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Phosphoinositide 3-kinase family in channel catfish and their regulated expression after bacterial infection

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ABSTRACT

The phosphoinositide-3-kinase (PI3Ks) family of lipid kinases is widely conserved from yeast to mammals. In this work, we identified a total of 14 members of the PI3Ks from the channel catfish genome and transcriptome and conducted phylogenetic and syntenic analyses of these genes. The expression profiles after infection with *Edwardsiella ictaluri* and *Flavobacterium columnare* were examined to determine the involvement of PI3Ks in immune responses after bacterial infection in catfish. The results indicated that PI3Ks genes including all of the catalytic subunit and several regulatory subunits genes were widely regulated after bacterial infection. The expression patterns were quite different when challenged with different bacteria. The PI3Ks were up-regulated rapidly at the early stage after ESC infection, but their induced expression was much slower, at the middle stage after columnaris infection. RNA-Seq datasets indicated that PI3K genes may be expressed at different levels in different catfish differing in their resistance levels against columnaris. Future studies are required to confirm and validate these observations. Taken together, this study indicated that PI3K genes may be involved as a part of the defense responses of catfish after infections, and they could be one of the determinants for disease resistance.

1. Introduction

Phosphoinositol 3-kinases (PI3Ks) belong to a family of intracellular lipid kinases that phosphorylate the 3′-hydroxyl group of phosphatidylinositol (also known as PI or *PtdIns*) and phosphoinositides in cellular membranes either acting constitutively or in response to extracellular stimuli such as growth factors and hormones. The lipid products of PI3Ks serve multiple functions in the cell by regulating cellular membrane trafficking and acting as second messengers. Cytosolic PH-domain containing signaling proteins recruited to the membrane could activate diverse signal transduction pathways which in turn act synergistically to mediate a number of cell behaviors and properties in both normal and pathological conditions, including cell growth, proliferation, differentiation, survival, metabolism, vesicular trafficking, degranulation, cytoskeletal rearrangement, and motility [1–3]. PI3Ks are an evolutionarily conserved family of enzymes; based on their structural features, *in vitro* lipid substrate specificity, tissue distribution, mechanism of activation, and function, they were classified into three distinct classes, Class I to III [4]. Their structural components and domains were well depicted in the review by Vanhaesebroeck et al. [4].

Class I enzymes exist as a heterodimer consisting of one catalytic subunit and one regulatory subunit. Notably, members of this class are activated by various cell surface receptors, leading to further subdivision of this class into subfamilies IA and IB. Members of the subclass IA are activated by receptor tyrosine kinase, whereas those of subclass IB are activated by G-protein coupled receptors (GPCRs) [1,3,4]. Class IA PI3Ks enzymes include p110α (PI3KCA), p110β (PI3KCB), and p110δ (PI3KCD), which can pair with one of the five regulatory subunits p85α, p55α, p50α (alternatively spliced from PIK3R1), P85β (PIK3R2), and p55γ (PIK3R3) [5–7]. In contrast, Class IB PI3K enzymes consist of the p110γ (PI3KCG) catalytic subunit which forms heterodimers with either p101 (PIK3R5) or p87 (PIK3R6) [2,8,9]. The regulatory subunits seemed to be primarily responsible for spatiotemporal control of PI3Ks activation [10]. For example, the regulatory subunits p85 of class IA PI3Ks acts to localize the catalytic subunit p110 to the plasma membrane in response to growth factor stimulation and activation of growth

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factor receptors such as the platelet derived growth factor receptor (PDGFR) [3]. All Class I PI3K enzymes have been shown to phosphorylate phosphatidylinositol (PI), PI(4)P, and PI(4,5)P2 to produce the lipid products PI3P, PI(3,4)P2, and PI(3,4,5)P3, respectively *in vitro*. However, phosphorylation by class I PI3Ks was observed *in vivo* only with PI(4,5)P2 to produce PI(3,4,5)P3 [6,11,12].

Class II PI3Ks have been identified based on structural homology with Class I PI3Ks but have received relatively little attention compared with their class I counterparts. Unlike class I PI3Ks, class II PI3Ks are monomers with only a single catalytic domain without regulatory subunit, and are distinguished by a carboxy-terminal C2 domain [13,14]. There are three isoforms of class II PI3K, named C2α, C2β, and C2γ [5,11]. Class II PI3Ks have well-recognized *in vitro* substrates, but their *in vivo* targets are still being identified. PI(4)P is the substrate for the generation of PI(3,4)P2, and PI is the substrate for the generation of PI(3)P [9].

Class III PI3Ks has a single member, the vacuolar protein-sorting 34 (VPS34, also called PIK3C3) that was first identified in mammals in 1995 [15]. It is the oldest PI3K and is the only one found in yeast and plants as well as in metazoans and is highly conserved among yeast, plants, and mammals [3]. Class III PI3K enzymes are structurally more similar to class I PI3Ks than the class II PI3Ks, since they consist of a catalytic (VPS34) and regulatory subunit p150 (also called VSP15 or PIK3R4). VPS34 enzymes are unique among PI3Ks in that they only use phosphatidylinositol as the substrate. VPS34 could therefore share protein effectors with the class II PI3Ks, but it is not clear whether the functions of class II and class III PI3Ks overlap [16]. The most recognized function of class III PI3Ks is the regulation of vesicular trafficking in the endosomal/lysosomal system [17–21]. Class III PI3Ks also activate additional mechanisms in mammalian cells, such as endocytosis and phagocytosis. Additionally, VPS34 kinase may also play a role in autophagy and protein synthesis through an mTOR-dependent mechanism [22]. As such, class III PI3Ks may be highly relevant to disease defense responses.

