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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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A B S T R A C T

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.

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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

to determine the conserved syntenic pattern in RTK gene loci among human, chicken, zebrafish, tilapia and channel catfish. The upstream and downstream genes surrounding the putative RTK genes were identified from the channel catfish scaffolds by FGENESH program (Solovyev et al., 2006). BLASTP was used to annotate these neighboring genes by searching against NCBI nr database.

2.4. Expression analysis after bacterial infection and hypoxic stress

Expression profiles of RTK genes in response to bacterial infection and hypoxic stress were determined using currently available RNA-Seq datasets. These included RNA-Seq data generated from the intestines collected at 3 h, 24 h and 3 days after *E. ictaluri* infection (Li et al., 2012), and the RNA-Seq data generated from gill tissues of catfish that were tolerant or intolerant to hypoxic stress (Feng et al., 2013). RNA-Seq analyses were conducted using CLC Genomics Workbench package. The RNA-Seq short reads were first mapped with the reference transcript sequences (Liu et al., 2012) including RTKs with at least 95% of the reads being aligned with the reference sequence and a maximum of two mismatches. The total number of mapped reads for each transcript was determined, and then normalized to calculate RPKM (Reads Per Kilobase of exon model per Million mapped reads). The proportion-based Kal's tests were conducted to identify differentially expressed genes between control and treatment groups with a statistical significance of P-value < 0.05 after false discovery rate (FDR) adjustment. Transcripts with total number of mapped reads greater than 5 and absolute fold change values greater than 1.5 (FDR adjusted P-value < 0.05) were determined as significant differentially expressed genes.

3. Results

3.1. Identification and analysis of RTK genes in the channel catfish genome

A total of 26 RTK genes were identified in the channel catfish genome. Characteristics of the RTK transcripts, including lengths of open reading frames, theoretical isoelectric points, completeness of the coding sequences, and their GenBank accession numbers, were provided in Table 1.

The 26 RTK genes were grouped into four subfamilies, EPH, TIE, INSR, and ErbB, based on phylogenetic relationships (Fig. 1). Fourteen genes belonging to the EPH subfamily were identified including EPHB1, EPHB2a, EPHB2b, EPHB3a, EPHB3, EPHB4b, EPHB4a, EPHA2a, EPHA2, EPHA3, EPHA4b, EPHA4a, EPHA4 and EPHA5. Of these, full-length coding sequences were obtained for 11 of the 14 catfish EPH genes except for EPHA3, EPHA5, and EPHB2a (Table 1). Two genes belonging to the TIE subfamily (TIE1 and TIE2) were identified in the channel catfish genome. Full-length coding sequence was obtained for TIE2, and partial coding sequence was obtained for TIE1 (Table 1). Six genes of the ErbB subfamily were identified in the channel catfish genome including EGFR, ErbB3a, ErbB3b, ErbB4, ErbB4a and ErbB4b. Full-length coding sequences were obtained for three of the six catfish ErbB genes including EGFR, ErbB3a, and ErbB3b (Table 1). Four INSR genes were identified including IGF1Ra, IGF1Rb, INSRa, and INSRb. Full-length coding sequences were obtained for all four INSR genes (Table 1).

3.2. Phylogenetic analysis of RTK genes

Phylogenetic analysis of RTK genes conducted separately within each of the four subfamilies supported the gene annotation. For

