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Identification and expression analysis of 26 oncogenes of the receptor tyrosine kinase family in channel catfish after bacterial infection and hypoxic stress

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ABSTRACT

Receptor tyrosine kinases (RTKs) are high-affinity cell surface receptors for many polypeptide growth factors, cytokines and hormones. RTKs are not only key regulators of normal cellular processes, but are also involved in the progression of many types of tumors, and responses to various biotic and abiotic stresses. Catfish is a primary aquaculture species in the United States, while its industry is drastically hindered by several major diseases including enteric septicemia of catfish (ESC) that is caused by *Edwardsiella ictaluri*. Disease outbreaks are often accompanied by hypoxic stress, which affects the performance and survival of fish by reducing disease resistance. In this study, we identified 26 RTK oncogenes in the channel catfish genome, and determined their expression profiles after ESC infection and hypoxic stress. The 26 RTK genes were divided into four subfamilies according to phylogenetic analysis, including TIE (2 genes), ErbB (6 genes), EPH (14 genes), and INSR (4 genes). All identified RTKs possess a similar molecular architecture including ligand-binding domains, a single transmembrane helix and a cytoplasmic region, which suggests that these genes could play conserved biological roles. The expression analysis revealed that eight RTKs were significantly regulated after bacterial infection, with dramatic induction of insulin receptor genes including INSRb, IGF1Ra, and IGF1Rb. Upon hypoxic stress, EPHB3a, EGFR, ErbB4b, and IGF1Rb were expressed at higher levels in the tolerant catfish, while EPHA2a, EPHA2, TIE1 and INSRa were expressed at higher levels in the intolerant catfish. These results suggested the involvement of RTKs in immune responses and hypoxic tolerance.

1. Introduction

Receptor tyrosine kinases (RTKs) are high-affinity cell surface receptors for many polypeptide growth factors, cytokines, and hormones (Schlessinger, 2000). The human RTK family contains 58 members that are grouped into 20 subfamilies (Blume-Jensen and Hunter, 2001). The overall topology of RTKs, the mechanism of activation, and the key components of the intracellular signaling pathways that they trigger, are evolutionarily conserved from nematodes to humans, suggesting that they play critical regulatory roles (Lemmon and Schlessinger, 2010). The RTK family includes a group of oncogenes and candidate proto-oncogenes, including epidermal growth factor receptor (EGFR/ErbB family), ephrin receptors (EPHs), angiopoietin receptors (TIEs) and insulin receptors (INSRs).

RTKs are activated by induced receptor dimerization through the binding of their cognate ligands (Ullrich and Schlessinger, 1990). Following activation, RTKs selectively form phosphotyrosine residues by autocatalytic modifications. Trans-autophosphorylation then initiates further responses through cascades of posttranslational modifications and generation of lipid second messengers. RTKs not only play key roles in regulating normal cellular processes such as proliferation and differentiation, cell survival, cell migration, and cell-cycle control (Ullrich and Schlessinger, 1990; Blume-Jensen and Hunter, 2001), but also play critical roles in regulating development and progression of many types of cancers. For instance, ErbB2 (human epidermal growth factor receptor 2) is a known proto-oncogene. Approximately 20% of breast cancers exhibit ErbB2 gene amplification/overexpression, resulting in an aggressive tumor phenotype and reduced survival (Slamon et al., 1987, 1989). TIEs (TIE1 and TIE2) are involved in modulating cell–cell and cell–matrix interactions which are required for vascular remodeling and maturation (Pawson, 1995; Shewchuk et al., 2000). The cellular responses to EPH receptor stimulation by their ephrin ligands are important in mediating a wide range of biological activities, including angiogenesis, cell segregation, cell attachment, shape, and motility. Several EPH/ephrin molecules are expressed in vascular systems, with the EPHB4 and its ligand EPHRINB2 being found as the

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most significantly expressed genes (Hasina et al., 2013). EPH/ephrin signaling has been identified to play roles in many human cancers, such as lung cancer, breast cancer, and prostate cancer, as well as melanoma and leukemia (Kullander and Klein, 2002). Moreover, a number of recent studies provide an updated picture of INSR/IGF1R-mediated signaling events (Taniguchi et al., 2006; LeRoith and Accilli, 2008). INSR is a vital mediator of metabolic responses, whereas IGF1R is primarily involved in mitogenesis, differentiation, and anti-apoptotic activities. Cross-talk between insulin, IGFs, and their receptors is a common event in many organs and processes. Hence, the role of INSR in mitogenesis and cell motility lays the foundation for its involvement in cancer development and progression (Belfiore et al., 2009).

A common phenomenon in human solid tumors and inflamed tissues is hypoxia. Hypoxia occurs when the balance between oxygen supply and demand is disturbed, which is an important feature at sites of acute and chronic tissue inflammation under pathogen challenge, such as wound healing and tumor growth (Lund-Olesen, 1970). Clinical studies in human head and neck, soft tissue, and uterine cervical cancers have demonstrated that patients with highly hypoxic tumors have a significantly higher risk of developing metastases and often have a poorer clinical outcome than patients with fewer or non-hypoxic tumors (Hockel et al., 1993, 1996; Brizel et al., 1996, 1997). The lack of oxygen in the inner core of tumors, primarily due to increased distance of tumor cells from blood vessels and aberrant formation of blood vessels resulting in poor blood flow, is believed to contribute to tumor progression, as well as resistance to chemotherapy and radiotherapy (Janssen et al., 2005). In tumors, irregular tumor vessel formation is the main cause of tumor hypoxia. Studies have shown that various hypoxia-induced proteins are capable of regulating tumor cell growth, autophagy, angiogenesis (e.g., vascular endothelial growth factor [VEGF]), metastasis (e.g., EGFR and c-MET), tumor cell metabolism (e.g., glucose transporter [GLUT1]), and pH (carbonic anhydrase IX [CA9]). These molecular changes during hypoxic conditions may aid tumor cell adaptation to hypoxic stress and possibly lead to disease progression (Hong et al., 2013).