Catfish industry, the most prominent aquaculture industry in the United States, has encountered great challenges including devastating diseases which cause large economic losses. In particular, bacterial diseases, enteric septicemia of catfish (ESC) and columnaris disease, cause huge losses to the catfish industry [23,24]. Recently, a third bacterial disease, caused by *Aeromonas hydrophila*, emerges to be another devastating disease to the catfish industry [25]. With columnaris, the most frequently occurring bacterial disease, a recent QTL study indicated that the PI3K pathway is highly related to the host resistance against this disease [26]. As the first step to understand the mechanism of resistance against columnaris disease, the goal for this study is to identify and annotate PI3Ks genes in channel catfish, and determine their expression after infection with *Edwardsiella ictaluri* and *Flavobacterium columnare*.

2. Materials and methods

2.1. Gene identification and sequence analysis

To identify the PI3K genes, the channel catfish transcriptome database [27–29] and the whole genome database of channel catfish (unpublished data) were searched using available PI3Ks amino acid sequences from teleost fish, including zebrafish (*Danio rerio*), stickleback (*Gasterosteus aculeatus*), medaka (*Oryzias latipes*), tilapia (*Oreochromis niloticus*), fugu (*Takifugu rubripes*), turtle (*Pelodiscus sinensis*), lizard (*Anolis carolinensis*), chicken (*Gallus gallus*), mouse (*Mus musculus*), and human (*Homo sapiens*) as query sequences. TBLASTN was performed by searching against the channel catfish transcriptome database [29]. The e-value was set at an intermediately stringent level of e−10 for collecting potential PI3Ks.

The ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) was used to predict the open reading frames of retrieved sequences. The predicted ORFs were verified by BLASTP against NCBI non-redundant protein database. Simple Modular Architecture Research Tool (SMART http://smart.embl-heidelberg.de) was used to predict the conserved domains based on sequence homology. To determine copy numbers of PI3Ks genes in channel catfish genome, BLASTN was used to search against channel catfish draft whole genome assembly database. FGENESH [30] were used to predict amino acid sequence from genomic sequences.

2.2. Phylogenetic analysis

The full-length amino acid sequences of PI3Ks from several representative vertebrates including those from human, mouse, chicken, lizard, and several fish species such as zebrafish, tilapia, medaka and stickleback were retrieved from NCBI and Ensembl databases for phylogenetic analysis. Multiple protein sequences alignments were conducted using the Clustal W2 program [31]. The maximum likelihood method was used to conduct phylogenetic analysis using MEGA 5.2 [32]. Jones–Taylor–Thornton (JTT) and gamma distributed rate within variant sites (G + I) model were chosen based on the alignment result [33]. Gaps were removed by pair-wise deletion and 1000 bootstrap replicates were performed in phylogenetic analysis.

2.3. Syntenic analysis

The homologies of the PI3Ks, and their neighboring genes in channel catfish were examined through comparing those among human, zebrafish, and tilapia, to provide additional evidence for gene identification and orthology. The genome information for each species was retrieved from Ensembl and Genomicus. The upstream and downstream genes surrounding the putative PI3Ks genes were identified from the channel catfish scaffolds by FGENESH program [30]. BLASTP was used to annotate these neighboring genes by searching against NCBI database. The catfish PI3Ks genes were named following the Zebrafish Nomenclature Guidelines [34].

2.4. Expression analysis using available RNA-Seq datasets

The Illumina-based RNA-Seq reads were retrieved from bacterial challenge experiments in catfish: intestine samples challenged with *E. ictaluri* (SRA Accession SRP009069) [35], and gill samples challenged with *F. columnare* (SRA Accession SRP012586, SRP017689.) [36,37]. For ESC studies, fish were challenged with a concentration of 4×10^8 CFU/ml in 30 L aquaria for 2 h by immersion exposure. At 3 h, 24 h and 3 d after challenge, 30 fish were collected from each of the appropriate control and treatment aquaria at each time point and euthanized with MS-222 (300 mg/L). The entire intestinal tracts from 10 fish were dissected, bisected and gently washed and pooled together for RNA extraction [35]. For columnaris, Challenge experiments were conducted by immersion exposure for 2 h at a final concentration of the bacteria at 3×10^6 CFU/mL. At 4 h, 24 h, and 48 h after challenge, 18 fish from both control and treatment were randomly selected and divided into 3 replicate pools (6 fish each) respectively. Gill tissues in the 3 replicates were placed into 5 ml RNA later™ (Ambion, Austin, TX, USA) for RNA extraction [36]. The gill samples of resistant and susceptible families differing in their susceptibility to *F. columnare* were collected at 0-h, 1-h, 2-h and 8-h after *F. columnare* infection, as previously reported [37]. Trimmed high quality reads were mapped onto the catfish PI3Ks genes using CLC Genomics