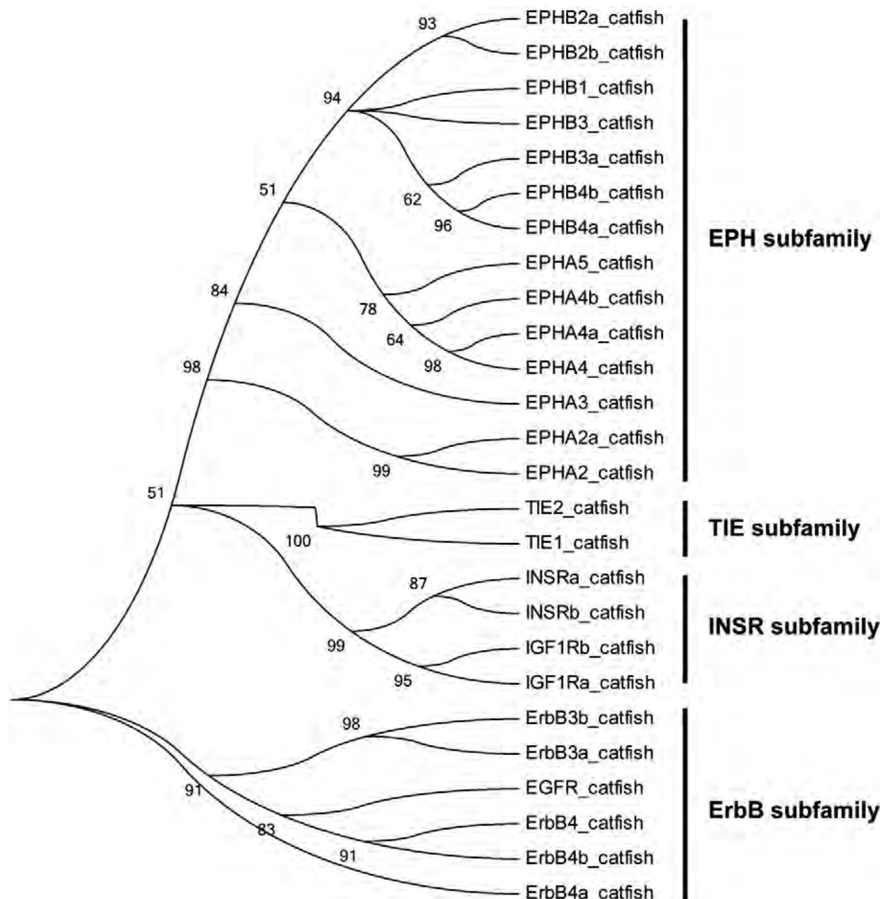

Fig. 1. Phylogenetic analysis of catfish RTK genes. The 26 genes were divided into four subfamilies based on phylogenetic relationships.

