Technical Report

Channel catfish, *Ictalurus punctatus* Rafinesque 1818, tetraspanin membrane protein family: Characterization and expression analysis of CD81 cDNA

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**ABSTRACT**

CD81, also known as the target of antiproliferative antibody 1 (TAPA-1) in human, is a member of tetraspanin integral membrane protein family. This protein plays many important roles in immune and other physiological functions. In this report, we characterized and analyzed expression of the channel catfish CD81 transcript. The full-length of channel catfish CD81 cDNA comprised of 1130 nucleotides, including an open reading frame which appears to encode a putative peptide of 234 amino acid residues. By comparison with the human counterpart, the channel catfish CD81 peptide could be divided into domains, including four transmembrane domains, three intracellular domains, and one of each small and large extracellular loops. The degree of conservation of the channel catfish CD81 amino acid sequence to that of mammalian counterparts ranged from 65% to 67%. The large extracellular domain shows the least conservation between fish and mammals. However, the characteristic Cys159-Cys160-Gly161 motif and Cys176/188 in this domain were conserved. The channel catfish CD81 transcript was detected by RT-PCR in spleen, head kidney, liver, intestine, skin and gill. This result provides important information for further elucidating CD81 functions in channel catfish.

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Channel catfish production is the most important aquacultural industry in the southeastern U.S., generating over 450 million dollars in value annually (USDA, 2007). During studies on the pathogenesis of *Edwardsiella ictaluri*, we found that a battery of channel catfish gene transcripts is up-regulated at the early stage of infection (unpublished data). One of these transcripts is CD81.

CD81, also known as the target of antiproliferative antibody 1 (TAPA-1) in human, is a member of tetraspanin integral membrane protein family (Hemler, 2005; Berditchevski and Odintsova, 2007; Levy and Shoham, 2005a,b), was first identified, cloned and characterized in a human lymphoma cell line (Oren et al., 1990). CD81 plays many important roles in immunological and pathophysiological processes in host. CD81 often associated with CD19 is required for humoral immune response to antigens (Maecker and Levy, 1997; Miyazaki et al., 1997; Tsitsikov et al., 1997; Shoham et al., 2003), which event needs CD81 be palmitoylated for lipid raft-dependent receptor signaling (Cherukuri et al., 2004a,b; Clark et al., 2004). After that, the CD81/CD19 complexes associate with the complement receptor CD21 to activate B cells (Fearon and Carroll, 2000; Levy and Shoham, 2005a). Another study demonstrated that CD81 has been dynamically redistributed at the central zone of T–B cell immune synapses, indicating CD81 is involved...
### Alignment

<table>
<thead>
<tr>
<th>Domain</th>
<th>Transmembrane</th>
<th>Small Extracellular</th>
</tr>
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<tr>
<td>Intracellular</td>
<td>Transmembrane</td>
<td>Small Extracellular</td>
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<tr>
<td><strong>Human</strong></td>
<td>MG--VEGCTKICYLLFVPNVPFWLAVGLGVIALGVALRHLDPQNTTLNLYLE 48</td>
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</tr>
<tr>
<td><strong>Rhesus monkey</strong></td>
<td>MG--VEGCTKICYLLFVPNVPFWLAVGLGVIALGVALRHLDPQNTTLNLYLE 48</td>
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<tr>
<td><strong>Cotton-top tamarin</strong></td>
<td>MG--VEGCTKICYLLFVPNVPFWLAVGLGVIALGVALRHLDPQNTTLNLYLE 48</td>
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<tr>
<td><strong>Chinese tree shrew</strong></td>
<td>MG--VEGCTKICYLLFVPNVPFWLAVGLGVIALGVALRHLDPQNTTLNLYLE 48</td>
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<tr>
<td><strong>Cattle</strong></td>
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<td></td>
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<tr>
<td><strong>Pig</strong></td>
<td>MG--VEGCTKICYLLFVPNVPFWLAVGLGVIALGVALRHLDPQNTTLNLYLE 48</td>
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<tr>
<td><strong>Mouse</strong></td>
<td>MG--VEGCTKICYLLFVPNVPFWLAVGLGVIALGVALRHLDPQNTTLNLYLE 48</td>
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<tr>
<td><strong>Rat</strong></td>
<td>MG--VEGCTKICYLLFVPNVPFWLAVGLGVIALGVALRHLDPQNTTLNLYLE 48</td>
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<tr>
<td><strong>African clawed frog</strong></td>
<td>MG--VEGCTKICYLLFVPNVPFWLAVGLGVIALGVALRHLDPQNTTLNLYLE 48</td>
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<tr>
<td><strong>Channel catfish</strong></td>
<td>MG--VEGCTKICYLLFVPNVPFWLAVGLGVIALGVALRHLDPQNTTLNLYLE 48</td>
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<tr>
<td><strong>Zebrafish</strong></td>
<td>MG--VEGCTKICYLLFVPNVPFWLAVGLGVIALGVALRHLDPQNTTLNLYLE 48</td>
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**Spotted green pufferfish**

| MA--VENCTKICYLCPFNVPFWLAVGLGVIALGVALRHLDPQNTTLNLYLE 48

| Identical amino acids among species are denoted by asterisks (*) below the sequences. GenBank accession numbers of each sequence are as follows: African clawed frog, NP_001080082; cattle, NP_001030271; channel catfish, FJ205473; Chinese tree shrew, ABQ52430; cotton-top tamarin, Q9N0J9; human, NP_004347; mouse, NP_598416; pig, NP_001072147; rat, AAH60583; rhesus monkey, XP_001093228; spotted green pufferfish, CAG05519; and zebrafish, NP_571593.

