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Transcriptomic signatures of attachment, NF-κB suppression and IFN stimulation in the catfish gill following columnaris bacterial infection

Fanyue Sun [1], Eric Peatman [*,1], Chao Li, Shikai Liu, Yanliang Jiang, Zunchun Zhou, Zhanjiang Liu

The Fish Molecular Genetics and Biotechnology Laboratory, Department of Fisheries and Allied Aquacultures and Program of Cell and Molecular Biosciences, Aquatic Genomics Unit, Auburn University, Auburn, AL 36849, USA

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ABSTRACT

Outbreaks of columnaris disease (*Flavobacterium columnare*) are common in wild and cultured freshwater fish worldwide. Disease occurrences, particularly those caused by virulent genomovar II isolates, in aquaculture species such as channel catfish can be devastating. In contrast to other important aquaculture pathogens, little is known about host immune responses to columnaris. Adhesion of *F. columnare* to gill tissue has been correlated in some previous studies to virulence and host susceptibility. Here, therefore, we conducted the first transcriptomic profiling of host responses to columnaris following an experimental challenge. We utilized Illumina-based RNA-seq expression profiling to examine transcript profiles at three timepoints (4 h, 24 h, and 48 h) in catfish gill after bath immersion infection. Enrichment and pathway analyses of the differentially expressed genes revealed several central signatures following infection. These included the dramatic upregulation of a rhamnose-binding lectin, with putative roles in bacterial attachment and aggregation, suppression of NF-κB signalling via IκBs, BCL-3, TAX1BP1, and olfactomedin 4, and strong induction of IFN-inducible responses including iNOS2b, IFI44, and VHSV genes. Fifteen differentially expressed genes with varying expression profiles by RNA-seq, were validated by QPCR (correlation coefficients 0.85–0.94, p-value <0.001). Our results highlight several putative immune pathways and individual candidate genes deserving of further investigation in the context of development of therapeutic regimens and laying the foundation for selection of resistant catfish lines against columnaris.

1. Introduction

Mucosal epithelial surfaces act as dynamic interfaces between the external environment and the internal milieu. While mediating physiological functions such as nutrient and oxygen absorption and waste secretion, the epithelial barrier and associated immune actors in the periphery are responsible for sensing, sampling and screening pathogens while maintaining homeostasis in the presence of common and/or commensal microbes and foreign matter (Cerutti et al., 2011). The mucosal barriers of fish (gills, skin and intestine) are considered to be critical points of pathogen entry (Shephard, 1994; Dickerson and Clark, 1998). Early studies on mucosal immune responses have been conducted in several fish species including rainbow trout (Swan et al., 2008; Komatsu et al., 2009; Perez-Sanchez et al., 2011; Sheikhzadeh et al., 2012), Atlantic salmon (Niklasson et al., 2011), Atlantic cod (Caipang et al., 2010), and carp (Ryo et al., 2010). While most studies have been limited to examining *a priori* gene targets, a few have utilized microarray technology to capture broader transcriptional profiles.

Catfish (*Ictalurus* spp.) is the dominant aquaculture species in the United States accounting for more than 80% of US aquaculture production, in spite of a recent downward trend in production (Hanson and Sites, 2012). Production efficiency continues to be hindered by disease losses to several bacterial pathogens including *Edwardsiella ictaluri*, *Edwardsiella tarda*, *Aeromonas hydrophila*, and *F. columnare*. Of these, *E. ictaluri* has been the most thoroughly characterized with studies examining both pathogen (e.g. Rogge and Thune, 2011; Williams et al., 2012) and host responses (Baoprasertkul et al., 2004, 2006; Bao et al., 2005; Chen et al., 2005; Peatman et al., 2006; Sha et al., 2008, 2009; Takano et al., 2008; Liu et al., 2010a,b, 2011; Rajendran et al., 2011; Zhang et al., 2012). Recently, we utilized RNA-seq technology to capture the intestinal mucosal transcriptional profiles of channel catfish following infection with *E. ictaluri* (Li et al., 2012). Rather than relying on hybridization to capture transcripts of interest, RNA-seq utilizes the throughput capacity of next-generation sequencing to sequence transcripts and quantify expression levels. Where a reference genome exists, these transcripts can be mapped back to the genome. In species such as catfish, where a reference genome is not available, a reference transcriptome can be generated *de novo*, followed by mapping of individual reads and transcript read quantification. Due to rapidly declining sequencing prices, the ability of

* Corresponding author. Tel.: +1 334 844 9319; fax: +1 334 844 4694.
E-mail address: peatmer@auburn.edu (E. Peatman).
[1] Both these authors contributed equally to the manuscript.

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RNA-seq to capture rare transcripts and splicing variants, and developed algorithms for assessing gene expression levels, use of RNA-seq for transcriptome profiling is increasing (Nagalakshmi et al., 2010; Malone and Oliver, 2011).

In comparison with *E. ictaluri*, our knowledge of columnaris, caused by *F. columnare*, is limited. *F. columnare* is a Gram-negative bacterium implicated in disease outbreaks in freshwater fish species throughout the world (Decostere et al., 1999a). In the catfish industry, columnaris is the most frequently occurring disease in catfish (MSU, 2010), with in-pond mortality reported from both juvenile and adult foodfish. The disease often begins as a local infection impacting the skin, fins, and gills and can proceed rapidly to cause significant gill necrosis, fin erosion, and saddleback skin lesions within days after infection. While research has been conducted on the modes and dynamics of columnaris adhesion (Decostere et al., 1999a; Olivares-Fuster et al., 2011) and the virulence of different genomic groups or genomovars (Shoemaker et al., 2008), almost nothing is known about host responses to columnaris infection (Pridgeon and Klesius, 2010; Niu et al., 2011). Understanding these responses can highlight mechanisms of pathogen entry and immune evasion and suggest strategies and targets for vaccine development and selection of resistant strains and lines of catfish. To begin to understand catfish responses to columnaris at the transcriptomic level, here we examined the transcriptional profiles of the catfish gill at three timepoints following experimental infection. Utilizing high-througput RNA-seq technology, we identified 2,605 differentially expressed genes with critical roles in pathogen recognition, cytoskeletal dynamics, cell junction integrity, oxidative stress responses, apoptosis, lysosomal processes, and pro- and anti-inflammatory pathways.

2. Materials and methods

2.1. Experimental fish, bacteria challenge and sample collection

All procedures involving the handling and treatment of fish used during this study were approved by the Auburn University Institutional Animal Care and Use Committee (AU-IACUC) prior to initiation. One-year-old channel catfish of both sexes, weighing 48.6 ± 2.5 g, were reared at the hatchery of the Auburn University Fish Genetics Research Unit at 25 °C. All fish were maintained in the laboratory for at least 2 weeks prior to experimental use. Fish were randomly divided into 6 rectangular 30 L aquaria of which 3 aquaria were designated control (4 h, 24 h and 48 h) and the other 3 were designated challenge groups (4 h, 24 h and 48 h).