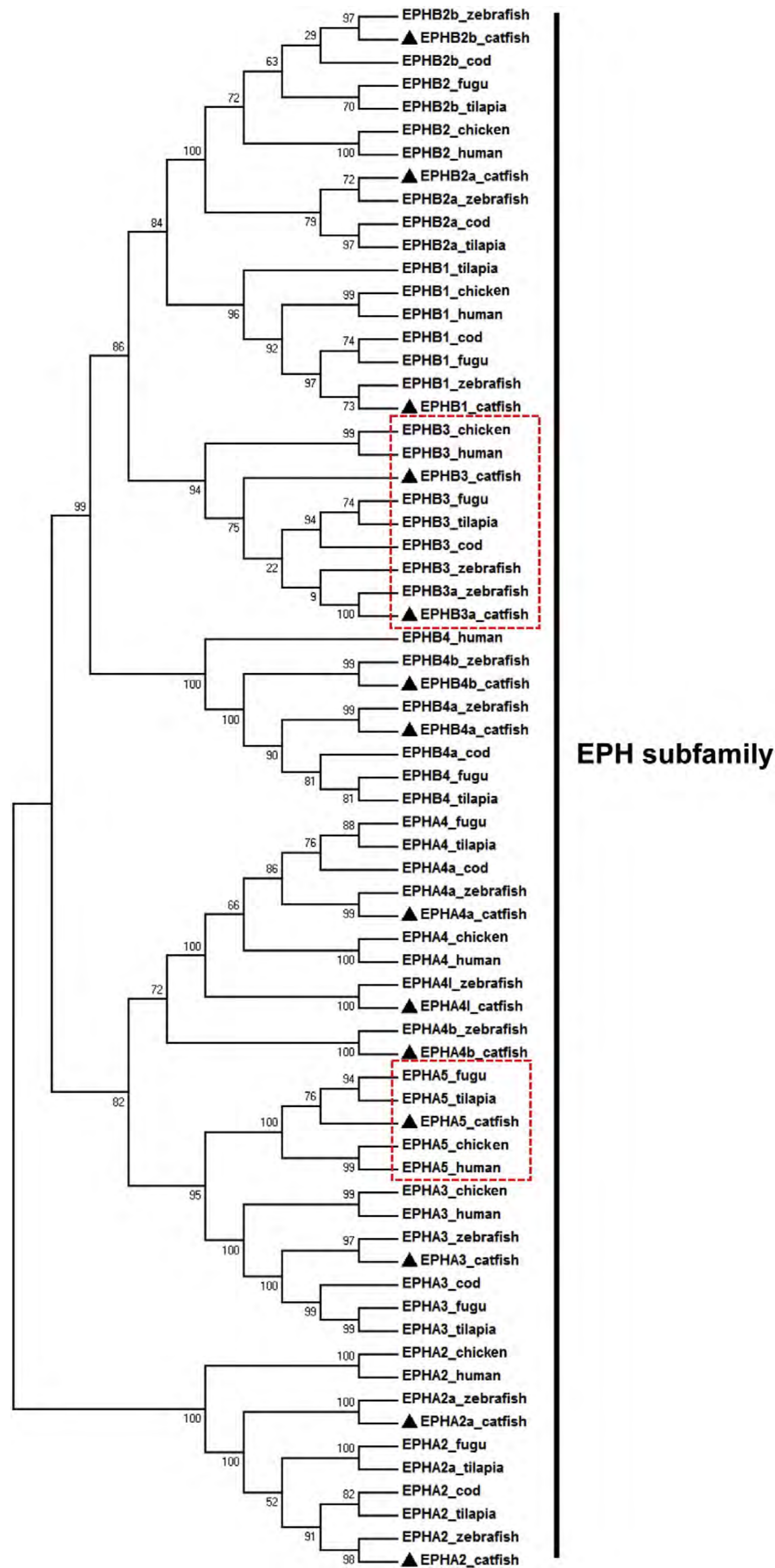

Fig. 2. Phylogenetic analysis of EPH subfamily genes. Multiple amino acid sequences were aligned using ClustalW. The phylogenetic tree was constructed using the maximum likelihood method in MEGA 5.2. The statistical robustness of the tree was estimated by bootstrapping with 1000 replicates. Bootstrap values were indicated at the nodes.

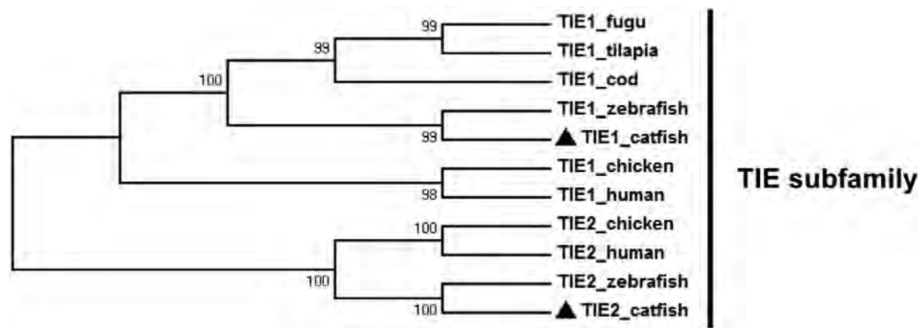

Fig. 3. Phylogenetic analysis of TIE subfamily genes. Multiple amino acid sequences were aligned using ClustalW. The phylogenetic tree was constructed using the maximum likelihood method in MEGA 5.2. The statistical robustness of the tree was estimated by bootstrapping with 1000 replicates. Bootstrap values were indicated at the nodes.

subfamily EPH, phylogenetic analysis provided strong evidence for annotation of 11 of the 14 genes (Fig. 2). All EPH genes, with the exception of EPHB3, EPHB3a, and EPHA5, fell into clades that were consistent with the phylogenetic relationships of the organisms being investigated, indicating that the identities of the EPH genes were properly determined. For EPHB3, EPHB3a, and EPHA5, phylogenetic analysis alone was not sufficient to provide strong support for their annotation, and therefore, syntenic analysis was conducted for these genes (see below).

For TIE subfamily genes, phylogenetic analysis allowed proper annotation of TIE subfamily (Fig. 3), with strong bootstrap support. Similarly, phylogenetic analysis allowed proper annotation of INSR subfamily (Fig. 4), with strong bootstrap support. For ErbB subfamily genes, phylogenetic analysis allowed annotation of ErbB3a, ErbB3b, ErbB4, ErbB4a, ErbB4b, and EGFR. However, the relationship of ErbB4, ErbB4a and ErbB4b, could not be resolved based on phylogenetic analysis alone (Fig. 5).

3.3. Syntenic analysis of channel catfish RTK genes

Syntenic analysis conducted for EPHA5, EPHB3, EPHB3a, ErbB4, ErbB4a and ErbB4b provided additional evidence of orthologies to support gene annotation. As shown in Fig. 6(A–C), conserved syntenies were observed for all these genes, with well-conserved neighboring genes as compared with those of zebrafish, chicken and human, suggesting their orthologies. For instance, the cluster of genes (tctex1d2–nmur1–hykk–EPHB3–rnf13) on the zebrafish chromosome 22 were highly conserved with that of the channel catfish (Fig. 6B).

3.4. Conserved motifs of RTK genes

RTK genes are characteristic of their molecular architectures, with ligand-binding domains in the extracellular region, a single transmembrane helix, and a cytoplasmic region that contains the protein tyrosine kinase (TK) domain, plus additional carboxy (C-) terminal TyrKc and juxtamembrane regulatory region. Based on sequence homology by searching against SMART and PFAM databases, conserved domains were identified in all RTKs except for ErbB4, ErbB4a and ErbB4b with partial coding sequences (Fig. 7).

