the expression level remained high until 6 days post-infection. The up-regulation of RIG-I in response to bacterial infection observed in the present study corroborates the finding that regulatory function of RIG-I is broad in that it has a role not only in antiviral responses but in antibacterial responses as well (Kong et al., 2009). Further, Monroe et al. (2009) have provided genetic evidence that the RNA-sensing proteins, RIG-I and MDA5 participate in the IFN response to *Legionella pneumophila*, a Gram-negative bacterial pathogen. Similarly, a regulatory role of LGP2 in response to intracellular bacterial pathogens has been demonstrated in mice by Pollpeter et al. (2011). According to their study, using LGP2-deficient mice infected with *Listeria monocytogenes*, they have observed reduced levels of type I IFN and IL12 resulting in increased bacterial growth in infected animals with greater colonization of both spleen and liver. Nevertheless, as far as is known, there are no reports on the antibacterial response of RLR genes in teleost fish.

It has been hypothesized that RLRs are an ancient group of PRRs, with ancestors among invertebrates such as sea urchins and amphioxus (Huang et al., 2008; Zou et al., 2009).The origin of RLR system of viral detection developed earlier in evolution, possibly prior to the appearance of vertebrates (Zou et al., 2009; Hansen et al., 2011). This is the first report of RLR genes, a group of key cytosolic receptors that have proven antiviral responsiveness, in catfish. To date, as RIG-I has been identified only in cyprinids and salmonids (Biacchesi et al., 2009; Zou et al., 2009; Huang et al., 2010), the discovery of a full-length putative RIG-I in catfish is suggestive of its presence in other teleost species as well.

In conclusion, channel catfish, *I. punctatus*, which represents a diverse and ancient lineage of fishes, possesses a full repertoire of ancient cytosolic viral recognition receptors. Furthermore, all the identified genes are functional and appear to have innate immune functions against viral and bacterial pathogens. Nevertheless, a detailed functional characterization of the RLRs in catfish is needed to elucidate the exact PAMPs of individual RLR genes and their interactions in catfish.

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References

Ablasser, A., Bauernfeind, F., Hartmann, G., Latz, E., Fitzgerald, K.A., Hornung, V., 2009. RIGI-dependent sensing of poly (dA:dT) through the induction of an RNA polymerase III-transcribed RNA intermediate. Nat. Immunol. 10, 1065–1072.

Akira, S., Uematsu, S., Takeuchi, O., 2006. Pathogen recognition and innate immunity. Cell 124, 783–801.

Andrejeva, J., Childs, K.S., Young, D.F., Carlos, T.S., Stock, N., Goodbourn, S., et al., 2004. The V proteins of paramyxoviruses bind the IFN-inducible RNA helicase, MDA-5, and inhibit its activation of the IFN-{beta} promoter. PNAS 101, 17264–17269.

Antao, A.B., Wilson, M., Wang, J., Bengtén, E., Miller, N.W., Clem, L.W., Chinchar, V.G., 2001. Genomic organization and differential expression of channel catfish MHC class I genes. Dev. Comp. Immunol. 25, 579–595.

Bao, B., Peatman, E., Li, P., He, C., Liu, Z., 2005. Catfish hepsidin gene is expressed in a wide range of tissues and exhibit tissue-specific upregulation after bacterial infection. Dev. Comp. Immunol. 29 (11), 939–950.

Bao, B., Peatman, E., Peng, X., Baoprasertkul, P., Wang, G., Liu, Z., 2006. Characterization of 23 CC chemokine genes and analysis of their expression in channel catfish (*Ictalurus punctatus*). Dev. Comp. Immunol. 30 (9), 783–796.

Baoprasertkul, P., Peatman, E., Somridhivej, B., Liu, Z., 2006. Toll-like receptor 3 and TICAM genes in catfish: species-specific expression profiles following infection with *Edwardsiella ictaluri*. Immunogenetics 58 (10), 817–830.