The bacteria *F. columnare* were provided by the Aquatic Microbiology Laboratory, Auburn University. Several fish were experimentally infected with a virulent isolate (BGFS-27; genomovar II; Olivares-Fuster et al., 2011) and bacteria were re-isolated from a single symptomatic fish and confirmed visually and biochemically to be *F. columnare*. A single colony was inoculated into modified Shieh broth and grown for 24 h in a shaker incubator (100 rpm) at 28 °C. Challenge experiments were then conducted by immersion exposure for 2 h at a final concentration 3×10^6 CFU/mL. After the challenge, fish were incubated in aquaria with flow through water turned on. Control fish were treated with identical procedures except that they were not exposed to the bacteria, but exposed to sterile modified Shieh broth.

Gill tissues were collected at 4 h, 24 h, and 48 h timepoints post challenge. At each time point, 18 fish from both control and treatment were randomly selected and divided into 3 replicate pools (6 fish each) respectively. The fish were euthanized with tricaine methanesulfonate (MS 222) at 300 mg/L (buffered with sodium bicarbonate) before tissues were collected. Gill tissues in the 3 replicates were placed into 5 mL RNA later™ (Ambion, Austin, TX, USA). After 1 day of temporary storage at 4 °C, samples immersed in the RNA later™ were transferred to a −80 °C ultra-low freezer until preparation of RNA. During the challenge, symptomatic treatment fish and control fish were collected and confirmed to be infected with *F. columnare* and pathogen-free, respectively, at the Fish Disease Diagnostic Laboratory, Auburn University.

2.2. RNA isolation, library construction and Illumina sequencing

Prior to RNA extraction, samples were removed from the −80 °C freezer and ground with sterilized mortar and pestle in the presence of liquid nitrogen to a fine powder. Total RNA was extracted from tissue powder using the RNeasy Plus Kit (Qiagen) following manufacturer's instructions and treated with RNase-free DNase I (Qiagen) to remove genomic DNA. RNA concentration and integrity of each sample was measured on an Agilent 2100 Bioanalyzer using a RNA Nano Bioanalysis chip. For each timepoint, equal amounts of RNA from the three treatment replicates were pooled for RNA-seq library construction. For the control samples, the replicate pools spanned each of the three timepoints (4 h, 24 h, and 48 h). A master pool composed of equal amounts of each replicate control pool was formed for use in RNA-seq.

RNA-seq library preparation and sequencing was carried out by HudsonAlpha Genomic Services Lab (Huntsville, AL, USA) as previously described by Li et al. (2012). cDNA libraries were prepared with ~2.5 μg of starting total RNA following the protocols of the Illumina TruSeq RNA Sample Preparation Kit (Illumina). The libraries were amplified with 15 cycles of PCR and contained TruSeq indexes within the adaptors, specifically indexes 1–4. The final libraries had an average fragment size of ~270 bp and final yields of ~400 ng. After KAPA quantitation and dilution, the libraries were clustered 4 per lane and sequenced on an Illumina HiSeq 2000 instrument with 100 bp paired end (PE) reads.

2.3. De novo assembly

Raw sequencing reads were used for *de novo* assembly after removing adaptor sequences, ambiguous nucleotides ('N' at the end of reads), low quality sequences (quality score less than 20), and short read length sequences (length below 30 bp) with CLC Genomics Workbench (version 4.8; CLC bio, Aarhus, Denmark). The remaining high-quality reads were used in the following assembly. The assembly was performed using the *de Brujin* graph approach with ABySS (version 1.2.6) (Simpson et al., 2009) and Trans-ABySS version 1.2.0 for *de novo* assembly to obtain accurate and reliable consensus contigs as a reference assembly result (Robertson et al., 2010). Briefly, continuous multiple k-mers ranging from 50 to 96 were used in ABySS, and then all 47 assemblies from ABySS were merged into one assembly to generate the transcriptome assembly using Trans-ABySS. Afterwards, CAP3 (Huang and Madan, 1999) was utilized to remove redundancy and resulting contigs that were >200 bp were regarded as final non-redundant transcripts.

2.4. Transcriptome annotation and gene ontology

The assembled contigs were used as queries for BLAST searches against the zebrafish RefSeq protein database, UniProtKB/Swiss-Prot, and the NCBI non-redundant (nr) protein database, respectively. Searches were conducted using the BLASTX program with an *E*-value cut-off of 1e-5 and matching to the top hits. The Gene ontology (GO) analysis was carried out using the BLAST results from zebrafish RefSeq in Blast2GO version 2.5.0 (http://

www.blast2go.org/) and the annotation output was categorized by molecular function, biological process and cellular component, as described in Li et al. (2012).

2.5. Differential expression analysis

Differential expression analysis was performed using the "RNA-seq analysis" and "Expression analysis" modules within CLC Genomics Workbench version 4.8.1 after mapping the sequence reads to the Trans-ABySS reference assembly. Mapping parameters were set to $\geqslant$95% of the bases in perfect alignment and $\leqslant$2 mismatches. The total mapped reads number for each transcript was determined, and then normalized to detect RPKM (Reads Per Kilobase of exon model per Million mapped reads). The proportions-based test was used to identify the differently expressed genes between control and 4 h, 24 h and 48 h with p-value <0.05. The proportions-based test method, which was originally developed for SAGE data, allows an estimation of differential expression based on single measurements of tag/read counts for two conditions (Kal et al., 1999). After quantile normalization of the RPKM values, fold changes were calculated. Transcripts with absolute fold change values of larger than 1.5 and total read number larger than 10 were included in analysis as differently expressed genes. Gene pathway analysis of significantly differentially expressed genes was carried out based on GO annotation and KEGG pathway analysis, as well as on manual literature review.

2.6. GO term enrichment analysis

Statistical analysis for overrepresentation of Gene Ontology (GO) terms in sets of differentially expressed genes from the zebrafish RefSeq result was performed using Ontologizer 2.0 (Bauer et al., 2008) using parent–child method to which a Benjamini–Hochberg correction (Grossmann et al., 2007) for multiple testing was applied. The 'study set' corresponded to the frequency of GO terms in the differentially expressed genes set, while the 'population' corresponded to the whole catfish gill transcriptome.

2.7. Quantitative real-time PCR validation

In order to validate the reliability of the RNA-seq data, quantitative real-time PCR was performed on 15 representative differentially expressed genes. RNAs were transcribed into cDNAs with the iScript™ cDNA Synthesis Kit (Bio-Rad) according to the manufacturer's protocol and the cDNAs were amplified by PCR using specific primers designed based on RNA-seq contig sequences (Supplementary Table 1). Real-time PCR was performed in a total volume of 20 μl, and cycling conditions were 94 °C for 5 s, followed by 40 cycles of 94 °C for 5 s, 60 °C for 5 s, and 65 °C to 95 °C for 5 s following each step. The reactions were performed in biological triplicates on a CFX96 real-time PCR Detection System (Bio-Rad Laboratories, Hercules, CA) using the SsoFast™ EvaGreen® Supermix. The results were expressed relative to the expression levels of 18S rRNA in each sample using the Relative Expression Software Tool version 2009 (Pfaffl et al., 2002). The triplicate fluorescence intensities of the control and treatment products for each gene, as measured by crossing-point (C_t) values, were compared and converted to fold differences by the relative quantification method and assuming 100% efficiencies. Expression differences between control and treatment groups were assessed for statistical significance using a randomization test in the REST software. The mRNA expression levels of all samples were normalized to the levels of 18S ribosomal RNA gene in the same samples. Expression levels of 18S were constant between all samples (<0.30 change in Ct). A no-template control was run on all plates (Li et al., 2012).

