Accepted Manuscript

Biological characterization, expression, and functional analysis of tumor necrosis factor receptor-associated factor 6 in nile tilapia (*Oreochromis niloticus*)

Zhiwen Wang, Yu Huang, Yuan Li, Bei Wang, Yishan Lu, Liqun Xia, Jufen Tang, JiChang Jian

PII: S1050-4648(18)30377-2

DOI: 10.1016/j.fsi.2018.06.036

Reference: YFSIM 5379

To appear in: *Fish and Shellfish Immunology*

Received Date: 22 March 2018

Revised Date: 13 June 2018

Accepted Date: 18 June 2018

Please cite this article as: Wang Z, Huang Y, Li Y, Wang B, Lu Y, Xia L, Tang J, Jian J, Biological characterization, expression, and functional analysis of tumor necrosis factor receptor-associated factor 6 in nile tilapia (*Oreochromis niloticus*), *Fish and Shellfish Immunology* (2018), doi: 10.1016/j.fsi.2018.06.036.

Biological characterization, expression, and functional analysis of tumor necrosis factor receptor-associated factor 6 in Nile tilapia (*Oreochromis niloticus*)

Zhiwen Wang [a,b,c,1], Yu Huang [a,b,c,1], Yuan Li [a,b,c], Bei Wang [a,b,c], Yishan Lu [a,b,c,*], Liqun Xia [a,b,c], Jufen Tang [a,b,c], JiChang Jian [a,b,c]

[a] College of Fishery, Guangdong Ocean University, Zhanjiang, 524025, China

[b] Guangdong Provincial Key Laboratory of Pathogenic Biology and Epidemiology for Aquatic Economic Animals, Zhanjiang, 524025, China

[c] Key Laboratory of Control for Disease of Aquatic Animals of Guangdong Higher Education Institutes, Zhanjiang, 524025, China

[*] Corresponding author. Fisheries College, Guangdong Ocean University, No. 1 of Haida Road, Huguang Yan East, Mazhang District, Zhanjiang, Guangdong Province 524088, China. Tel./fax: +86 759 2383507.

E-mail address: fishdis@163.com (Y. Lu).

[1] These authors contributed to the work equally and should be regarded as co-first authors.

Abstract

Nile tilapia (*Oreochromis niloticus*) is a pivotal economic fish that has been plagued by *Streptococcus agalactiae* infections for many years. Tumor necrosis factor receptor-associated factor 6 (TRAF6) is a crucial adaptor molecule of the interleukin-1 receptor/Toll-like receptor (IL-1/TLR) superfamily, which can trigger downstream signaling cascades involved in innate immunity. In this study, the full-length cDNA of *TRAF6* was cloned from *O. niloticus* (named *On-TRAF6*), which has an open reading frame of 1716 bp, and encode a polypeptide of 571 amino acids. The predicted amino acid sequence of On-TRAF6 contained the characteristic motifs of TRAF proteins, including a Zinc finger of RING-type, two Zinc fingers of TRAF-type, and a MATH (meprin and TRAF homology) domain. Multiple sequence alignment revealed that On-TRAF6 shares a relatively high level of identity with those of other fishes (64-98%). In healthy tilapia, mRNA expression of *On-TRAF6* could be detected in all the examined tissues and the highest expression occurred in the spleen. Moreover, we found that *On-TRAF6* was involved immune response of Nile tilapia following the stimulation with *Streptococcus agalactiae* and polyinosinic: polycytidylic acid (Poly I:C) when determined by using qPCR. The result of subcellular localization showed that On-TRAF6 distributed in the cytoplasm, and over-expression of On-TRAF6 could strongly activated NF-κB pathway in HEK293T cells. These findings indicated that On-TRAF6 may play an important role in the immune response to intracellular bacteria in Nile tilapia.

Key words: *Oreochromis niloticus*; TRAF6; Infection; mRNA expression