Baoprasertkul, P., Peatman, E., Abernathy, J., Liu, Z., 2007a. Structural characterization and expression analysis of Toll-like receptor 2 gene from catfish. Fish Shellfish Immunol. 22, 418–426.

Baoprasertkul, P., Xu, P., Peatman, E., Kucuktas, H., Liu, Z., 2007b. Divergent Toll-like receptors in catfish (*Ictalurus punctatus*): TLR5S, TLR20, TLR21. Fish Shellfish Immunol. 23, 1218–1230.

Barber, M.R., Aldridge Jr., J.R., Webster, R.G., Magor, K.E., 2010. Association of RIGI with innate immunity of ducks to influenza. Proc. Natl. Acad. Sci. USA 107, 5913–5918.

Barker, K.S., Khayat, M., Miller, N.W., Wilson, M., Clem, L.W., et al., 2002. Immortal and mortal clonal lymphocyte lines from channel catfish: comparison of telomere length, telomerase activity, tumor suppressor and heat shock protein expression. Dev. Comp. Immunol. 26, 45–51.

Barker, K.S., Quiniou, S.M., Wilson, M.R., Bengtén, E., Stuge, T.B., et al., 2000. Telomerase expression and telomere length in immortal leukocyte lines from channel catfish. Dev. Comp. Immunol. 24, 583–595.

Baum, A., Sachidanandam, R., Garcia-Sastre, A., 2010. Preference of RIG-I for short viral RNA molecules in infected cells revealed by next-generation sequencing. Proc. Natl. Acad. Sci. USA 107, 16303–16308.

Bengtén, E., Quiniou, S.M., Stuge, T.B., Katagiri, T., Miller, N.W., et al., 2002. The IgH locus of the channel catfish, *Ictalurus punctatus*, contains multiple constant region gene sequences: different genes encode heavy chains of membrane and secreted IgD. J. Immunol. (Baltimore, 1950) 169, pp. 2488–2497.

Biacchesi, S., LeBerre, M., Lamoureux, A., Louise, Y., Lauret, E., Boudinot, P., et al., 2009. Mitochondrial antiviral signaling protein plays a major role in induction of the fish innate immune response against RNA and DNA viruses. J. Virol. 83, 7815–7827.

Bilodeau, A.L., Waldbieser, G.C., 2005. Activation of TLR3 and TLR5 in channel catfish exposed to virulent *Edwardsiella ictaluri*. Dev. Comp. Immunol. 29 (8), 713–721.

Burge, C., Karlin, S., 1997. Prediction of complete gene structures in human genomic DNA. J. Mol. Biol. 268, 78–94.

Clem, L.W., Bly, J.E., Ellsaesser, C.F., Lobb, C.J., Miller, N.W., 1990. Channel catfish as an unconventional model for immunological studies. J. Exp. Zoolog. Suppl. 4, 23–25.

Clem, L.W., Bly, J.E., Wilson, M.R., Chinchar, V.G., Stuge, T.B., et al., 1996. Fish immunology: the utility of immortalized lymphoid cells–a mini review. Vet. Immunol. Immunopathol. 54, 137–144.

Chen, G., Shaw, M.H., Kim, Y.G., Nunez, G., 2009. NOD-like receptors: role in innate immunity and inflammatory disease. Ann. Rev. Pathol. Mech. 4, 365–398.

Choi, M.K., Wang, Z., Ban, T., Yanai, H., Lu, Y., Koshiba, R., et al., 2009. A selective contribution of the RIG-I-like receptor pathway to type I interferon responses activated by cytosolic DNA. Proc. Natl. Acad. Sci. USA 106, pp. 17870–5.

Feng, H., Liu, H., Kong, R., Wang, L., Wang, Y., Hu, W., Guo, Q., 2011. Expression profiles of carp IRF-3/-7 correlate with the up-regulation of RIG-I/MAVS/TRAF3/TBK1, four pivotal molecules in RIG-I signaling pathway. Fish Shellfish Immunol. 30, 1159–1169.