3. Results

3.1. Sequencing of short expressed reads from catfish gill

The artificial challenge with virulent *F. columnare* (genomovar II) resulted in mortality beginning around 24 h and becoming widespread by 36 h after exposure. No control fish manifested symptoms of *F. columnare*, and randomly selected control fish were confirmed to be negative for *F. columnare* by standard diagnosis procedures. Dying fish manifested external signs associated with *F. columnare* infection including skin lesions and erosions of fins. *F. columnare* bacteria were successfully isolated from randomly selected treatment fish.

Illumina-based RNA-sequencing (RNA-seq) was carried out on samples from the gills of both control group and fish infected with columnaris (4 h, 24 h and 48 h post-exposure). Reads from time-point-specific samples were distinguished through the use of multiple identifier (MID) tags. One lane of sequencing on an Illumina HiSeq 2000 instrument yielded a total of 203.2 million 100 bp PE reads. Each library generated more than 48 million reads. Ambiguous nucleotides, low-quality sequences (quality scores <20) and short reads (length <30 bp) were removed, and the remaining high-quality reads (97.9%) were carried forward for assembly and analysis (Table 1).

3.2. De novo assembly of the catfish gill transcriptome with ABySS & Trans-ABySS

In order to obtain an optimized reference for mapping of high quality reads and accurate capture of differentially expressed genes after challenge, we utilized ABySS & Trans-AbySS, previously demonstrated to provide a superior assembly when compared with Velvet and CLCbio (Li et al., 2012). Use of Trans-ABySS to merge ABySS multi-k-assembled contigs, resulted in approximately 1.2 million contigs with average length of 337 bp and N50 size of 1658 bp. A total of 235,634 contigs with lengths greater than 200 bp were carried forward for additional analysis. CAP3 was employed to remove redundancy that was generated during multi-k assemblies. Following CAP3, 126,289 contigs with an average length 1,080 bp and N50 size of 2,014 bp were used as the reference catfish gill transcriptome in the following steps of analysis including transcriptome annotation and gene expression profiling.

3.3. Catfish gill transcriptome annotation

The assembled contigs were used as queries in BLASTX searches against the reference proteins available at NCBI zebrafish RefSeq, UniProt protein and nr databases. A total of 55,388 assembled contigs had significant (1e-5) hits to the zebrafish RefSeq database corresponding to 15,774 unique proteins. In searches against the UniProt database, 48,272 catfish contigs had significant hits, accounting for a total of 19,554 unique proteins. Searches against the nr database were most informative resulting in hits for 58,009 contigs and matching 22,864 unique proteins. Cumulatively, a total of 56,892 catfish contigs had at least one significant hit against the three queried databases.

3.4. Identification and analysis of differentially expressed genes

Using a 1.5-fold change cut-off, a total of 3536 contigs were found to exhibit significant differential expression post challenge. Of these, 2605 contigs could be annotated based on significant BLAST hits against at least one database (Supplementary Table 2). At all three examined timepoints following experimental infection, more than twice as many differentially expressed contigs were

Table 1
Summary of Illumina sequencing results of barcoded libraries representing control and post- *F. columnare* challenge samples from channel catfish gill.

	0 h	4 h	24 h	48 h
No. of reads	54,327,678	50,127,430	48,926,940	49,782,122
Average read length (bp)	101	101	101	101
Number of reads after trimming	53,133,196	49,084,349	47,875,496	48,708,535
Percentage retained	97.8%	97.92%	97.85%	97.84%
Average read length after trimming (bp)	93.6	93.8	93.5	93.6

Table 2
Significantly differentially expressed contigs with BLAST identities based on hits against the three searched databases ($p < 0.05$, total gene reads >10, *E*-value ⩽1e-5, fold change ⩾1.5).

	4 h	24 h	48 h
Upregulated	752	1374	581
Downregulated	169	451	288
Total	921	1825	869

upregulated than were down-regulated. This imbalance was greatest at the 4 h timepoint where greater than four times as many contigs were upregulated as down-regulated. The largest number of differentially expressed contigs was observed at 24 h following infection (1825), with roughly equivalent numbers of contigs found at 4 h and 48 h, 921 and 869 contigs, respectively (Table 2).

3.5. Enrichment and pathway analysis

Differentially expressed contigs/genes were classified into different biological and functional gene ontology categories according to Blast2GO (Supplementary Fig. 1). Use of the Ontologizer program revealed 103 significantly over-represented GO terms (FDR-corrected cut-off $p < 0.1$) that were enriched in response to the bacterial challenge. Of these, 17 higher level GO terms were retained as informative for further pathway analysis, including proteasome complex, cytoskeletal protein binding, MHC protein complex, and immune response (Table 3). As described previously (Li et al., 2012), GO term analysis and downstream pathway analysis were frustrated by the incomplete annotation and unique nomenclature characteristics of fish proteins. We used a combination of KEGG pathway analysis, manual re-annotation based on the nr database, and manual literature searches to identify eight functional categories of genes observed to be differentially expressed in the catfish gill following infection with *F. columnare*. These categories included: (1) pathogen/antigen recognition; (2) actin cytoskeleton/entry; (3) junctional regulation/permeability; (4) oxidative stress response; (5) apoptosis; (6) autophagy/lysosome/phagosome (7) anti-inflammatory/immunosuppressive and (8) pro-inflammatory responses. Table 4 lists key, non-redundant gene components of these categories. A particular pattern of NF-κB repression and IFN stimulation was observed in pathway results (Fig. 1).

3.6. Validation of differentially expressed genes using quantitative real-time PCR

In order to validate the differentially expressed genes identified by RNA-seq, we selected 15 representative genes for QPCR confirmation, randomly selected from those with differential expression patterns. DNase I-treated, column-purified total RNA samples from control, and 4 h, 24 h and 48 h following challenge (3 biological replicate sample pools ($n = 6$ for each pool)) were used for QPCR. Primers were designed based on contig sequences (Supplementary Table 1). Melting-curve analysis revealed a single product for all tested genes. Fold changes from QPCR were compared with the RNA-seq expression analysis results. As shown in Fig. 2, QPCR results were significantly correlated with the RNA-seq results at each timepoint (correlation coefficients 0.85–0.94, *p*-value <0.001). In general, the RNA-seq results were confirmed by the QPCR results, indicating the reliability and accuracy of the Trans-ABySS reference assembly and RNA-seq expression analysis.

4. Discussion

Outbreaks of columnaris disease (*F. columnare*) are common in wild and cultured freshwater fish worldwide. Disease occurrences in the catfish aquaculture industry can be particularly devastating (Plumb, 1999; Wagner et al., 2002) and much effort has been

Table 3
Selected summary of GO term enrichment result of significantly expressed genes in channel catfish following *F. columnare* challenge. The 2605 differentially expressed genes were analyzed as the study set in comparison to a total of 27,327 catfish unigenes. *p*-Value ⩽0.1 was considered significant. Count = study count/population count where population count is the number of genes associated with the term in the population set and study count is the number of genes associated with the term in the study set.