The EPH subfamily proteins possess unique functional domains, including an ephrin receptor ligand binding domain (EPH_lbd), two fibronectin type III (FN3) domains and a sterile alpha motif (SAM) (Fig. 7A). The ligand-binding domain of Eph receptors is unique to this family of RTKs and shares no significant amino-acid sequence homology with other known proteins. The conserved 180-amino-acid N-terminal ligand-binding domain is both necessary and sufficient for bindings of the receptors to their ephrin ligands.

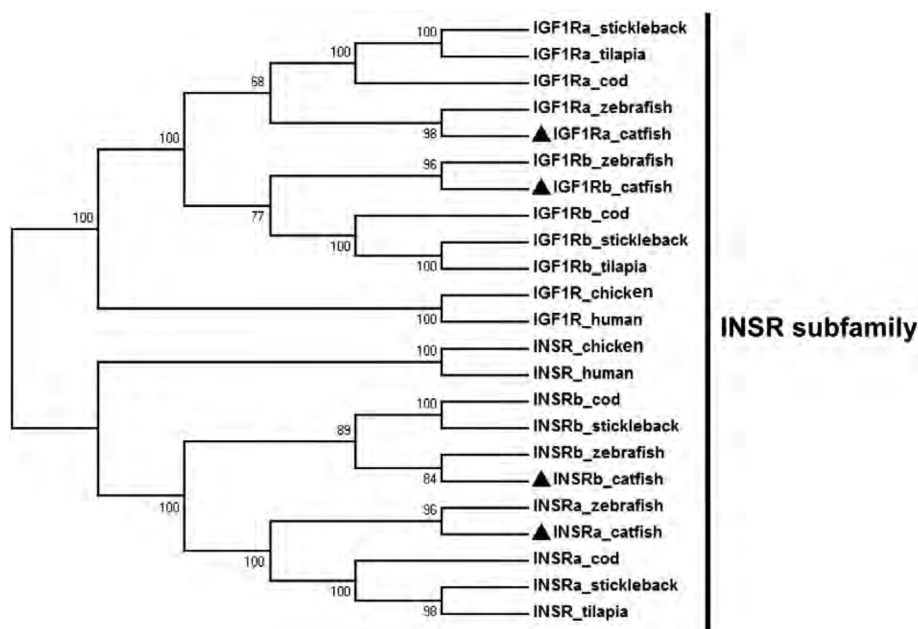

Fig. 4. Phylogenetic analysis of INSR subfamily genes. Multiple amino acid sequences were aligned using ClustalW. The phylogenetic tree was constructed using the maximum likelihood method in MEGA 5.2. The statistical robustness of the tree was estimated by bootstrapping with 1000 replicates. Bootstrap values were indicated at the nodes.

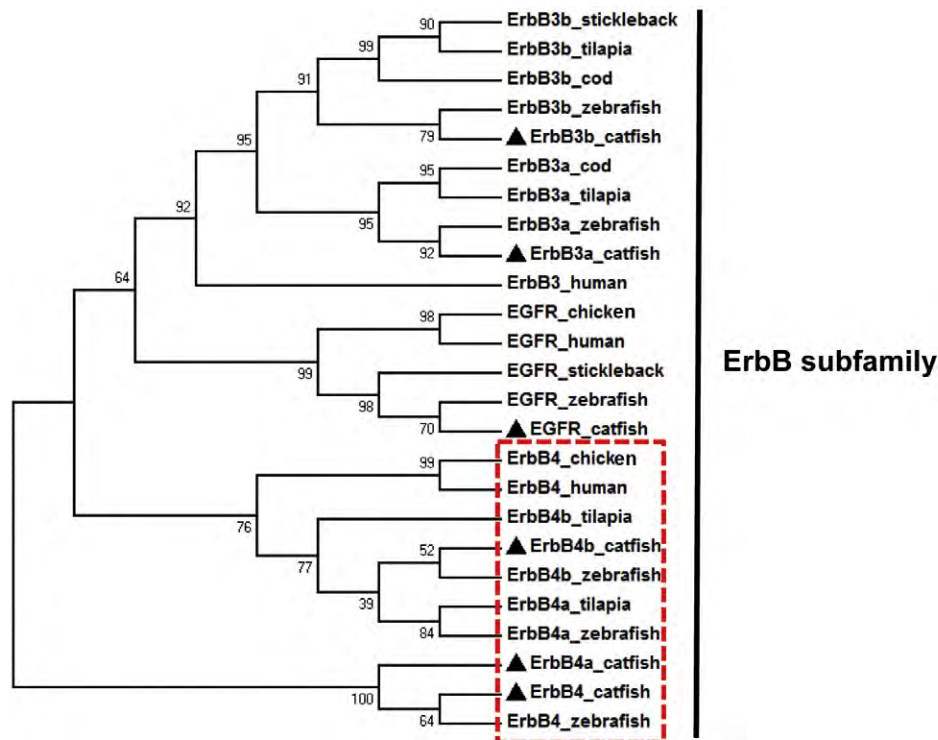

Fig. 5. Phylogenetic analysis of ErbB subfamily genes. Multiple amino acid sequences were aligned using ClustalW. The phylogenetic tree was constructed using the maximum likelihood method in MEGA 5.2. The statistical robustness of the tree was estimated by bootstrapping with 1000 replicates. Bootstrap values were indicated at the nodes.

