Interleukin-8 involved in Nile Tilapia (*Oreochromis niloticus*) against bacterial infection

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Abstract

Interleukin 8 (IL8) is vital in promoting inflammation and is a crucial mediator in various physiopathological processes while influencing immunological function. The effect of IL8 on the immunological response to acute bacterial infections in Nile tilapia (*Oreochromis niloticus*) remains unknown. This work found an *IL8* gene from Nile tilapia (On-IL8). It includes a 285 bp open reading frame and codes for 94 amino acids. The transcript levels of *On-IL8* were highest in the head-kidney tissue and sharply induced by *Streptococcus agalactiae* and *Aeromonas hydrophila*. Besides, in vitro experiments revealed that On-IL8 regulated a variety of immunological processes, such as up-regulating *IL-1β* and *TNFα*, down-regulating *IL-10* and *TGFβ*, inhibiting *P38* and *P65*, promoting *MyD88* and *STAT3*, and promoting inflammatory responses. These significant findings serve as the basis for further investigation into how IL8 confers protection to bony fish in opposition to bacterial infections.

Introduction

IL8, a member of the chemokine family, is a short soluble peptide with pro-inflammatory effects involved in many immune-related diseases. It is mainly produced by monocyte macrophages. Its biological function is to chemotaxis neutrophils, T-lymphocytes and basophils to the lesion site in response to inflammation, and to promote wound healing by activating the angiogenic response and triggering endothelial cell proliferation, survival and recruitment.