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**Fig. 1.** Multiple alignments of CD81 amino acid sequences from various species. Gaps introduced in the sequences are indicated as (-). Structural domains of CD81 are indicated above the sequences.
in the T–B lymphocyte collaboration required for the cell activation (Mittelbrunn et al., 2002). On the other hand, CD81 has been identified as receptors for two important human pathogens. Silvie et al. (2003) demonstrated that CD81 on the cell surface of hepatocytes is required for Plasmodium sporozoite infectivity. CD81 is an entry coreceptor on the cell surface of hepatocytes for the hepatitis C virus envelope protein E2 (Cormier et al., 2004; Pileri et al., 1998).

In teleost fish, the zebrafish (Danio rerio) CD81 gene has been mapped to LG7 (Yoder and Litman, 2000), but its immunological/pathophysiological functions have not been explored. In the course of studying pathogenesis of E. ictaluri in channel catfish, we observed that CD81 expressed sequence tag (EST) was up-regulated at the early stage of infection (unpublished data). This observation prompted us to speculate that CD81 may play a role in early stages of E. ictaluri infection. In this report, we describe the isolation, characterization and analysis of expression of the channel catfish CD81 transcript.

The NWAC 103 strain of channel catfish was used in this study as per the Guidelines for the Use of Fish in Research (Nickum et al., 2004). The protocol of animal use was approved by the Institutional Animal Care and Use Committee, Aquatic Animal Health Research Unit, Agricultural Research Service, U.S. Department of Agriculture in Auburn, AL. Tissues were aseptically excised.

Total RNA from tissues was isolated by using a Tri reagent (Molecular Research Center, Inc., Cincinnati, OH) as described previously (Yeh and Klesius, 2008a,b). After total RNA isolation, channel catfish CD81 cDNA was generated by rapid amplification of cDNA ends (RACE) by using a GeneRacer kit (Invitrogen, Carlsbad, CA) per manufacturer’s instructions. Primers for PCR amplification are as follows: GeneRacer 5′ primer (Invitrogen), 5′-CGACTGAGCCAAGGACTGA-3′; GeneRacer 3′ primer (Invitrogen), 5′-GCTGTCAGAGATCGCATTACG-3′; CD81-45F, 5′-TGCTGCGCTCTGACATCTGTCAT-3′; and CD81-203R, 5′-GAGCAGCCCCATCTTCTTGC-3′. The PCR products were ligated into the pSC-A cloning vector (Agilent Technologies, Santa Clara, CA). The ligated plasmids were transformed into Escherichia coli by heat-shock. After culture enrichment at 37 °C in SOC medium, the cells were streaked on LB plates containing 30 μg/ml of kanamycin and incubated at 37 °C overnight. Colonies were randomly picked and cultured in WU medium. No reverse transcriptase added in reactions was included in experiments to ensure that no amplification was from residual genomic DNA.

The DNA sequencing reactions were carried out at the USDA ARS MidSouth Area Genomics Laboratory with an ABI 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA) as described previously (Yeh and Klesius, 2008a,b). More than six clones of each PCR product were sequenced on both strands. chromatograms were edited and trimmed to remove the vector sequences by using Phred and Lucy software, respectively (Ewing and Green, 1998; Li and Chou, 2004; Ewing et al., 1998). Amino acid sequences were deduced from nucleic acid sequences by using Transeq software (Rice et al., 2000) and aligned with other CD81 amino acid sequences by using ClustalW2 (Larkin et al., 2007) (http://www.ebi.ac.uk/services/index.html). ExPASy server (Gasteiger et al., 2005) was used to calculate the CD81 molecular mass and pI. Transmembrane topology and signal peptide of the channel catfish CD81 peptide were predicted via the Phobius web server (Käll et al., 2007). Phylogenetic relationships of CD81 from various species were analyzed by the MEGA 4.0 software (Tamura et al., 2007) based on the ClustalW2 alignment results.

RT-PCR assays for CD81 gene transcript in channel catfish tissues were performed by a two-step procedure routinely used in our laboratory (Yeh and Klesius, 2008a,b). Primers (CD81-45F and CD81-203R) were also used in these assays. Primers for β-actin were β-actin F, 5′-GACCTGGAGCAGGAGATGGG-3′ and β-actin R, 5′-AACCTCTCATGCAATGGT-3′. These amplified products were analyzed in 2% agarose gel electrophoresis and stained with ethidium bromide. Images were recorded by a KODAK Gel Logic 440 Imaging System and processed with Adobe Photoshop (v. 7.0.1., Adobe Systems Incorporated, San Jose, CA).