The TIE subfamily proteins have common EGF, immunoglobulin (IG) and FN3 domains, but TIE2 possesses special functional domains, the TIE-2 Ig-like domain (Ig_Tie2_1) and ligands of the Delta/Serrate/Lag-2 (DSL) family (Fig. 7B). The angiopoietin-binding site is harbored by the N-terminal two immunoglobulin-like (Ig-like) domains of TIE2. The INSR subfamily proteins possess several furin-like repeats (FU) domains and fibronectin type III (FN3) (Fig. 7C). These domains facilitate interaction with the activated tyrosine-phosphorylated insulin receptor. The ErbB subfamily proteins possess several furin-like repeats (FU) domains and leucine-rich repeats (LRR) (Fig. 7D). The furin-like cysteine rich region is involved in signal transduction by receptor tyrosine kinases, which involves receptor aggregation. The leucine-rich repeats consist of 2–45 motifs of 20–30 amino acids in length that generally fold into an arc or horseshoe shape.

3.5. Expression analysis after bacterial infection and hypoxic stress

The expression profiles of all 26 RTK genes after bacterial infection with *E. ictaluri* indicated that mRNA abundance levels of eight RTK genes were significantly altered (|fold change| > 1.5 and *P*-value < 0.05), while mRNA abundance of the remaining 18 genes did not significantly change (Table 2). The regulation of gene expression after acute bacterial infection was reflected in the levels of mRNA abundance alteration. Specifically, five RTK genes were up-regulated after ESC infection. These included INSRb, IGF1Ra, IGF1Rb, EPHA5 and TIE1. The levels of up-regulation varied among genes, with the most highly induced gene being INSRb (3.16 fold to 3.83 fold depending on the timing after infection, Table 2), followed by IGF1Ra (2.49 fold to 3.27 fold), IGF1Rb (2.25 fold to 3.35 fold), EPHA5 (1.74 fold to 2.21 fold) and TIE1 (1.69 fold). Three genes were down-regulated, including EPHB4b, EPHA2a, and EPHB4a (Table 2). The levels of down-regulation varied among genes, with the most highly down-regulated gene being EPHA2a (1.79 fold to 1.93 fold depending on the timing after infection, Table 2), followed by EPHB4b (1.57 fold), and EPHB4a (1.54 fold).

Comparison of mRNA abundance of the 26 RTK genes between catfish that were tolerant and intolerant to hypoxic stress indicated that several RTKs were involved in oxygen regulation. As summarized in Table 3, significant differences in mRNA abundance were observed in eight genes between the tolerant and intolerant catfish upon hypoxic stress. Specifically, EPHB3a, EGFR, ErbB4b, and IGF1Rb were expressed at higher levels in the tolerant catfish than in intolerant catfish, while INSRa, EPHA2, EPHA2a, and TIE1 were expressed at lower levels in tolerant catfish than in intolerant catfish (Table 3).

4. Discussion

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 tumor progression during hypoxic conditions. A group of RTKs are oncogenes and candidate proto-oncogenes that have been extensively studied from various organisms (Drakenberg et al., 1993; Gutierrez et al., 1993, 1995; Parrizas et al., 1995; Chan et al., 1997; Lyons et al., 1998; Elies et al., 1999; Greene and Chen, 1999; Maures et al., 2002; Wang and Ge, 2004). In the present study, we identified, annotated, and characterized a set of 26 RTK oncogenes in the channel catfish genome, including fourteen EPHs, two TIEs, four INSRs, and six ErbBs. Phylogenetic analysis allowed grouping of all identified RTKs into four subfamilies, and supported annotation of 20 of the 26 genes. The orthologies of EPHA5, EPHB3, EPHB3a, ErbB4, ErbB4a and ErbB4b could not be established by phylogenetic analysis alone. Therefore, conserved synteny analysis was conducted for these genes. Combining phylogenetic analysis and syntenic analysis allowed for correct annotation of all 26 RTK genes in channel catfish.