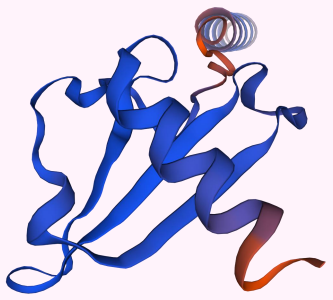

Figure 1. Tertiary structure model of On-IL8 protein

Results

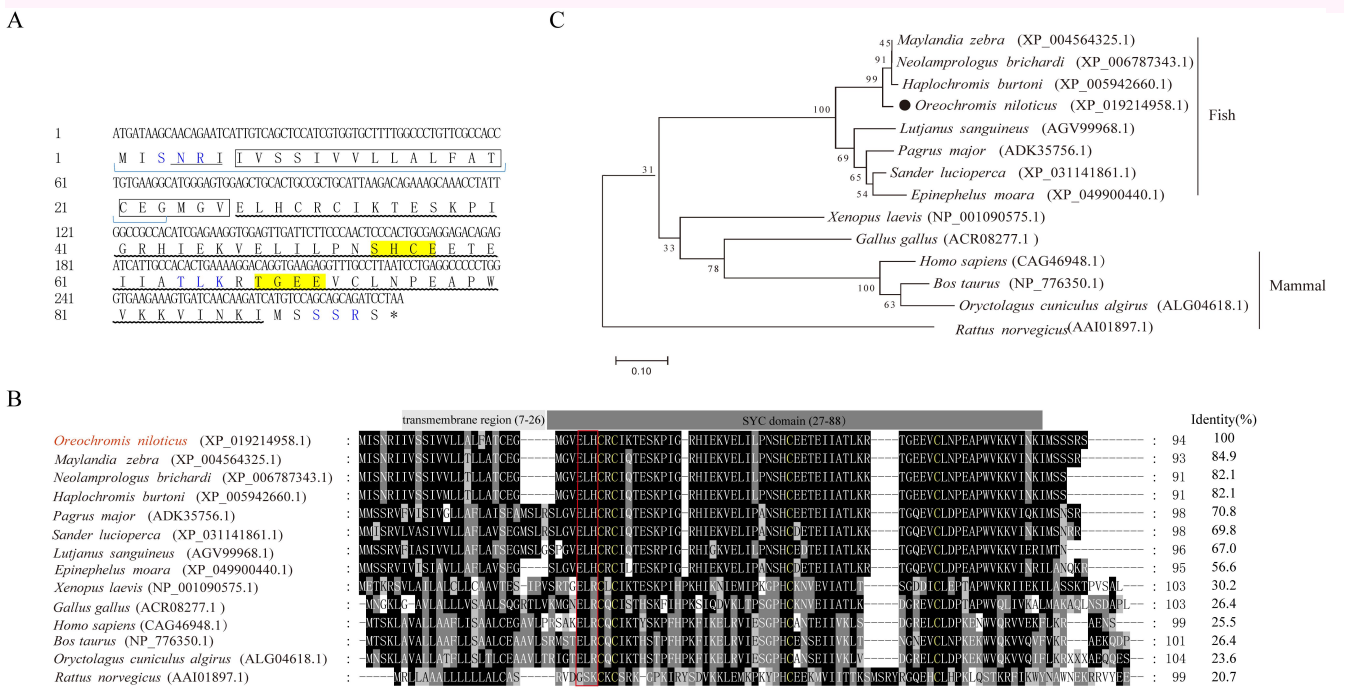

Figure 2. (A) Amino acid sequence and functional site of IL8 in Nile tilapia. Blue brackets: signal peptide region;Blue font: protein kinase C phosphorylation site; Yellow shading: casein kinase II phosphorylation site;"—":microbodies C-terminal targeting signal; "▭": transmembrane region; "〰": small cytokine C-X-C; "*": stop codon. (B)Multiple sequence alignments of IL8 among different species. Following each amino acid sequence, the percentage number reveals the total sequence identity between On-IL8 and other sequences. Similar residues are highlighted in grey, while consensus residues are highlighted in black.The definition of the SYC domain is the intercrine alpha family (small cytokine C-X-C). The ELR motif is circled with the red box. Cysteine residues, important in forming disulfide bonds, are marked with the yellow font. (C) The neighbour-joining approach was used to generate a phylogenetic tree of On-IL8 among various species. The numbers at the branches' nodes are the bootstrap confidence levels with 1000 replicates.

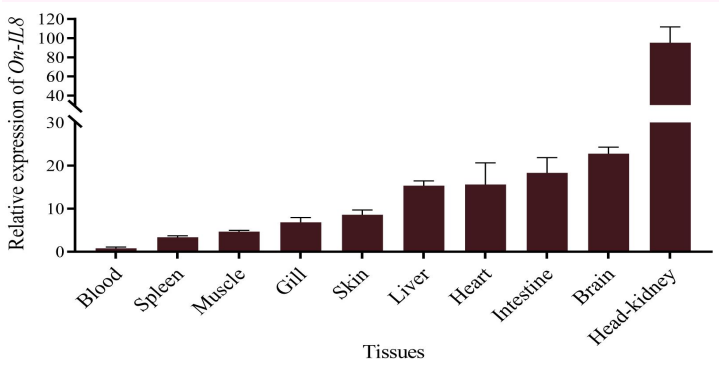

Figure 3. qRT-PCR was employed to measure the expression of On-IL8 mRNA in different tissues of healthy Nile tiapia. All values are mean standard deviations (n = 3). The amount of On-IL8 expression in the blood was set at 1.

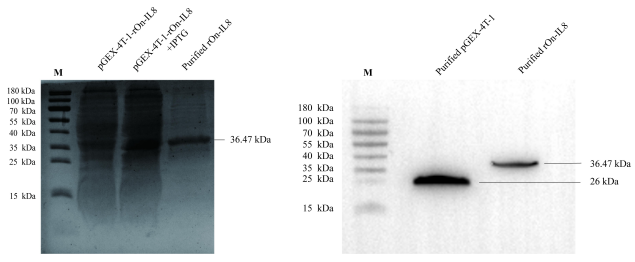

Figure 4. SDS-PAGE of total proteins from uninduced and induced bacterial strains shows pGEX-4T-1-rOn-IL8 prokaryotic recombinant vector and purified recombinant protein with expected molecular weight kDa (left). The purified pGEX-4T-1 and rOn-IL8 were identified with an anti-GST-tag antibody in a Western blot (right).