GO ID	Name	Adjusted *p*-value	Count
GO:0000502	Proteasome complex	3.04e-18	36/42
GO:0070003	Threonine-type peptidase activity	1.45e-07	19/21
GO:0008233	Peptidase activity	2.51e-06	76/268
GO:0006508	Proteolysis	0.000602	84/303
GO:0008092	Cytoskeletal protein binding	0.000627	38/143
GO:0045111	Intermediate filament cytoskeleton	0.000975	21/36
GO:0022890	Inorganic cation transmembrane transporter activity	0.00550	31/111
GO:0005509	Calcium ion binding	0.00637	51/279
GO:0042611	MHC protein complex	0.00841	8/10
GO:0006950	Response to stress	0.0205	56/319
GO:0015002	Heme-copper terminal oxidase activity	0.0257	10/18
GO:0019882	Antigen processing and presentation	0.0297	8/14
GO:0006955	Immune response	0.0345	20/82
GO:0044092	Negative regulation of molecular function	0.0747	10/43
GO:0030117	Membrane coat	0.0826	13/35
GO:0031316	Canonical Wnt receptor signaling pathway	0.0826	2/2
GO:0050918	Positive chemotaxis	0.0886	9/25

Table 4
Key channel catfish genes differentially expressed in gill post *F. columnare* challenge. Bold fold change values indicate timepoints where the gene was significantly changed relative to the control.

Gene name	Contig ID	4 h	24 h	48 h
Pathogen/antigen recognition				
C-type mannose receptor 2-like	Contig19917	**2.26**	**3.44**	**2.12**
Killer cell lectin-like receptor subfamily B member 1A	Contig26465	1.81	1.36	**2.11**
Leukocyte immune-type receptor TS32.15 L1.1a	Contig28202	**2.18**	1.78	**2.15**
MFAP4	Contig23123	**2.97**	**1.83**	**1.55**
MHC class I alpha chain	Contig23553	**26.93**	**16.95**	**21.26**
MHC class II beta chain	Contig26540	**6.7**	**7.01**	**4.33**
NCAMP-1	Contig26707	**−1.21**	**−3.89**	**−2.06**
NITR10	Contig26726	**−2.17**	−1.64	**−2.89**
NITR2	Contig17433	2.66	2.33	**3.64**
NLR3-like	Contig27483	**3.75**	3.23	**4.67**
Rhamnose-binding lectin	k52:34654	**105.66**	**2.69**	**1.81**
Actin cytoskeleton/entry				
AHNAK	k77:475342	**4.14**	**3.83**	**3.43**
Alpha-actinin-1	Contig10502	1.43	**3.1**	**2.13**
ARP2/3 5b subunit	Contig21476	**1.71**	**2.37**	1.08
Cadherin 1, epithelial	k96:90812	**2.61**	**2.55**	**1.79**
Catenin beta-1	Contig21348	**4.24**	2.59	**5.1**
Cortactin	k84:404849	**5.05**	**6.98**	**9.75**
Dynein light chain 2, cytoplasmic	k52:128164	1.33	**2.98**	**2.96**
ECAM	k96:274473	**3.84**	**10**	**13.24**
EMILIN-2-like	Contig13018	**1.9**	1.59	**3.97**
F-actin-capping protein subunit alpha-1	Contig4296	**7.08**	**5.79**	**5.45**
Filamin-A	Contig1571	**3.35**	**3.07**	**3.45**
Integrin, beta 4	Contig15875	2.89	**4.32**	**3.85**
Keratin, type I cytoskeletal 50 kDa	k96:275217	**4.26**	2.07	**4.92**
MLCK, smooth muscle	Contig22462	**5.65**	1.15	**5.61**
Myosin-9	k65:684723	1.56	**4.43**	**6.09**
Plectin	Contig20511	4.07	**6.85**	4.2
RAC1	k76:500844	3.02	**4.33**	3.51
Septin 2	k74:540910	**6.29**	3.69	**4.96**
Zyxin-like	Contig11369	3.5	4.53	**5.86**
Junctional regulation and permeability				
Aquaporin 3a	Contig26595	**3.53**	**3.01**	**2.91**
Claudin 28b	Contig28076	1.31	**5.18**	1.21
Claudin 8-like	Contig24859	**3.48**	**4.71**	**4.11**
Desmocollin 2-like	k92:221828	**−2.31**	**−1.42**	**−1.57**
NCAM-1	k94:177085	**6.48**	1.47	**17.78**
Pendrin	Contig372	**5.64**	**11.4**	4.12
VCAM-1	Contig11711	2.12	**4.19**	1.69
Oxidative Stress Response				
AHSA1	k72:568882	**4.58**	**8.11**	1.82
ARMET (MANF)	Contig26999	**1.98**	**4.52**	1.42
ATPase inhibitor, mitochondrial-like	k68:647858	**4.99**	**10.1**	**10.88**
Calreticulin like precursor	Contig11018	**1.51**	**3.55**	**1.58**
Calreticulin precursor	Contig933	1.73	**5.47**	1.52
Endoplasmin precursor (GRP94)	Contig1697	1.66	**5.62**	**2.48**
Glutamate–cysteine ligase catalytic subunit (GCLC)	Contig23844	4.47	**8.85**	4.36
Glutathione peroxidase 4 precursor	Contig15152	**−3.72**	**−3.16**	−1.46
Glutathione S-transferase P	Contig11730	1.54	**4.7**	**2.84**
GRP78	Contig4179	**3.67**	**9.15**	2.51
HSP70-like	Contig24589	**3.97**	**3.91**	**5.89**
Hypoxia inducible gene domain family member 1A	Contig18205	**2.38**	**3.77**	**1.54**
Hypoxia up-regulated protein 1 precursor	Contig11951	1.84	**4.51**	1.93
Procollagen-lysine,2-oxoglutarate 5-dioxygenase 2 (PLOD2)	k92:226308	**5.68**	3.33	**5.09**
Protein disulfide-isomerase A6 precursor (PDIA6)	k94:177118	1.51	**4.68**	1.75
PTEN-induced putative kinase 1 (PINK1)	k59:295794	**4.75**	3.16	3.41
Sestrin-1	Contig21944	**6.29**	**5.12**	1.35
Apoptosis				
Anti-				
14–3-3b	Contig16248	**3.99**	1.89	−1.34
Apoptosis inhibitor 5	Contig20789	1.49	**1.97**	1.55
Defender against cell death 1	Contig24842	1.52	**2.6**	1.22
Glutamate [NMDA] receptor-associated protein 1 (GRINA)	Contig12897	1.2	**12.6**	−1.54
Grainyhead-like 3	Contig27008	**3.51**	1.96	3.05
G3BP1	Contig21172	**−31.83**	**−32.4**	**−33.03**
Jun B proto-oncogene	k75:522068	**5.03**	1.71	−1.02
Pro-				
Asc	k84:406733	−1.19	**3.11**	1.39
Butyrate response factor 1	k68:19590	**3.94**	**2.46**	1.62
Calpain 8	k73:546003	**6.36**	**5.39**	2.4

(*continued on next page*)

Table 4 (*continued*)