Duplications of RTK genes were observed in the channel catfish genome. For instance, three copies of ErbB4 genes were identified in channel catfish. This was also observed in other teleost fish. In zebrafish, three copies of ErbB4 genes were present on different chromosomes with ErbB4a on chromosome 1, and ErbB4b and ErbB4 on chromosome 9, suggesting that the duplication of RTKs could be derived from the teleost-specific whole genome duplication, then followed by lineage-specific tandem duplication and gene loss (Rytkonen et al., 2013). The

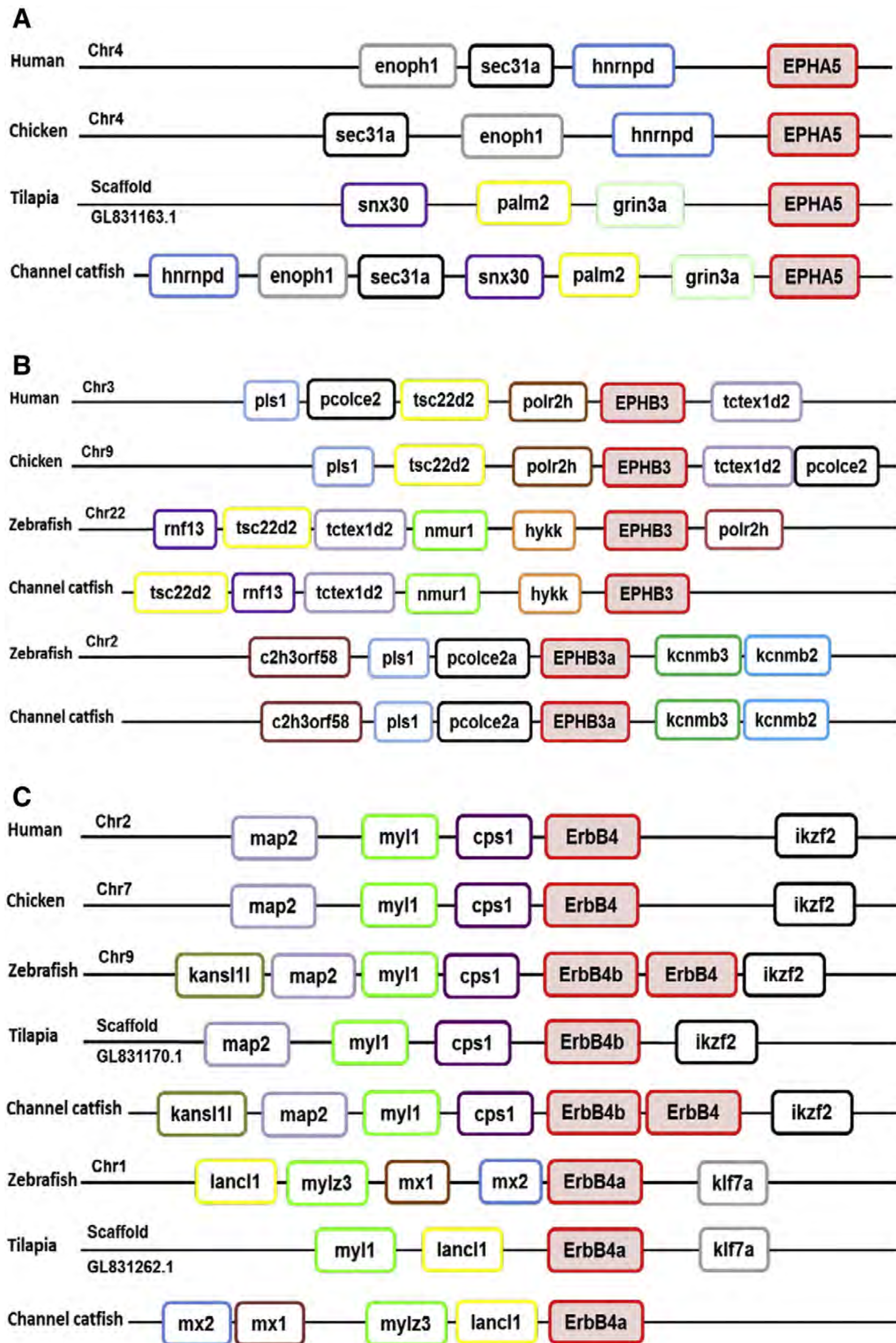

Fig. 6. Conserved synteny analysis of partial channel catfish EPH subfamily genes with corresponding homologous genes that were retrieved from zebrafish, tilapia, chicken, and human genomes. A) EPHA5, B) EPHB3, C) rbB4.

syntenic analysis indicated that three channel catfish ErbB4 genes showed a high level of orthology with zebrafish ErbB4 genes (Fig. 6C). Based on this orthology relationship, the three channel catfish ErbB4 genes were annotated as ErbB4, ErbB4a and ErbB4b following the zebrafish gene nomenclature, respectively.