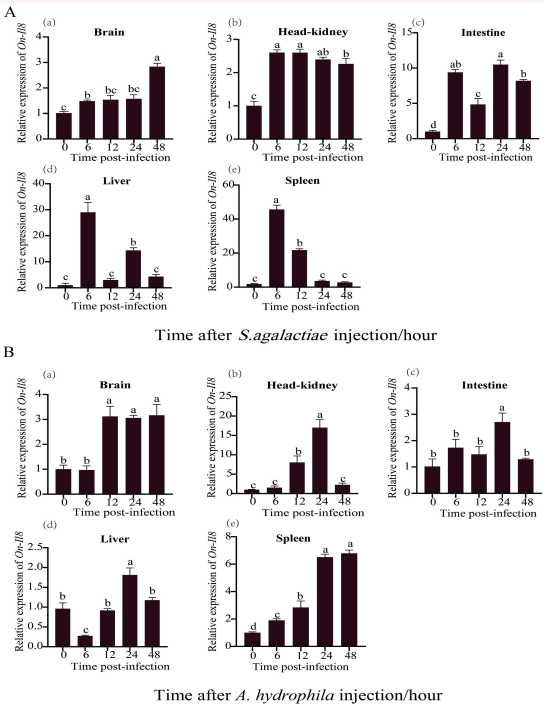

Figure 5. qRT-PCR was applied to detect the expression trends of *On-IL8* mRNA in several tissues (brain, head-kidney, intestine, liver, and spleen) following *S. agalactiae* (A) and *A. hydrophila* (B) injection. For calculating the relative expression of the other time points, the expression level of *On-IL8* at 0 h was set to 1.00. All values are mean standard deviations (n = 3). The use of different letters showed a significant difference. ($p < 0.05$).

Materials and Methods

1）Fish feeding and sample preparation: The 500 Nile tilapia(50 ± 5 g) used in this experiment were obtained from a commercial aquaculture farm in Zhanjiang in Guangdong Province, China.Fish were cultured in 1000 L tanks (for 14 days acclimatization period), fed twice daily (08:00 and 16:00) at 3% body weight with a commercial diet (purchased from Zhanjiang Aohua Feed Co. Ltd., Guangdong, China), and maintained at a water temperature of 28 ± 0.5 °C, dissolved oxygen of 5.0 to 6.0 mg/mL, and pH of 7.2 to 7.7.Three healthy fish were randomly selected and RNA from each of the ten tissues (blood, brain, gill, head-kidney, heart, intestine, liver, muscle, skin and spleen) was extracted to prepare cDNA templates.

2) Cloning and sequence analysis of On-IL8 : The NCBI Primer designing tool was used to create all the primers utilized in this research .The predicted gene sequence of On-IL8 was acquired from NCBI (XM_019359413.2), and the polymerase chain reaction (PCR) was used to amplify the entire open reading frame (ORF) sequence using head-kidney cDNA and specific primers.

3) Pathogen infection and sample preparation: Nile tilapia were challenged using *S. agalactiae* and *A. hydrophila* and cDNA templates were prepared by reverse transcription of RNA extracted from individual tissues at different time points of infection.

4) Quantitative real-time PCR (qRT-PCR): Following the manufacturer's instructions, TB Green® Premix Ex Taq TM II (Tli RNaseH Plus) (TaKaRa, Dalian, China) and QuantStudio 6 and 7 Flex Real-Time PCR systems (Thermo Fisher Scientific, Waltham, USA) were used to assess where On-IL8 transcripts were found in healthy Nile tilapia and how they changed after being challenged.

5) Preparation of recombinant On-IL8 protein (rOn-IL8) : Recombinant plasmid was constructed by pGEX-4T-1 and cloned fragment IL8, and recombinant protein was obtained using IPTG induction.

6) Preparation of cDNA from On-IL8 effect on Nile tilapia HKLs with different stimulation: Tilapia head-kidney lymphocytes were isolated using the percoll separation method, and then HKLs were stimulated with LPS, LTA, and rOn-IL8, respectively, and then the cDNA templates were prepared by collecting the cells after stimulation at different time points, respectively.Finally, the indicators were tested by qPCR.