We previously identified a channel catfish CD81 expressed sequence tag by subtractive suppression

<table>
<thead>
<tr>
<th>Human</th>
<th>ELFSQKLYLIGIAMIVAVIMFEMILSMVLCGIRNSVY</th>
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<td>Rhesus monkey</td>
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<td>Cotton-top tamarin</td>
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<td>Chinese tree shrew</td>
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<td>Cattle</td>
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<td>Pig</td>
<td>ELFSQKLYLIGIAMIVAVIMFEMILSMVLCGIRNSVY</td>
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<td>Mouse</td>
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<td>Zebrafish</td>
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<td>Spotted green pufferfish</td>
<td>ELPSQKLYLIGIAMIVAVIMFEMILSMVLCGIRNSVY</td>
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<tr>
<td>**</td>
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Fig. 1. (Continued).
hybridization (unpublished data). Based on this EST, we
continued to clone, sequence and characterize the
channel catfish CD81 cDNA with the RACE method
(Frohman et al., 1988). The full-length of the channel
catfish CD81 cDNA had 1130 nucleotides (GenBank
accession numbers FJ205473), including 5'- and 3'-
untranslated region (UTR), and an open reading frame
(ORF). The ORF appears to encode a 234-amino acid
peptide with a calculated molecular mass of 25,993 Da
and pI of 6.09. The 5'-UTR contained a TATA box
sequence (TATAAA) at positions −39 to −34, and a Kozak
sequence (ACCATGG) at positions −3 to +4 upstream of
the translation start codon. The 3'-UTR had a poly-
adenylation tail. Recently, similar EST of channel catfish
CD81 was deposited in the GenBank’s EST database
(www.ncbi.nlm.gov/dbEST/).

We further analyzed the deduced CD81 amino acid
sequence, and we found that, like the mammalian
counterparts (Hemler, 2005; Levy and Shoham,
2005a,b), channel catfish CD81 is a transmembrane
protein, which can be structurally divided into four
transmembrane domains, three intracellular domains
and two (small and large) extracellular loops (Fig. 1).
Unlike among mammals shared more than 85% homol-
ogy (Cho et al., 2007), the deduced channel catfish CD81
amino acid sequence shared 79% identity with zebrafish,
and 65–67% identity with the mammals. In zebrafish,
Yoder and Litman (2000) also demonstrated that the
CD81 peptide of zebrafish is 66% and 65% identical to that
of human and mouse, respectively. As shown in Fig. 1,
we observed that although the large extracellular loops
showed the least conservation between fish and mam-
mals, the characteristic Cys159-Cys160-Gly161 motif and
Cys176/188 (numbering after channel catfish) (Kitadokoro
et al., 2001) of the large extracellular loops among
species examined were conserved, suggesting that the
three-dimensional structure of the large extracellular
loop of CD81 may be conserved via disulfide linkages
throughout the evolution (Fletcher et al., 1994; Rush-
mere et al., 1994). Other key conserved feature includes
the potential palmitoylation at the Cys6-Thr7-Lys8-Cys9
motif and at the Cys225-Cys226 sites in intracellular
domains.

A phylogenetic tree was generated using the ClustalW2
alignment results (Fig. 1). As seen in Fig. 2, mammalian
CD81 formed a very closely supported clade, distinguish-
able from that of fish counterparts, which are hetero-
genous groups of over 27,300 species (Helfman, 2007).
These results are in agreement with our previous findings
in other channel catfish genes (Yeh and Klesius, 2007a,b,
2008a,b,c).

The expression profile of channel catfish CD81 was
examined in spleen, head kidney, liver, intestine, skin and
gill with multiplex RT-PCR amplification. The amplified
CD81 and β-actin products had 159 and 203 nucleotides,
respectively. As seen in Fig. 3, the channel catfish CD81
transcript was detected in all tissues of fish examined.
These results are in agreement with reports for the
mammalian counterparts that CD81 is ubiquitous on
animal cell surfaces (Hemler, 2005; Levy and Shoham,
2005a).

In summary, the channel catfish CD81 cDNA transcript
was cloned, sequenced, and characterized. The transcript
was constitutively expressed in all tissues examined.
Experiments for the CD81 expression in E. coli
and production of polyclonal antisera that will be used to
further explore the channel catfish CD81 functions are
underway.
Fig. 3. Tissue distribution of channel catfish CD81 transcript (n = 4). Total RNA from various tissues was used for RT-PCR assays (Yeh and Klesius, 2008a,b). The amplified products were analyzed by agarose gel electrophoresis and stained with ethidium bromide. The sizes of amplified CD81 and β-actin were 159 and 203 nucleotides, respectively. Spleen (lanes 1, 7, 13, and 19), head kidney (lanes 2, 8, 14, and 20), liver (lanes 3, 9, 15, and 21), intestine (lanes 4, 10, 16, and 22), skin (lanes 5, 11, 17, and 23), and gill (lanes 6, 12, 18, and 24). Lane 25, negative control and lane 26, 100 bp molecular weight markers (500, 400, 300 and 200 nucleotides).

Acknowledgments

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References


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