Fish & Shellfish Immunology 58 (2016) 42–49

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

# Estrogen regulation of gene expression in the teleost fish immune system

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ARTICLE INFO

*Article history:*
Received 9 July 2016
Received in revised form
1 September 2016
Accepted 10 September 2016
Available online 12 September 2016

*Keywords:*
Sex hormones
Immune system
Teleosts
microRNAs
Estrogen receptors

ABSTRACT

Elucidating the mechanisms of estrogens-induced immunomodulation in teleost fish is of great importance due to the observed worldwide continuing decrease in pristine environments. However, little is know about the immunotoxicological consequences of exposure to these chemicals in fish, or of the mechanisms through which these effects are mediated. In this review, we summarize the results showing estrogens (natural or synthetic) acting through estrogen receptors and regulating specific target genes, also through microRNAs (miRNAs), leading to modulation of the immune functioning. The identification and characterization of miRNAs will provide new opportunities for functional genome research on teleost immune system and can also be useful when screening for novel molecule biomarkers for environmental pollution.



**Contents**



## 1. Introduction

There is a bi-directional communication between the endocrine and immune systems in teleosts [1–3], while the endocrine system is known to modulate the function of immune components [1–4] through circulating hormones, including those critical to the reproductive system [1,5]. Indeed, in many cases changes in plasma hormone levels correspond with alterations in the immune status and health of the fish [1]. These circulating hormone concentrations can be influenced by exogenous factors (photoperiod, temperature, pH, xenobiotic compounds), and therefore the immune system functions can also be accordingly regulated [1,6,7].

To date, estrogens are recognized as modulators of both reproduction and immune response in fish [8], and there is a great interest in understanding their effect on the immune system [5,9]. This has been in part by the observed increase presence of endocrine disrupting chemical (EDCs) in aquatic environment [10–12], predominantly introduced via wastewater systems and husbandry runoff [10,13]. A wide-ranging of EDCs compounds includes pesticides, detergents and surfactants, phthalates, alkylphenols, and natural or synthetic estrogens. Adverse effects of EDCs include population decline in wildlife, an increasing incidence of cancer,

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http://dx.doi.org/10.1016/j.fsi.2016.09.006
1050-4648/

inhibition of reproductive function, and developing disruption of the immune and nervous systems [14–16].

Exposure to elevated levels of 17<beta>-estradiol (E2), the most potent natural estrogen [17], could alter components of the immune system, inducing immunosuppressive effects and increased disease susceptibility in individuals [18–20], and may have significant implications at the population level of fish [4]. E2 may also have immunostimulatory effects during the reproductive season [21], modulating leukocyte functions at the end of gametogenesis, spawning, and postspawning stages [22]. Besides, E2 appears to promote the infiltration of acidophilic granulocytes and cause a resumption of gonad cell proliferation [21,22]. Between the EDCs, the 17α-ethynylestradiol (EE2), a potent synthetic estrogenic compound even more than E2 [23,24], seems to have the capacity to alter fish immune function, and decrease pathogen resistance [25], which may have important implications to fish survival and population growth [20,26]. Since estrogen receptors (ERs) are known to exist in the immune system organs [27–31], their actions are executed by the classical genomic pathway [32], through nuclear translocation of estrogen-ER complexes and estrogen response elements (EREs)-mediated regulation of transcription [33], and by a rapid non-genomic pathway, which involves phosphorylation cascade through G protein-coupled receptors [34].

Current evidence indicates that microRNAs (miRNAs), a group of small non-coding RNAs, have been increasingly implicated in the modulation of the innate and acquired immune response in vertebrates [35–37]. MiRNAs are associated in the development of hematopoietic cell lineages [38], regulation of host-pathogen interactions [39–41], and regulation of signal transduction in immune cells [35], thus indicating that proper regulation of miRNA expression is crucial for disease prevention [37]. Transcriptional regulation by nuclear receptors (NRs) is considered to be a primary level of control for miRNA expression [42]. These NRs, i.e. ERs, can regulate the miRNA biogenesis at different levels (Fig. 1); in the nucleus, by regulating the microprocessor complex activity in the processing of pri-miRNA to pre-miRNA. Both ER<alpha> and ER<beta> can inhibit the activity of Drosha complex, thereby preventing the conversion of pri-miRNAs to pre-miRNAs [43]. In the cytoplasm, at the step of maturation from pre-miRNA to mature miRNA [44]. Moreover, there are evidences to indicate a regulation of NR signalling by miRNAs targeting mRNAs of NR co-regulators and target genes [45]. Therefore, variation in miRNAs expression profiles, which are known to be associated with different ER expression patterns [46,47], can fine-tune immune development and response and their misregulation can also lead to autoimmune diseases and cancer [48–50].

The evidence suggesting an immunomodulatory role of estrogens in fish comes from the following findings: (a) estrogen receptors are expressed in fish immune organs [27–31], (b) pathogen susceptibility of fish increases under estrogen exposure [25,32], and (c) immune gene expression may be modulated by estrogen-induced miRNA variation [40,41,51,52].