Franchi, L., Munoz-Planillo, R., Reimer, T., Eigenbrod, T., Nunez, G., 2010. Inflammasomes as microbial sensors. Eur. J. Immunol. 40, pp. 595–653.

Gack, M.U., Nistal-Villan, E., Inn, K.-S., Garcia-Sastre, A., Jung, J.U., 2010. Phosphorylation-mediated negative regulation of RIG-I antiviral activity. J. Virol. 84, 3220–3229.

Ghaffari, S.H., Lobb, C.J., 1993. Structure and genomic organization of immunoglobulin light chain in the channel catfish. An unusual genomic organizational pattern of segmental genes. J. Immunol. (Baltimore, 1950) 151, pp. 6900–6912.

Gitlin, L., Barchet, W., Gilfillan, S., Cella, M., Beutler, B., Flavell, R.A., et al., 2006. Essential roleof mda-5 in type I IFN responses to polyriboinosinic:polyribocytidylic acid and encephalomyocarditis picornavirus. PNAS 103, 8459–8464.

Godwin, U.B., Flores, M., Quiniou, S.M., Wilson, M.R., Miller, N.W., et al., 2000. MHC class II A genes in the channel catfish (*Ictalurus punctatus*). Dev. Comp. Immunol. 24, 609–622.

Hansen, J.D., Vojtech, L.N., Laing, K.J., 2011. Sensing disease and danger: a survey of vertebrate PRRs and their origins. Dev. Comp. Immunol. 35, 886–897.

Hawke, N.A., Yoder, J.A., Haire, R.N., Mueller, M.G., Litman, R.T., et al., 2001. Extraordinary variation in a diversified family of immune-type receptor genes. Proc. Natl. Acad. Sci. USA 98, 13832–13837.

Hibino, T., Loza-Coll, M., Messier, C., Majeske, A.J., Cohen, A.H., Terwilliger, D.P., Buckley, K.M., Brockton, V., Nair, S.V., Berney, K., Fugmann, S.D., Anderson, M.K., Pancer, Z., Cameron, R.A., Smith, L.C., Rast, J.P., 2006. The immune gene repertoire encoded in the purple sea urchin genome. Dev. Biol. 300, 349–365.

Hogan, R., Waldbieser, G., Goudie, C., Antao, A.B., Godwin, U., et al., 1999. Molecular and immunologic characterization of gynogenetic channel catfish (*Ictalurus punctatus*). Mar Biotechnol. (N. Y.) 1, 317–327.

Holm, G.H., Zurney, J., Tumilasci, V., Leveille, S., Danthi, P., Hiscott, J., Sherry, B., Dermody, T.S., 2007. Retinoic acid-inducible gene-I and interferon beta promoter stimulator-1 augment proapoptotic responses following mammalian reovirus infection via interferon regulatory factor-3. J. Biol. Chem. 282, 21953–21961.

Hornung, V., Ellegast, J., Kim, S., Brzozka, K., Jung, A., Kato, H., et al., 2006. 5′-triphosphateRNA is the ligand for RIG-I. Science 314, 994–997.

Huang, S., Yuan, S., Guo, L., Yu, Y., Li, J., Wu, T., Liu, T., Yang, M., Wu, K., Liu, H., Ge, J., Huang, H., Dong, M., Yu, C., Chen, S., Xu, A., 2008. Genomic analysis of the immune gene repertoire of amphioxus reveals extraordinary innate complexity and diversity. Genome Res. 18, 1112–1126.

Huang, T., Su, J., Heng, J., Dong, J., Zhang, R., Zhu, H., 2010. Identification and expression profiling analysis of grass carp *Ctenopharyngodon idella* LGP2 cDNA. Fish Shellfish Immunol. 29, 349–355.