The RTKs are a large and important group of receptors in signal transduction (Schlessinger, 2000), and play conserved and critical biological roles in normal cellular processes as well as pathogenesis. The expression analysis of RTK oncogenes in catfish after *E. ictaluri* infection showed that five genes were greatly up-regulated after

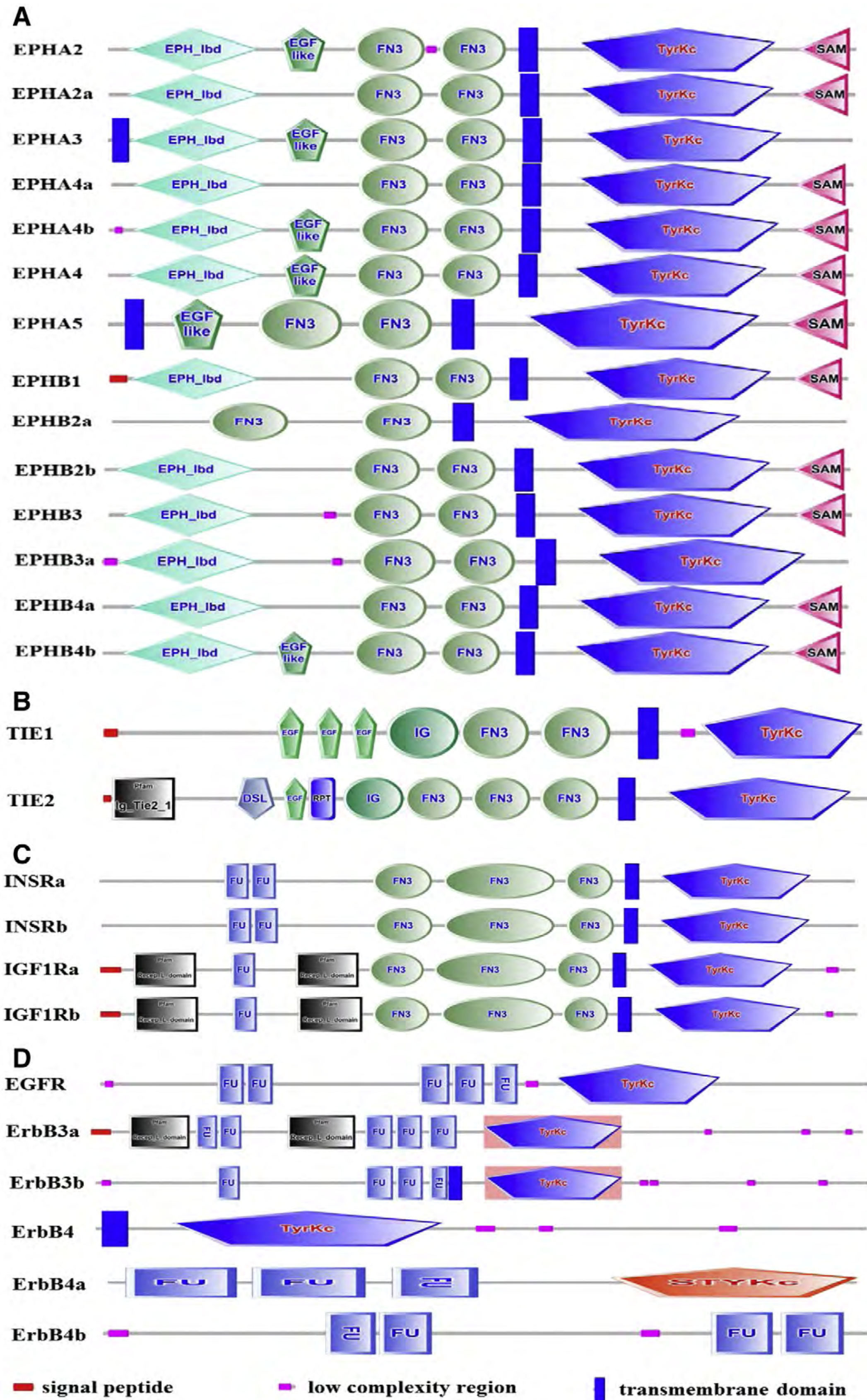

Fig. 7. Schematic presentation of domain architectures of the channel catfish RTK genes. A) EPH, B) TIE, C) INSR, and D) ErbB subfamily genes.