Gene name	Contig ID	4 h	24 h	48 h
Caspase 8	Contig7489	1.85	**2.35**	1.45
Caspase-1-like	Contig23680	−1.45	**−2.7**	−1.25
Caspase-3	Contig20383	1.13	**1.73**	−1.27
Cellular tumor antigen p53	Contig26773	1.47	**2.12**	1.45
Cyclophilin D	k51:845817	**7.61**	**6.15**	2.95
DNA damage-inducible transcript 3	k75:500186	**8.23**	2.69	2.06
DNA damage-inducible transcript 4 protein-like	Contig13221	**3.94**	**5.93**	**4.08**
Autophagy/lysosome/phagosome				
Cathepsin B preproprotein	k93:201804	**4.92**	**10.86**	**9.73**
Cathepsin L precursor	k96:223936	**2.17**	**4.39**	1.31
Cathepsin Z precursor	k51:845948	1.14	**2.66**	1.27
CD63 (LAMP3)	Contig20601	**5.33**	1.37	**5.76**
Cystatin B	Contig25482	1.42	**3.78**	1.35
Legumain	Contig11987	**3.55**	**4.36**	−1.49
Lysosomal protective protein	k95:325093	**2.69**	**4.22**	1.64
Lysozyme c precursor	Contig25856	−1.06	**5.04**	1.18
Lysozyme g	k51:837552	1.03	**4.32**	1.38
Nucleolin	Contig24287	**5.87**	**7.12**	**3.96**
SEC61-alpha like 1	Contig4375	2.13	**2.99**	1.75
Anti-inflammatory/immunosuppressive				
BCL-3	k59:790437	**8.3**	2.45	1.12
Elf-1	Contig25691	**−5.7**	1.44	−1.17
Interferon regulatory factor 2 (IRF2)	Contig11871	1.33	**1.67**	1.19
Interferon regulatory factor 4 (IRF4)	Contig11211	**2.43**	1.04	1.98
IRF2-binding protein 2	k94:170235	**1.53**	−1.02	1.17
Leukocyte elastase inhibitor (SERPINB1)	Contig13475	**2.78**	**3.22**	−1.17
Lymphocyte activation gene 3 precursor	Contig12238	**4.06**	**4.14**	2.84
NF-kappaB inhibitor alpha-like protein A	Contig11128	**3.08**	**2.37**	**2.12**
NF-kappaB inhibitor alpha-like protein B	Contig23508	**1.91**	**1.81**	1.25
NF-kappa-B inhibitor epsilon (IKBE)	Contig11028	1.47	**1.86**	1.13
NF-kappa-B inhibitor zeta (IKBZ)	Contig22301	**1.82**	1.34	1.33
Olfactomedin-4-like	Contig3033	1	**4.33**	1.47
SOCS1	k55:599137	**2.55**	1.24	−1.18
SOCS3	k95:322784	**3.87**	1.92	−1.48
TAX1BP1	k73:548324	2.52	**4.05**	**7.71**
TNFAIP3-interacting protein 1 (TNIP1/ABIN)	Contig24388	**1.52**	1.16	1.03
Toll-interacting protein (TOLLIP)	Contig6886	**1.81**	**1.56**	−1.08
TRAF2	Contig22190	3.31	**6.12**	2.89
Pro-inflammatory				
ADAM10	Contig18914	**3.79**	2.49	2.25
B7-H3 (CD276)	k61:767462	**4.59**	2.34	1.84
Bactericidal permeability-increasing protein	k75:514795	1.7	1.79	**7.77**
Canopy 4	Contig15172	1.93	**4.43**	2.95
CC chemokine SCYA101	Contig4718	1.21	**4.27**	**−4.3**
CC chemokine SCYA106	Contig24149	**2.62**	**6.05**	−1.16
CC chemokine SCYA109	k78:114741	**3.01**	**5.1**	−1.24
CC chemokine SCYA113	Contig21020	**3.73**	**2.89**	1.08
CD59 glycoprotein-like	Contig23655	**5.86**	**5.01**	2.02
Complement component C7	Contig3539	1.4	**4.14**	1.25
Complement factor D	Contig24178	1.47	**3.81**	**2.74**
Complement receptor-like	k96:274658	**3.95**	2.7	**3.37**
Gp130/IL-6RB	Contig354	**1.85**	1.48	1.32
Immunoresponsive gene 1 (IRG1)	Contig25081	**4.28**	**3.42**	−2.11
Interferon-induced GTP-binding protein Mx	Contig24160	−2.26	−1.15	**−10.26**
Interferon-induced protein 44 (IFI44)	Contig24755	**15.65**	**5.27**	**7.55**
Interferon-induced very large GTPase 1-like	Contig2272	**2.4**	−1.93	**3.26**
Interferon regulatory factor 1 (IRF1)	Contig13038	**2.03**	**1.38**	−1.14
Interferon regulatory factor 8 (IRF8)	k69:617771	**3.42**	1.58	1.31
Interleukin enhancer-binding factor 3 homolog	Contig21945	3.27	**9.4**	**6.42**
Interleukin-17 receptor A	Contig9978	**1.88**	1.56	1.32
JAK1	Contig15970	**2.1**	1.59	1.36
Mannan-binding lectin serine protease 2	k61:766103	2.69	**4.88**	**4.24**
MBL serine protease 2	Contig26941	**3.06**	**3.51**	**2.5**
MMP9	Contig2927	**1.91**	**6.46**	1.24
NF-kappa-B p100 subunit	Contig1335	**1.75**	1.26	1.01
Nitric oxide synthase 2b, inducible (iNOS2b)	Contig8464	**12.19**	2.1	1.28
SAM domain and HD domain-containing protein 1	Contig18760	**21.27**	2.23	**12.08**
Signal peptide peptidase-like 2A precursor	k92:224821	**18.16**	**17.35**	**16.39**
STAT1	Contig2842	**2.31**	2.07	1.25
TANK-binding kinase 1 (TBK1)	Contig1889	**4.35**	**3.89**	2.26
Transmembrane protease serine 9-like	Contig22068	**13.96**	**6.12**	**7.02**
VHSV-induced protein	k96:275209	**9.77**	**4.33**	**4.92**

focused over the last decade in understanding routes of infection (Bullard et al., 2011), adhesion dynamics and their relation to virulence (Decostere et al., 1999a,b; Kunttu et al., 2009; Olivares-Fuster et al., 2011), and treatment and prevention strategies (Olivares-Fuster and Arias, 2011; Shoemaker et al., 2011). However, columnaris remains one of the primary pathogens encountered in aquaculture settings and additional approaches are needed to lessen the impact of the disease in the commercial industry. One such approach is to focus on selection of lines and strains of fish with higher resistance to columnaris. However, little is known about genetic variation in host susceptibility or the underlying molecular mechanisms governing resistance. Accordingly, we conducted the first global measurement of transcriptomic responses to *F. columnare* in the catfish gill to begin to understand host-pathogen interactions and downstream immune events triggered by infection. Over 2600 annotated RNA-seq contigs were differentially expressed at three timepoints following infection, including genes with putative roles in facilitating pathogen adhesion and invasion and the concomitant host immune response. These candidate genes will provide a foundation of valuable biomarkers for evaluating immune responses following vaccination, comparing families and strains of fish with differing susceptibilities, and examining individual variation in host defense mechanisms to *F. columnare* infection.