Kang, D.C., Gopalkrishnan, R.V., Wu, Q., Jankowsky, E., Pyle, A.M., Fisher, P.B., 2002. MDA-5:an interferon-inducible putative RNA helicase with double-stranded RNA-dependent ATPase activity and melanoma growth-suppressive properties. PNAS99, pp. 637–42.

Kato, H., Takeuchi, O., Sato, S., Yoneyama, M., Yamamoto, M., Matsui, K., et al., 2006. Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature 441, 101–105.

Kato, H., Takeuchi, O., Mikamo-Satoh, E., Hirai, R., Kawai, T., Matsushita, K., Hiiragi, A., Dermody, T.S., Fujita, T., Akira, S., 2008. Length-dependent recognition of double stranded ribonucleic acids by retinoic acid-inducible gene-I and melanoma differentiation-associated gene 5. J. Exp. Med. 205, 1601–1610.

Khayat, M., Stuge, T.B., Wilson, M.R., Bengtén, E., Miller, N.W., et al., 2001. Thioredoxin acts as a B cell growth factor in channel catfish. J. Immunol. (Baltimore, 1950) 166, pp. 2937–2943.

Komuro, A., Bamming, D., Horvath, C.M., 2008. Negative regulation of cytoplasmic RNA-mediated antiviral signaling. Cytokine 43, 350–358.

Kong, L., Sun, L., Zhang, H., Liu, Q., Liu, Y, Qin, L., et al., 2009.An essential role for RIG-I in toll-like receptor-stimulated phagocytosis. Cell Host Microbe 6, pp. 150–61.

Lauksund, S., Svingerud, T., Bergan, V., Robertsen, B., 2009. Atlantic salmon IPS-1mediates induction of IFNa1 and activation of NF-kB and localizes to mitochondria. Dev. Comp. Immunol. 33, 1196–1204.

Liu, S., Zhou, Z., Lu, J., Sun, F., Wang, S., Liu, H., Jiang, Y., Kucuktas, H., Kaltenboeck, L., Peatman, E., Liu, Z.J., 2011. Generation of genome-scale gene-associated SNPs in catfish for the construction of a high-density SNP array. BMC Genomics 12, 53.

Liu, S., Zhang, Y., Zhou, Z., Waldbieser, G., Sun, F., Lu, J., Zhang, J., Jiang, Y., Zhang, H., Wang, X., Rajendran, K.V., Kucuktas, H., Peatman, E., Liu, Z., 2012. Comprehensive annotation of the transcriptome of channel catfish (*Ictalurus punctatus*) by paired-end RNA-Seq. Abstract of Plant & Animal Genome XX, 2012 January14–18, San Diego.

Lu, J, Peatman, E, Yang, Q, Wang, S, Hu, Z, Reecy, J, Kucuktas, H, Liu, Z. 2011. The catfish genome database cBARBEL: an informatic platform for genome biology of ictalurid catfish. Nucleic Acids Res. 2011 39 (Database issue): pp. D815–21.

Lu, R., Yigit, E., Li, W.X., Ding, S.W., 2009. An RIG-I-Like RNA helicase mediates antiviral RNAi downstream of viral siRNA biogenesis in *Gaenorhabditis elegans*. PLoS Pathog. 5, p. e1000286.

Magor, B.C., Wilson, M.R., Miller, N.W., Clem, L.W., Middleton, D., et al., 1994. Heavy Chain Enhancer. J. Immunol. 156, pp. 2488–2497.

Meylan, E., Tschopp, J., Karin, M., 2006. Intracellular pattern recognition receptors in the host response. Nature 442, 39–44.

Miller, N.W., Rycyzyn, M.A., Wilson, M.R., Warr, G.W., Naftel, J.P., et al., 1994. Development and characterization of channel catfish long term B cell lines. J. Immunol. (Baltimore, 1950) 152, pp. 2180–2189.

