Expression and characterization of Toll-like receptor 3 (TLR3) in turbot (*Scophthalmus maximus*)

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Abstract: Toll-like receptor (TLR) 3 is an important member of the TLR family, recognizes invading pathogens specifically double-stranded RNA viruses. In this paper, turbot (*Scophthalmus maximus*) TLR3 gene (smTLR3) was cloned and its expression profiles were analyzed with RT-PCR and *In situ* hybridization. Then its expression was investigated in cultured peripheral blood leukocytes (PBLs) simulated with poly I:C, peptidoglycan (PGN), or lipopolysaccharides (LPS); in liver, spleen, gill and kidney injected with *Streptococcus iniae*, *Edwardsiella tarda* hirame rhabdovirus virus (HRV), poly I:C, PGN, or LPS using real-time PCR. The recombinant protein of smTLR3 (rp-smTLR3) and the polyclonal antibody(Pab) was produced, then the reactions of Pab to tissues was studied by Western-blotting, the binding of rp-smTLR3 to all the stimulants was detected using ELISA. The results showed the full length of smTLR3 cDNA consists of about 3100bp, which has the typical construction of the TLR family with 2883 bp of the open reading frame encoding 961 amino acids. It was highly expressed in spleen, liver, gill, skin, intestine, and PBLs, mainly distributed in epithelial cells. SmTLR3 expression was significantly up-regulated by PolyI: C both *in vitro* and *in vivo*, no significant change in PGN and LPS groups; SmTLR3 significantly increased in liver and spleen after *S. iniae* infection with the maximum of 3.6 times and 3.3 times; in liver and kidney after *E. tarda* infection with the maximum of 3.4 times and 4.1 times; and then in gills and kidney after HRV infection by 3.5 and 4.7 times. The reaction binds of Pab in liver,

spleen, gills and kidney was 120kDa, 90kDa and 40kDa. The rp-smTLR3 could bind to all the ligands *in vitro*. The data suggested the expression of smTLR3 in the turbot and could recognize the ligands more than dsRNA.

Key Words: *Scophthalmus maximus*, Toll-like receptor 3, gene expression, binding feature