In the present work, we used a virulent genomovar II isolate of *F. columnare* for our experimental challenge which resulted in widespread fish mortality by 36 h following infection and 90% mortality by day 7 (challenge endpoint). Genomovar II has been convincingly shown to be the isolates with highest virulence in catfish and the strain most likely to be implicated in large-scale columnaris mortality events in commercial aquaculture ponds (Arias et al., 2004; Shoemaker et al., 2008). Given the high, rapid mortality observed in our challenge, the expression profiles obtained likely represent the expression signatures of a highly susceptible host whose immune defenses have been manipulated and overcome by a virulent pathogen. Expression profiles of resistant catfish lines or catfish challenged with genomovar I are likely to differ significantly from those obtained here and we anticipate making these informative comparisons in follow-up studies.

Use of RNA-seq-based expression profiling continues to gain popularity as a robust method to assess transcriptional responses to differing experimental conditions (Oshlack et al., 2010). As in our recent work (Li et al., 2012), use of 100 bp PE reads and the Trans-ABySS multiple-k assembly algorithm, resulted in a high quality reference assembly containing 126,289 unique contigs with an average length of 1079.7 bp. By comparison, our recent intestinal transcriptome sequenced at approximately the same coverage (1 lane of Illumina HiSeq 2000), captured 176,481 contigs with average length of 893.7 bp (Li et al., 2012). In both studies, the contigs had significant matches to approximately 23,000 unique proteins in the most comprehensive database searched, the nr database. The capture of a comprehensive transcriptome from catfish gill provides the ability to examine the expression profiles of less abundant transcripts that would likely be missed in a microarray-based study.

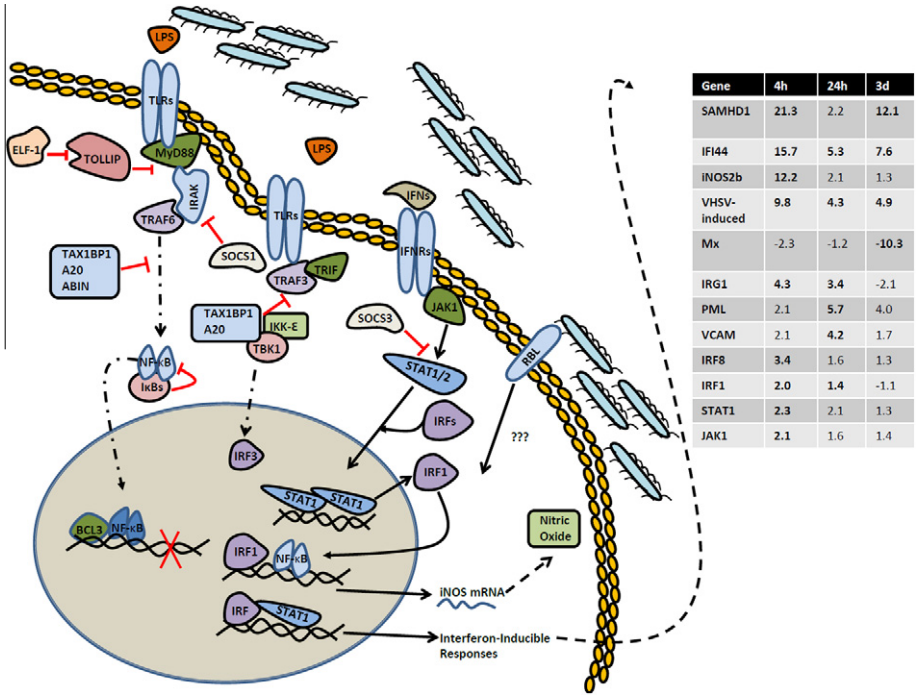
A portion of the transcript depth observed likely stems from the presence of a heterogeneous cell population including resident and circulating leukocyte populations. As discussed below, differentially expressed gene sets were indicative of transcriptional events not only in the gill epithelium but also in macrophages and dendritic cells. While differing proportions of these cell types throughout the extracted sample likely masked or complicated some transcript patterns, our approach captured broader expression responses to infection in the gill that should be reproducible in future studies aimed at whole tissue analysis.

Similarly, we used master-pooled samples, created by combining equal amounts of RNA from three replicate pools (6 fish per pool), to assess expression profiles at each timepoint following *F. columnare* infection. This approach likely masked some degree of individual variation in transcript levels among the challenged fish. However, we accepted this compromise to again initially focus on broad, shared responses to columnaris disease and to provide early insights into important host pathways and processes. Notably, no previous studies have examined host responses to columnaris on the transcriptome scale. Future studies will utilize target genes identified here to examine individual, strain, and family-level variation. We carried out real-time QPCR both to validate the RNA-seq results as well as to examine whether expression profiles in the master pools reflected those of the component replicate pools. Differentially expressed transcripts from each of the three timepoints and representing a variety of expression patterns were tested. There was significant correlation between QPCR and RNA-seq-based expression profiles ranging from 0.85 to 0.94 ($p < 0.001$; Fig. 2). In several cases, predominantly among low fold change genes, the direction of the differential expression differed among the two techniques, potentially indicating either differences in expression between replicate pools or inaccurate quantification by RNA-seq (e.g. see SCYA101 in Fig. 2A). By QPCR, a single product was amplified with all tested primer pairs, indicating that the contig assembly was largely accurate and did not produce a large number of chimeric transcripts.

A total of 2605 differentially expressed contigs with significant fold changes in at least one timepoint could be annotated by BLAST searches (Supplementary Table 2). We attempted to categorize the differentially expressed genes into broad functional categories based on GO annotation and manual imputation of putative function via literature search of studies in vertebrate model organisms (Table 4). Below we highlight several important pathways likely mediating the catfish response to columnaris infection.

4.1. Pathogen/antigen recognition

Several pathogenesis studies have shown correlations between the capacity of *F. columnare* to adhere to gill epithelium and virulence (Decostere et al., 1999a,b). Others, however, have found that adhesion dynamics alone cannot explain patterns of virulence (Kunttu et al., 2009; Olivares-Fuster et al., 2011). Decostere et al. (1999a) reported that treatment of either *F. columnare* bacterial cells or gill tissue with sodium metaperiodate (cleaves the C–C bond between vicinal hydroxyl groups of sugar) significantly reduced bacterial adhesion. Treatment of the bacteria with several sugar forms also reduced adherence. They hypothesized the interaction of a bacterial lectin with a host receptor that was carbohydrate in nature itself. Subsequent studies aimed at catfish found that mucus from skin and gills of catfish promoted chemotaxis of *F. columnare*, that the chemotactic effect was correlated with virulence (genomovar II vs. I), and that this effect was inhibited by pretreatment with sodium metaperiodate as well as by D-mannose, D-glucose, and N-acetyl-D-galactosamine (Klesius et al., 2008, 2010; LaFrentz and Klesius, 2009). Given these findings, we were interested in whether a carbohydrate receptor as postulated by Decostere et al. (1999a) and Klesius et al. (2008), could be detected in host expression signatures following infection. Intriguingly, a rhamnose-binding lectin (RBL) was by far the most highly up-regulated gene observed in our differentially expressed set, with expression increasing 105-fold by 4 h following infection (Table 4). This up-regulation had dramatically decreased at the later measured timepoints (24 h and 48 h), suggesting the importance of this gene during early infection events rather than in later downstream immune responses. RBLs have been reported from a variety of fish species as well as several invertebrates (Watanabe et al., 2009). They have well characterized roles in fish eggs as well as being pattern recognition receptors in innate immunity (Tateno et al., 2002a,b; Shiina