Miller, N.W., Wilson, M.R., Bengtén, E., Stuge, T.B., Warr, G., et al.,1998. Functional and molecular characterization of teleost leukocytes. Immunol. Rev. 166, pp. 187–197.

Monroe, K.M., McWhirter, S.M., Vance, R.E., 2009. Identification of host cytosolic sensors and bacterial factors regulating the type I interferon response to *Legionella pneumophila*. PLoS Pathog. 5 (11), e1000665. doi:10.1371/journal.ppat.1000665.

Ohtani, M., Hikima, J., Kondo, H., Hirono, I., Jung, T.S., Aoki, T., 2010. Evolutional conservation of molecular structure and antiviral function of a viral RNA receptor, LGP2, in Japanese Flounder, *Paralichthys olivaceus*. J. Immunol. 185, 7507–7517.

Ohtani, M., Hikima, J., Kondo, H., Hirono, I., Jung, T.S., Aoki, T., 2011. Characterization and antiviral function of a cytosolic sensor gene, MDA5, in Japanese flounder, *Paralichthys olivaceus*. Dev. Comp. Immunol. 35, 554–562.

Peatman, E., Bao, B., Peng, X., Baoprasertkul, P., Brady, Y., Liu, Z., 2006. Catfish CC chemokines: genomic clustering, duplications, and expression after bacterial infection with *Edwardsiella ictaluri*. Mol. Genet. Genomics 275 (3), 297–309.

Peatman, E., Liu, Z., 2007. Evolution of CC chemokines in teleost fish: a case study in gene duplication and implications for immune diversity. Immunogenetics 59 (8), 613–623.

Peatman, E., Baoprasertkul, P., Terhune, J., Xu, P., Nandi, S., Kucuktas, H., et al., 2007. Expression analysis of the acute phase response in channel catfish (*Ictalurus punctatus*) after infection with a Gram-negative bacterium. Dev. Comp. Immunol. 31 (11), 1183–1196.

Pichlmair, A., Reise Sousa, C., 2007. Innate recognition of viruses. Immunity 27 (3), 370–383.

Pichlmair, A., Schulz, O., Tan, C.P., Rehwinkel, J., Kato, H., Takeuchi, O., Akira, S., Way, M., Schiavo, G., Reise Sousa, C., 2009. Activation of MDA5 requires higher-order RNA structures generated during virus infection. J. Virol. 83, 10761–10769.

Pollpeter, D., Komuro, A., Barber, G.N., Horvath, C.M., 2011. Impaired cellular responses to cytosolic DNA or infection with *Listeria monocytogenes* and Vaccinia virus in the absence of the murine LGP2 protein. PLoS ONE 6 (4), e18842. doi:10.1371/journal.pone.0018842.

Pothlichet, J., Burtey, A., Kubarenko, A.V., Caignard, G., Solhonne, B., Tangy, F., et al., 2009. Study of human RIG-I polymorphisms identifies two variants with an opposite impact on the antiviral immune response. PLoS One 4, e7582.

Quiniou, S.M., Wilson, M.R., Bengtén, E., Waldbieser, G.C., Clem, L.W., et al., 2005. MHC RFLP analyses in channel catfish full-sibling families: identification of the role of MHC molecules in spontaneous allogeneic cytotoxic responses. Dev. Comp. Immunol. 29, 457–467.

Rajendran, K.V., Zhang, J., Liu, S., Kucuktas, H., Wang, X., Liu, H., Sha, Z., Terhune, J., Peatman, E., Liu, Z., 2012. Pathogen recognition receptors in channel catfish: I. Identification, phylogeny and expression of NOD-like receptors. Dev. Comp. Immunol. 37, 77–86.

Rehwinkel, J., Tan, C.P., Goubau, D., Schulz, O., Pichlmair, A., Bier, K., et al., 2010. RIG-I detects viral genomic RNA during negative-strand RNA virus infection. Cell 140, 397–408.