Table 2
Expression analysis of RTK genes in the intestine of catfish after *E. ictaluri* infection. The expressions were determined at three time-points: 3 h, 24 h and 3 days after bacterial infection. Asterisks indicated the time-points where the expression of the gene was significantly up- or down-regulated compared to the control (P-value < 0.05).

Gene name	3 h	24 h	3 days
INSRb	3.62*	3.16*	3.83*
IGF1Ra	3.27*	2.49*	3.10*
IGF1Rb	2.76*	3.35*	2.25*
EPHA5	2.21*	–	1.74*
TIE1	–	1.69*	–
EPHA2a	–	−1.93*	−1.79*
EPHB4b	–	−1.57*	–
EPHB4a	–	–	−1.54*

infection (Table 2). Notably, insulin receptor genes were highly induced, consistent with the observation in a previous study (Bilodeau et al., 2006), indicating their roles in immune response. TIE1 was also up-regulated, which may suggest the involvement of TIE1 in developmental and pathological angiogenesis. The TIE receptors are key players governing the generation of blood and lymphatic vessels because the growth of new blood and lymphatic vessels is a central element in many diseases (Jeltsch et al., 2013). The EPH receptors are involved in determining arterial versus venous identity (Adams and Eichmann, 2010). In this study, four EPH genes, EPHA5, EPHB4b, EPHA2a, and EPHB4a, were significantly regulated, indicating the important roles of the EPH subfamily in the immune response. Of these, EPHA5 was up-regulated, while the rest of three genes were down-regulated, suggesting their distinct roles in the same biological process. Despite the progress that has been made to understand the EPH/ephrin function in early developmental stages, further studies need to be conducted to understand the mechanisms and signaling processes in other physiological conductions.

The RTKs are also actively regulated during hypoxic stress, and involved in the progression of many types of tumors. Hypoxia has been well-linked to pathogenesis in humans including inflammatory diseases (Taylor and Sivakumar, 2005), cancers (Kondo et al., 2005; Phillips et al., 2005), and heart diseases (Covello and Simon, 2004). In this study, eight genes were differentially expressed between the catfish that are tolerant and intolerant to hypoxic stress. The IGF1Rb, EGFR, ErbB4b, and EPHB3a were expressed at higher levels in the tolerant catfish than in intolerant catfish (Table 3), indicating that these genes could contribute to hypoxia tolerance. IGF1R has been found to be required for cell transformation during hypoxia (Baserga, 1995), while the EGF receptor stimulation responses were found to be mediated via different signaling pathways during hypoxic conditions (Chen et al., 1994). The TIE1 was expressed at a lower level in tolerant catfish than in intolerant catfish. TIE genes are in an endothelial-specific RTK pathway that is involved in blood and lymphatic vessel development. The TIE receptors play context-dependent roles in the stabilization and remodeling of blood and lymphatic vessels (Jeltsch et al., 2013). These indicated that TIE1 was potentially involved in the hypoxia-sensing transcriptional system and played important roles in the angiogenesis machinery. The EPH subfamily genes showed different expression profiles in this study, indicating that EPH subfamily genes were involved in a complicated signaling network in response to hypoxic stress. Further investigation is required for functional analysis of the RTK genes in the catfish, which will be helpful to understand the molecular mechanism of disease progression and assist in developing proper prevention and treatment strategies for catfish diseases and hypoxic stress.

Table 3
Expression analysis of RTK genes in the gill of channel catfish after hypoxic stress. The gene expression was determined in the group of fish tolerant to hypoxia and intolerant to hypoxia, respectively. Asterisks indicated that the expression of genes was significantly up- or down-regulated in tolerant fish relative to the intolerant fish (P-value < 0.05). Minus indicated genes that were expressed at lower levels in tolerant fish than in intolerant fish.

Gene name	Tolerant vs intolerant
EPHB3a	2.20*
EGFR	1.87*
ErbB4b	1.80*
IGF1RB	1.67*
INSRa	−1.54*
EPHA2	−2.43*
TIE1	−2.43*
EPHA2a	−2.93*

5. Conclusions

In this study, we identified and annotated 26 RTK oncogenes in the channel catfish genome. Phylogenetic and syntenic analyses were conducted to clearly establish their orthologies, supporting their gene annotation. The 26 RTKs possessed similar molecular architectures in general, but each subfamily had their specific functional domains, indicating specific roles that they could play. The determination of expression profiles of RTKs in response to bacterial infection and hypoxic stress revealed their involvement in immune response and adaptation to low levels of oxygen environment. Further studies on functional analysis of RTKs in catfish warrant understanding of the molecular mechanisms underlying disease pathogenesis and hypoxia tolerance, providing proper strategies of prevention and treatment to control infectious diseases and hypoxia stress in aquaculture.

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