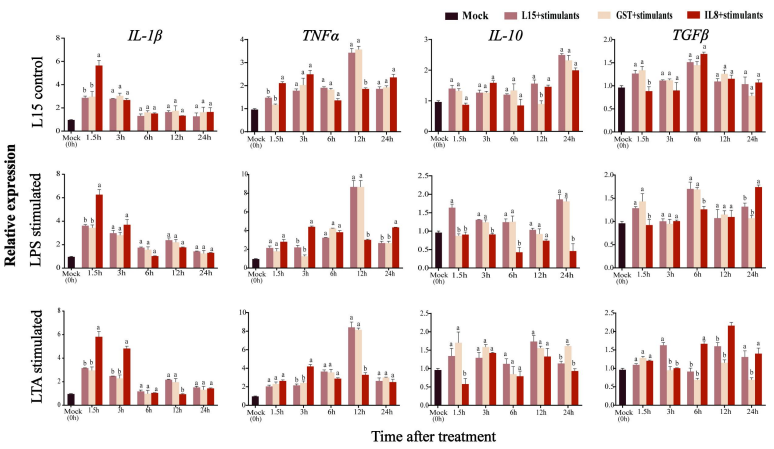

Figure 6. Expression trends of inflammatory factors (*IL-1β*, *TNFα*, *IL-10*, and *TGFβ*) in HKLs of tilapia as measured by qRT-PCR at various time points and treatments. All values are mean standard deviations (n = 3). Different letters indicate significant difference ($p < 0.05$).

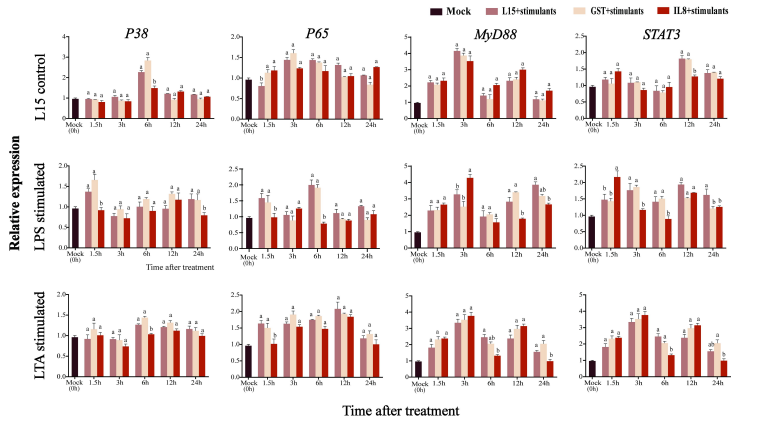

Figure 7. Expression trends of immune-related pathways (*P38*, *P65*, *MyD88*, and *STAT3*) in HKLs of tilapia as measured by qRT-PCR at various time points treatments. All values are mean standard deviations (n = 3). Different letters indicate significant difference ($p < 0.05$).

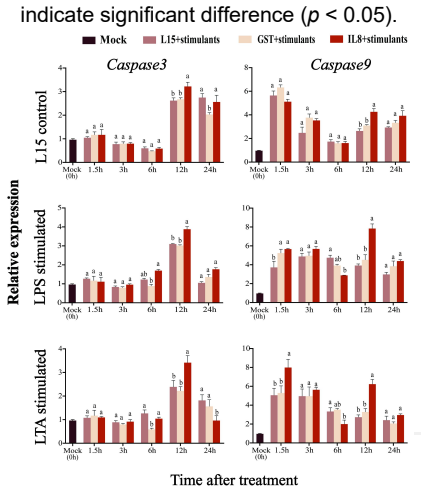

Figure 8. Expression trends of apoptosis factors (*Caspase3* and *Caspase9*) in HKLs of tilapia as measured by qRT-PCR at various time points treatments. All values are mean standard deviations (n = 3). Different letters indicate significant difference ($p < 0.05$).

Acknowledgment

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Conclusion

IL8 is widely distributed in various tissues in healthy Nile tilapia.
IL8 was induced during bacterial infection in Nile tilapia.
IL8 was partially involved in the immunomodulation of Nile tilapia head-kidney leukocytes in vitro.

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