Rothenfusser, S., Goutagny, N., Di Perna, G., Gong, M., Monks, B.G., Schoenemeyer, A., Yamamoto, A., Akira, S., Fitzgerald, K.A., 2005. The RNA helicase Lgp2 inhibits TLR-independent sensing of viral replication by retinoic acid-inducible gene-I. J. Immunol. 175, 5260–5268.

Saito, T., Hirai, R., Loo, Y.M., Owen, D., Johnson, C.L., Sinha, S.C., Akira, S., Fujita, T., Gale, M. Jr., 2007. Regulation of innate antiviral defenses through a shared repressor domain in RIG-I and LGP2. Proc. Natl. Acad. Sci. USA. 104, pp. 582–587. .

Satoh, T., Kato, H., Kumagai, Y., Yoneyama, M., Sato, S., Matsushita, K., Tsujimura, T., Fujita, T., Akira, S., Takeuchi, O., 2010. LGP2 is a positive regulator of RIG-I- and MDA5-mediated antiviral responses. Proc. Natl. Acad. Sci. USA 107, 1512–1517.

Sha, Z., Abernathy, J.W., Wang, S., Li, P., Kucuktas, H., Liu, H., Peatman, E., Liu, Z., 2009. NOD-like subfamily of the nucleotide-binding domain and leucine-rich repeat containing family receptors and their expression in channel catfish. Dev. Comp. Immunol. 33, 991–999.

Shen, L., Stuge, T.B., Zhou, H., Khayat, M., Barker, K.S., et al., 2002. Channel catfish cytotoxic cells: a mini-review. Dev. Comp. Immunol. 26, 141–149.

Shen, L., Stuge, T.B., Evenhuis, J.P., Bengtén, E., Wilson, M.R., et al., 2003. Channel catfish NK-like cells are armed with IgM via a putative FcμR. Dev. Comp. Immunol. 27, 699–714.

Shen, L., 2004. Identification and characterization of clonal NK-like cells from channel catfish (*Ictalurus punctatus*). Dev. Comp. Immunol. 28, 139–152.

Small, B.C., Murdock, C.A., Bilodeau-Bourgeois, A.L., Peterson, B.C., Waldbieser, G.C., 2008. Stability of reference genes for real-time PCR analyses in channel catfish (*Ictalurus punctatus*) tissues under varying physiological conditions. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 151 (3), 296–304.

Su, J., Huang, T., Dong, J., Heng, J., Zhang, R., Peng, L., 2010. Molecular cloning and immune responsive expression of MDA5 gene, a pivotal member of the RLR gene family from grass carp *Ctenopharyngodon idella*. Fish Shellfish Immunol. 28, 712–718.

Takahasi, K., Kumeta, H., Tsuduki, N., Narita, R., Shigemoto, T., Hirai, R., Yoneyama, M., Horiuchi, M., Ogura, K., Fujita, K., Inagaki, F., 2009. Solution structures of cytosolic RNA sensor MDA5 and LGP2 *C*-terminal domains: identification of the RNA recognition loop in RIG-I like receptors. J. Biol. Chem. 284, 17465–17474.

Takano, T., Sha, Z., Peatman, E., Terhune, J., Liu, H., Kucuktas, H., et al., 2008. The two channel catfish intellectin genes exhibit highly differential patterns of tissue expression and regulation after infection with Edwardsiella ictaluri. Dev. Comp. Immunol. 32(6), pp. 693–705.

Takeuchi, O., Akira, S., 2008. MDA5/RIG-I and virus recognition. Curr. Opin. Immunol. 20, 17–22.

Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599.

Vallejo, A.N., Miller, N.W., Clem, W., 1992. Antigen processing and presentation in teleost immune responses. Ann. Rev. Fish Dis. 2, 73–89.