Teleost fish are natural model systems for the study of hypoxia because oxygen availability is frequently altered in waters, both spatially and temporally. Life or death for fish depends on their ability to adapt to rapidly changing levels of oxygen in the aquatic environment. Indeed, much of the diversity of fishes can be attributed to the adoption of specialized anatomic, behavioral, and physiological strategies to compensate for particular aquatic oxygen conditions (Powell and Hahn, 2002; Nikinmaa et al., 2004; Geng et al., 2014). Channel catfish, *Ictalurus punctatus*, is highly tolerant to low oxygen. However, under intense aquaculture conditions, hypoxia frequently causes heavy mortalities. In addition, hypoxic stress often triggers major disease breakouts, such as enteric septicemia of catfish (ESC) disease, the most serious catfish disease that is caused by a bacterial pathogen, *Edwardsiella ictaluri*. As such, channel catfish have been extensively used for studies of abiotic stresses such as hypoxia (Feng et al., 2013; Geng et al., 2014), heat stress (Liu et al., 2013; Feng et al., 2014), and immune responses to bacterial infections (Li et al., 2012; Sun et al., 2012; Zhang et al., 2012, 2013). In this study, we sought to identify and characterize the RTK genes in the channel catfish genome, and determine their expression profiles after ESC disease infection and hypoxic stress.

2. Materials and methods

2.1. Identification and analysis of RTK genes

RTK genes were identified from the channel catfish genome by screening an RNA-Seq database (Liu et al., 2012) and whole genome sequence assembly of the channel catfish (unpublished data). The reported RTKs of various vertebrates including teleost fish (cod, tilapia, zebrafish, fugu, medaka, and stickleback), amphibian, turtle, chicken, mouse and human were collected and used as query sequences to search against the RNA-Seq database using local TBLASTN alignment tool with E-value cutoff of 1e − 5. The RNA-Seq database was generated from the transcriptome assembly of expressed short reads of a doubled haploid channel catfish, providing highly accurate assembled transcript sequences (Liu et al., 2012). The initial pool of transcript sequences of RTK genes was first obtained with E-value cutoff of 1e − 5 to include all potential homologous sequences in channel catfish, and then verified by aligning with the whole genome sequence assembly using BLASTN with E-value cutoff of 1e − 10. ORF (open reading frame) finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) was used to predict coding sequences from transcript sequences. FGENESH program (Solovyev et al., 2006) was used to predict coding sequences from the genome assembly. The predicted amino acid sequences were confirmed by BLASTP against NCBI non-redundant protein sequence database with E-value cutoff of 1e − 10. The theoretical isoelectric point (pI) values of RTK proteins were predicted using ExpAsy online tools (http://expasy.org/tools/). The simple modular architecture research tool (SMART, Letunic et al., 2012) was used to identify conserved domains based on sequence homology. Protein domains were further confirmed by conserved domain prediction from BLAST.

2.2. Sequence alignment and phylogenetic analysis

Phylogenetic analyses were conducted using the amino acid sequences of RTK genes identified from channel catfish, together with sequences from other species (including zebrafish, cod, tilapia, stickleback, chicken and human) retrieved from the Ensembl genome database (Release 68). Multiple protein sequence alignments were performed by ClustalW (Thompson et al., 2002). The phylogenetic trees were constructed using the maximum likelihood method with MEGA 5.2.1 (Tamura et al., 2011). Bootstrap testing was performed with 1000 resampling events to provide statistical support of the phylogenetic tree.

2.3. Conserved synteny analysis

To identify conserved syntenic blocks, genes upstream and downstream of RTK genes were identified. Ensembl Compara Database (Flicek et al., 2013) and Genomicus (Louis et al., 2013) were utilized

Table 1
Identification of RTK genes in the channel catfish genome.

Gene name	CDS (bp)	Deduced protein		CDS status	Accession number
		Length (aa)	pI		
EPHA2	2937	978	6.00	Complete	JT339582
EPHA2a	2958	985	6.37	Complete	JT340542
EPHA3	2916	971	6.97	Partial	JT414026
EPHA4	2970	989	6.84	Complete	JT409635
EPHA4a	2961	986	6.25	Complete	JT474057
EPHA4b	2937	978	6.16	Complete	JT415792
EPHA5	2472	823	7.92	Partial	JT343803
EPHB1	3048	1015	6.27	Complete	JT410849
EPHB2a	1877	625	6.06	Partial	KP126799
EPHB2b	2961	986	5.43	Complete	JT408020
EPHB3a	1452	483	5.63	Complete	JT400475
EPHB3	2946	981	5.74	Complete	JT477737
EPHB4b	2946	981	6.77	Complete	JT416389
EPHB4a	2982	993	7.47	Complete	JT410332
TIE2	3432	1143	7.38	Complete	JT406054
TIE1	2772	923	8.45	Partial	JT464126
INSRa	4026	1341	6.3	Complete	JT478824
INSRb	4029	1342	6.18	Complete	JT407578
IGF1RB	4074	1357	5.89	Complete	JT410602
IGF1RA	4149	1382	5.65	Complete	JT410011
EGFR	3600	1199	6.36	Complete	JT406164
ErbB3b	4257	1418	6.10	Complete	JT414000
ErbB3a	4335	1444	6.17	Complete	JT407415
ErbB4	2202	733	7.68	Partial	JT402114
ErbB4b	1857	618	6.71	Partial	JT268945
ErbB4a	1832	610	6.52	Partial	KP126798