Gene	4h	24h	3d
SAMHD1	21.3	2.2	12.1
IFI44	15.7	5.3	7.6
iNOS2b	12.2	2.1	1.3
VHSV-induced	9.8	4.3	4.9
Mx	-2.3	-1.2	-10.3
IRG1	4.3	3.4	-2.1
PML	2.1	5.7	4.0
VCAM	2.1	4.2	1.7
IRF8	3.4	1.6	1.3
IRF1	2.0	1.4	-1.1
STAT1	2.3	2.1	1.3
JAK1	2.1	1.6	1.4

Fig. 1. Putative pathways of TLR/NF-κB suppression and IFN-stimulated responses based on RNA-seq expression signatures in channel catfish gill. Lines with perpendicular ends indicate inhibitory signaling or functional effects. IFN-inducible genes and fold changes (subset of Table 4) are broken out in a table.

et al., 2002; Bah et al., 2011). The catfish RBL in our data shares highest similarities to well-characterized RBLs from amur catfish and rainbow (steelhead) trout STL1 (Tateno et al., 2001). Both the channel catfish RBL here and STL1 are Type I RBLs, meaning that they have three tandemly-repeated carbohydrate recognition domains. Immunohistochemical staining with antisera against STL1 in rainbow trout showed strong localization of expression in mucus (goblet) cells on the gill (Tateno et al., 2002a). This expression pattern is consistent with RBLs potentially playing a critical role in columnaris adhesion events given reports of aggregates of high virulence *F. columnare* around goblet cells in common carp (Decostere et al., 1999b) and catfish (Olivares-Fuster et al., 2011) using scanning electron microscopy (SEM) soon after infection. Follow-up studies to examine RBL expression in the context of columnaris isolates with differing virulence as well as in catfish families, strains, and species with differing susceptibilities are needed.

Several other known and putative pattern recognition receptor (PRR) molecules including NCAMP-1, found to be expressed on catfish nonspecific cytotoxic cells (Evans et al., 2005), several NLR3-like genes (Sha et al., 2009; Rajendran et al., 2011), several MFAP4 genes (Niu et al., 2011), and several novel immune type receptors (NITRs; Hawke et al., 2001) were differentially expressed following columnaris challenge (Table 4, Supplementary Table 2). However, all these PRR molecules showed modest fold changes in comparison with catfish RBL. Noticeably absent from the list were Toll-like receptors (TLRs) which we anticipated would be differentially expressed, particularly at the early timepoint (4 h). However, this result is consistent with our past transcriptomic profiling in catfish following ESC infection (Peatman and Liu, 2007; Peatman et al., 2008; Li et al., 2012). In those studies, TLR induction was modest, particularly in comparison with changes in lectins (intelectin, nattectin) and non-traditional immune factors (e.g. MFAP4). These emerging patterns may indicate the need to develop new paradigms in our understanding of pathogen recognition in teleosts that are not centered around TLRs (see Sepulcre et al., 2009). However, further profiling at different timepoints and in additional tissues is still needed for a more comprehensive perspective.

4.2. Inflammatory/immunosuppressive responses

While individual TLR genes were not captured, downstream signaling components traditionally associated with TLR signaling were found, including Elf-1, Canopy 4, and TOLLIP. Interestingly, the captured expression pattern among these genes and among other downstream pathway members was indicative of negative regulation of TLR and NF-κB signaling (Fig. 1). These inhibitory signals would be expected via Elf-1 and TOLLIP (Sugi et al., 2011) and suppressor of cytokine signaling (SOCS) genes (Strebovsky et al., 2012) which interrupt TRAF6-dependent signaling. Continued interruption of signaling via NF-κB would be expected through actions of TAX1BP1 and ABIN (Gao et al., 2011; Verstrepen et al., 2009), olfactomedin 4 (Liu et al., 2010b), and through induction of several IκBs which hold NF-κB subunits in the cytoplasm (Fig. 1, Table 4). Finally, BCL-3 can work in the nucleus to inhibit NF-κB-dependent gene transcription and diminish LPS responses (Carmody et al., 2007). Consistent with our findings, Sepulcre et al. (2009) reported that zebrafish TLR4 signaling potently inhibited NF-κB signaling. Given the acute nature of the infection, we speculate that negative regulation of one of the central innate immune signaling pathways may be the result of immune evasion/manipulation by the pathogen via secreted toxins. Inhibition of NF-κB signaling after *Yersinia* or *Mycobacterium* infections has been shown to decrease release of proinflammatory cytokines and increase apoptosis, allowing continued bacterial proliferation (Rosenberger and Finlay, 2003; Zheng et al., 2011).

Relatedly, we did not detect changes in several canonical inflammatory mediators such as TNF-α, IL-1, and IL-8. Other antibacterial genes such lysozyme g, bactericidal permeability-increasing protein (BPI), galectin 3/9, and metallothionein which were observed to be upregulated strongly in the gill of cod (Caipang et al., 2010), were only modestly changed in catfish gill following columnaris challenge. Several factors may contribute to these patterns. Given evidence of pathogen-regulated immune-disregulation, these factors may have been already suppressed by the 4 h timepoint. Alternately, a spike in these factors may have occurred