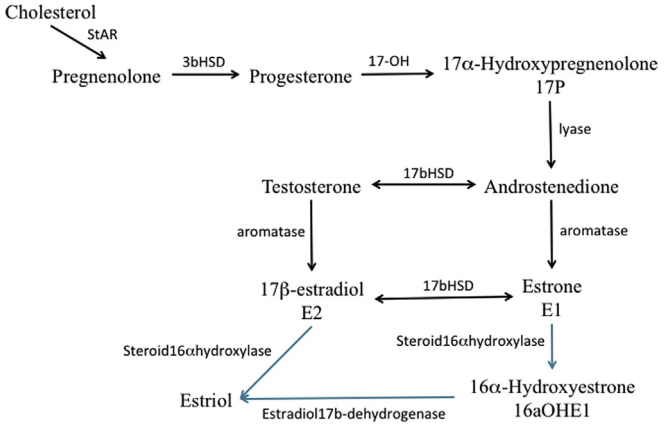


**Fig. 1. A**. Triggering of TLR3 receptor by virus activates downstream NF-κB signalling. **(1)** MyD88-dependent pathway: formation of MyD88-IRAK family (Myddosome) complex, autophosphorylation of IRAK1 forming TRAF6-TAK1 complex, and activation of the IKK-NF-κB complex pathway. IKK complex composed of subunits IKKα, IKKβ and, IKKγ (NEMO). **(2)** TRIF-dependent pathway: formation of TRIF-TRAF-RIP1 complex activates the TAK1complex, leading to activation of NF-κB [149]. **B**. Hypothetical deregulation of miRNA-462 biogenesis by estrogens (natural or synthetic). Overexpression of MYC by Estrogen-ER reducing transcription of pri-miRNAs. Estrogen-ERα suppresses Drosha-mediated processing of pri-miRNAs to pre-miRNAs. Myc can also indirectly lead to miRNAs repression, activating LIN28. EGFR: EGF receptor; ER: Estrogen receptor. **C**. Inhibition of NF-κB by Estrogen-ER(α/β) action. (a) Inhibition of IKK activity; (b) Inhibition of degradation of IkB; (c) Blocking DNA binding by NF-κB; (d) Binding to coactivators and compete with NF-κB for coactivator binding; and (e) Binding directly to DNA-bound NF-κB to inhibit NF-κB-mediated transcriptional activation [150].

## 2. Modulation of fish immune system by estrogens

Estrogens are recognized as modulators of immune responses in mammals and teleosts, regulating both the innate and adaptive immune system responses [1,8,17], so besides being considered as reproductive hormones, they can also physiologically act on many non-reproductive tissues [53]. Estrogens occur in three major natural forms (Fig. 2): estrone (E1), 17<beta>-estradiol (E2), and estriol (E3) [5,17], and their relevant concentrations can modulate several immune functions and alter specific and non-specific defence responses in mammals [54]. This endocrine-immune interface is partly mediated by interactions between circulating estrogens and their ERs found in leukocytes, although the molecular mechanisms by which they act are not completely understood [9].

Several ERs have been characterized in teleosts, ER<alpha>1, ER<alpha>2, ER<beta>1, ER<beta>2 [55,56], and an unrecognized form, ER<gamma> [55]. All forms have been detected in a variety of tissues including immune organs [28–31,57], explaining why the immune tissues are also putative targets for EDCs [58]. Recent evidence indicate that head kidney leukocytes (HKL) and peripheral blood leukocytes (PBL), mostly macrophages, acidophilic granulocytes (AG) and lymphocytes, express intracellular ERs [9], providing a signalling mechanism through which estrogens, mainly E2, may exert their effect over immune cells [4]. E2 seems to modulate the expression and functionality of the ER<alpha> and ER<beta>2 subtypes present in all leukocytes of channel catfish *Ictalurus punctatus*, participating in the regulation of the immune response, with possible negative effects on the responsiveness of PBL to mitogen [8]. Given the presence of ERs in immune organs during development [20,52,59], estrogens are likely to interfere with the maturation of primary immune organs and may alter the immune response of juveniles, modify the innate immune response, and exert detrimental effects on head kidney development as was reported in juvenile sea bass *Dicentrarchus labrax* [60]. In juvenile rainbow trout *Oncorhynchus mykiss*, four ER transcripts (ER<alpha>1, ER<alpha>2, ER<beta>1, ER<beta>2) were identified in HKL and PBL and altered during the E2 exposure period, demonstrating that physiologically relevant concentrations of E2 can modulate several immune functions in salmonids [4]. Hou et al. [18,19] reported an immunosuppressive effect of E2, at high concentration, on antibody activity of PBL, HKL, spleen and skin, and reduction in number of cells producing IgM.

Reproduction in female teleost fish is dominated by estrogens [61], where E2 increments its serum level and induces the synthesis of vitellogenin in the liver, and thus the oocyte growth [61,62].