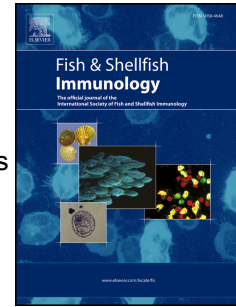


# Accepted Manuscript

Conservation of structural and interactional features of CD28 and CD80/86 molecules from Nile tilapia (*Oreochromis niloticus*)

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1 **Conservation of Structural and Interactional Features of CD28 and CD80/86**  
2 **Molecules from Nile Tilapia (*Oreochromis niloticus*)**

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16  
17 **Abstract**

18 Interaction of CD28 with CD80 or CD86 molecules provides a costimulatory  
19 signals required in T cell activation. In this study, we cloned and analyzed a CD28  
20 gene (On-CD28) and a CD80/86 gene (On-CD80/86) from Nile tilapia (*Oreochromis*  
21 *niloticus*). Sequence analysis revealed the typical characteristics of On-CD28 protein;  
22 for instance, the proline-based motif (<sup>117</sup>TYPPPL<sup>122</sup>) is essential in binding of CD28  
23 to CD80/86 ligands. Moreover, an extracellular Ig domain was found in On-CD80/86;  
24 this domain is responsible in binding of CD28 to CD80/86 receptors. Subcellular  
25 localization analysis showed that both On-CD28 and On-CD80/86 were distributed  
26 predominantly in the cytomembrane. Yeast two-hybrid assay showed that On-CD28  
27 directly interacted with On-CD80/86. On-CD28 and On-CD80/86 transcripts were  
28 detected in all the examined tissues of healthy Nile tilapia, and the highest expression  
29 levels of On-CD28 and On-CD80/86 were detected in the brain and heart, respectively.  
30 Following a bacterial challenge using *Streptococcus agalactiae* in vivo, On-CD28 and  
31 On-CD80/86 were upregulated in head kidney, spleen, intestines, and brain. However,  
32 they showed different expression profiles in response to stimulation with inactivated *S.*  
33 *agalactiae* in vitro. These findings indicated that the interaction of On-CD28 with  
34 On-CD80/86 provides a costimulatory signals that possibly play an important role in

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