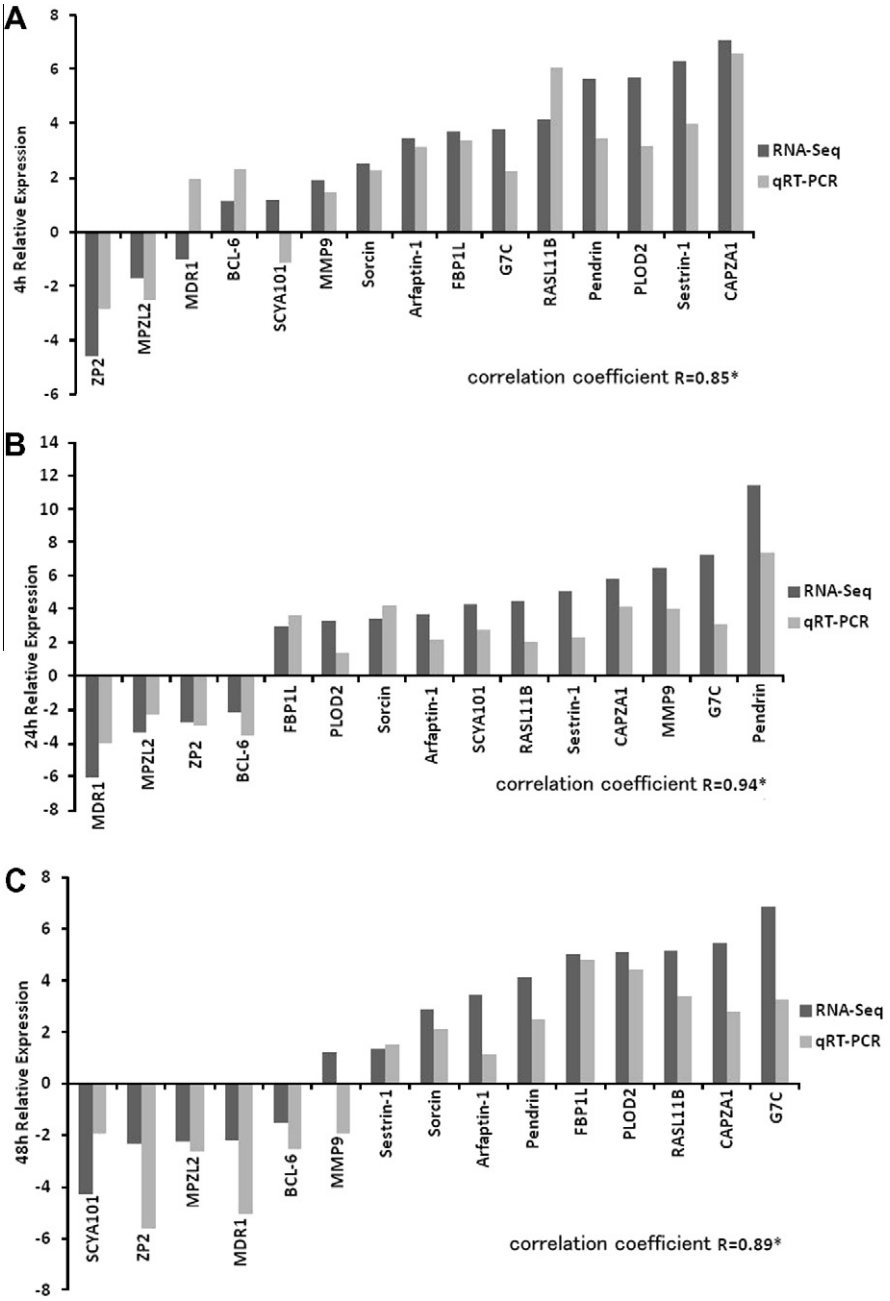


Fig. 2. Comparison of relative fold changes between RNA-seq and QPCR results in catfish gill at (A) 4 h; (B) 24 h; and (C) 48 h. Gene abbreviations are: SCYA101, CC chemokine SCYA101; MMP9, matrix metalloproteinase-9 precursor; CAPZA1, F-actin-capping protein subunit alpha-1; PLOD2, procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 isoform 2 precursor; RASL11B, Ras-like protein family member 11B; FBP1L, formin binding protein 1-like; MPZL2, myelin protein zero-like protein 2; MDR1, PREDICTED: multidrug resistance protein 1; BCL-6, B-cell lymphoma 6 protein; G7C, PREDICTED: protein G7c-like; ZP2, zona pellucida glycoprotein 2.3. Fold changes are expressed as the ratio of gene expression after *F. columnare* challenge to the control group as normalized with the 18S rRNA gene.

in the fairly broad gaps between timepoints. Further work is needed to determine whether these patterns may be related to the catfish host immune system, the pathogen, temporal/spatial regulation of immune responses, or interactions between several of these factors.

On the other hand, strong induction of inflammatory responses via interferon (IFN)-mediated pathways was captured in the RNA-seq results. While IFN and IFNR genes were not found among differentially expressed genes, many downstream pathway members were induced including JAK1 and STAT1, pro-inflammatory interferon regulatory factors (Ozato et al., 2007), and interferon-inducible genes including IFI44, VHSV-induced genes, and iNOS2b (Fig. 1). Once again, the fact that IFN and IFNR genes were not captured could have been caused by transient nature of up-regulation of these genes that were not reflected in the selected timepoints of this study. Inducible nitric oxide synthases (iNOS) generate nitric oxide (NO) from L-arginine. Often produced by macrophages, NO is a potent cytotoxic agent in immune defenses that can have beneficial antimicrobial activity, but which can also have far-reaching tissue-damaging effects (Bogdan, 2001). High levels of NO, for example, can induce apoptosis in leukocytes responding to sites of infection (Allione et al., 1999). Recently, Bah et al. (2011) demonstrated that a salmon RBL could induce nitric oxide production. Of interest here, iNOS2b shared the general pattern of induction of

the catfish RBL, with significant upregulation at 4 h, but not at later timepoints. Further work is needed to learn whether catfish RBLs can similarly induce iNOS production. More broadly, the debate is ongoing as to whether interferon plays a beneficial role in controlling bacterial infections (Decker et al., 2005; Trinchieri, 2010). In some cases, bacterially-triggered IFN responses can have death-sensitizing effects that increase macrophage and lymphocyte apoptosis and sustain infection (Al Moussawi et al., 2010; Stockinger et al., 2002; Stanley et al., 2007). Interestingly, similar patterns, either of NF-κB suppression or IFN-induced responses, were not apparent in expression profiles following *E. ictaluri* infection in catfish (Peatman and Liu, 2007; Peatman et al., 2008; Li et al., 2012), likely indicating the generation of pathogen-specific immune responses.

4.3. Oxidative stress/apoptosis

Consistent with high iNOS levels and apoptotic-promoting IFN responses, several members of oxidative stress responses and apoptotic pathways were strongly induced following *F. columnare* infection (Table 4). These include genes such as calreticulin, endoplasmin, glutathione S-transferase, GRP78, and HSP70-like likely involved in the attempt to limit cellular damage and detoxify free radicals (Higa and Chevet, 2012). Among more classic apoptotic factors such as calpain 8, caspase 8, cyclophilin D, and DNA damage-inducible transcripts that were upregulated, we also classified G3BP1 (Rasputin) as having a potential role in negative regulation of apoptosis. G3BP1 was highly down-regulated greater than 30-fold at all examined timepoints in contrast to most other differentially expressed genes which were upregulated or showed smaller decreases in expression (Table 4). While little is known about this gene in the context of bacterial infections (Irvine et al., 2004), it has been reported to interact with both parasite (Borth et al., 2011) and viral (Yi et al., 2011) pathogens. Further functional characterization of G3BP1 may aid in our understanding of catfish immune responses. In general, the oxidative stress/apoptosis pathways upregulated here after infection of columnaris were also upregulated after infection with ESC (Peatman and Liu, 2007; Peatman et al., 2008; Liu et al., 2010a,b, 2011), suggesting that the oxidative stress response is quite a general host response to bacterial infections.

5. Conclusions

Using Illumina RNA-seq technology, we provide the first transcriptomic-level analysis of teleost (channel catfish) mucosal immune responses to acute infection with *F. columnare*. Our analysis captured over 2600 differentially expressed transcripts in catfish gill, including a putative receptor for columnaris binding. Pathway analysis indicated suppression of NF-κB signaling accompanied by an IFN-dominated immune response, as well as highlighting individual candidate genes which may serve as biomarkers for future *F. columnare* studies. Building on this foundation, additional studies are needed to compare and contrast the expression profiles observed here with those captured following challenge of catfish families, strains, and species with differing columnaris susceptibilities with *F. columnare* isolates with varying virulence and adhesion profiles. Such expression candidates, along with positional candidate genes as determined by genome-wide association studies (GWAS), will eventually lead to the identification of genes responsible for resistance against important bacterial diseases such as ESC and columnaris.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.dci.2012.05.006.

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