Venkataraman, T., Valdes, M., Elsby, R., Kakuta, S., Caceres, G., Saijo, S., Iwakura, Y., Barber, G.N., 2007. Loss of DExD/H box RNA helicase LGP2 manifests disparate antiviral responses. J. Immunol. 178, 6444–6455.

Ventura-Holman, T., Lobb, C.J., 2002. Structural organization of the immunoglobulin heavy chain locus in the channel catfish: the IgH locus represents a composite of two gene clusters. Mol. Immunol. 38, 557–564.

Wilson, M.R., Marcuz, A., Ginkel, F., Miller, N.W., Clem, L.W., Middleton, D., Warr, G.W., 1990. The immunoglobulin M heavy chain constant region gene of the channel catfish, *Ictalurus punctatus*: an unusual mRNA splice pattern produces the membrane form of the molecule. Nucleic Acids Res. 18, 5227–5233.

Wilson, M.R., Bengtén, E., Miller, N.W., Clem, L.W., Pasquier, L.D., Warr, G.W., 1997. A novel chimeric Ig heavy chain from a teleost fish shares similarities to IgD. Proc. Natl. Acad. Sci. USA 94, 4593–4597.

Wolf, K., Darlington, R.W., 1971. Channel catfish virus: a new herpesvirus of ictalurid fish. J. Virol. 8, 525–533.

Xu, P., Bao, B., He, Q., Peatman, E., He, C., Liu, Z., 2007. Characterization and expression analysis of toll-like receptor 2 gene from catfish. Fish Shellfish Immunol. 29 (10), 865–878.

Yang, C., Su, J., Huang, T., Zhang, R., Peng, L., 2011. Identification of a retinoic acid-inducible gene I from grass carp (*Ctenopharyngodon idella*) and expression analysis *in vivo* and *in vitro*. Fish Shellfish Immunol. 30, 936–943.

Yoneyama, M., Kikuchi, M., Natsukawa, T., Shinobu, N., Imaizumi, T., Miyagishi, M., Taira, K., Akira, S., Fujita, T., 2004. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat. Immunol. 5, 730–737.

Yoneyama, M., Kikuchi, M., Matsumoto, K., Imaizumi, T., Miyagishi, M., Taira, K., Foy, E., Loo, Y.M., Gale Jr., M., Akira, S., Yonehara, S., Kato, A., Fujita, T., 2005. Shared and unique functions of the DExD/H-box helicases RIG-I, MDA5, and LGP2 in antiviral innate immunity. J. Immunol. 175, 2851–2858.

Yoneyama, M., Fujita, T., 2007. Function of RIG-I-like receptors in antiviral innate immunity. J. Biol. Chem. 282, 15315–15318.

Yoneyama, M., Fujita, T., 2008. Structural mechanism of RNA recognition by RIG-I like receptors. Immunity 29, 178–181.

Zhang, H., Peatman, E., Liu, H., Niu, D., Feng, T., Kucuktas, H., Waldbieser, G., Chen, L., Liu, Z., 2011. Characterization of a mannose-binding lectin from channel catfish (*Ictalurus punctatus*). Res. Vet. Sci. doi:10.1016/j.rvsc.2011.03.024.

Zhou, H., Stuge, T.B., Miller, N.W., Bengtén, E., Naftel, J.P., et al., 2001. Heterogeneity of channel catfish CTL with respect to target recognition and cytotoxic mechanisms employed. J. Immunol. (Baltimore, 1950) 167, pp. 1325–1332.

Zhou, H., Bengtén, E., Miller, N.W., Clem, L.W., Wilson, M.R., 2003. The T cell receptor beta locus of the channel catfish, Ictalurus punctatus, reveals unique features. J. Immunol. (Baltimore, Md: 1950) 170, pp. 2573–2581.

Zou, J., Chang, M., Nie, P., Secombes, C.J., 2009. Origin and evolution of the RIG-I like RNA helicase gene family. BMC Evol. Biol. 9, 85.