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Expression and localization study of pIgR in the late stage of embryo development in turbot (*Scophthalmus maximus*)

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ABSTRACT

The receptor responsible for maternofetal transmission of immunoglobulin (Igs) in the teleosts is not clear. Polymeric immunoglobulin receptor (pIgR) specifically binds with IgA and IgM and mediates the transcytosis of intracellular polymeric immunoglobulins (pIgs) at the mucosal surface to protect against pathogens. Hence there is a possibility that it may be involved in the transmission of maternal Igs. The aim of the present study was to detect the expression and localization of pIgR during embryonal development in turbot *(Scophthalmus maximus)*. pIgR gene was first cloned from eggs and embryos of turbot with or without parent immunization. The expression and distribution of pIgR in unfertilized egg and in embryos ranging from day 1 to day 5 after fertilization were analyzed using reverse transcriptase quantitative polymerase chain reaction and in situ hybridization. pIgR gene was detected in all eggs and embryos at different stages of development, with the highest level detected on the 5th day. pIgR mRNA was observed to be first located in the whole blastoderm and enveloped the yolk sac. Later, it was located around entoderm including primary digestive tract and pronephric tubule tract, and finally it was located at the joint of abdomen and vitelline membrane. Then, Eukaryotic expression plasmid carrying pIgR gene was constructed and transfected into HEK293T cells. Results showed mature pIgR protein located on the cellular membrane, and could bound IgM in vitro. Our findings provide information for studying the involvement of pIgR in maternal Igs transportation in turbot.

1. Introduction

The immune protection is crucial for fish embryos which are hatched in pathogen-rich aquatic environment. During the early development stages of fish embryos, the immune protection mainly depends on the specific and non-specific cytokines of maternal origin [1]. Maternal immunoglobulins (Igs), which are vital for protection of offspring in early development, are transported to the progenies through some specific receptors [2–5]. Polymeric immunoglobulin receptor (pIgR), identified in 1980, was primitively named as secretory component [6]. Which serves as an important function in immunoglobulin transportation, is produced by the epithelial cells of the skin,gastrointestinal tract, respiratory tract mucosa-associated lymphoid tissues, and glandular epithelial cells of the liver and breast. pIgR plays an important role in mammalian immuneresponse by transporting polymeric Ig across mucosal epithelial cells. pIgR can transport pIg from the basolateral surface onto the apical surface of epithelial cells by transcytosis [7]. It is one of the most important mucosal effectors which mediate the transcytosis of polymeric immunoglobulins (pIgs), such as polymeric IgA (pIgA) and pentameric IgM to protect the organisms [8]. In mammals, IgG is transported from mother to offspring by major histocompatibility complex (MHC) class I-related neonatal Fc receptor (FcRn) which binds IgG in a pH-dependent manner [9]. In birds and amphibians, IgY, the functional counterpart of IgG, is transported from mother to offspring across the yolk sac membrane into the embryonic bloodstream during embryonic development by FcRY, which is the functional counterpart of FcRn [10]. FcRY is a new class of Fc receptor which does not show sequence homology and architectural similarity to FcRn. Instead, FcRY is the homolog of phospholipase A2 receptor [11].

In higher animals, pIgR is mainly expressed in the epithelial cells and tract of the secretary glands, with its peak levels observed in the small and large intestines; normal levels are seen in kidney, pancreas,

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lung and endometrium [12]. In case of primate such as humans, FcRn is mainly expressed in the placenta and it is critical to the maternofetal transmission of IgG [11]. Several types of pIgR expressed in cellular membranes bind with the Fc fragment of the corresponding pIgs to perform their biological function. In lower animals, FcRY is mainly expressed in neonatal small intestine, fetal yolk sac, mammary gland, liver, vascular endothelial and respiratory endothelium. In teleost, pIgR is one of the most important effectors in the mucosal immune system of different types of fish, it may mediate the secretion of Igs. A single pIgR gene has been identified on zebrafish chromosome 2 along with a large multigene family consisting of pIgR -like genes [13]. The full-length cDNA of pIgR was firstly cloned in Qihe crucian carp (*Carassius auratus*), and it exhibited rapid immune response to A. hydrophila challenge and played an important role in the immune defense [14]. The full-length cDNA of pIgR was firstly cloned from flounder (*Paralichthys olivaceus*) in 2013, It's recombinant protein displayed binding capability to the purified mucus IgM and serum IgM of flounder by ELISA [15]. So far, the biogenesis of receptor responsible for maternofetal transmission of Igs in teleost remains unclear. Gamma subunit of Fc receptor of common carp (*Cyprinus carpio* L.), Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*) [16–18] have been cloned.

Turbot (*Scophthalmus maximus*) is one of the extensively maricultured species in China, but as a deep water fish, our knowledge about its natural breeding habits are still limited. It heavily depends on the artificial insemination in the industry, the survival rate of the fry is very low. In this regard, one of the most urgent requirements is to better understand the mechanism and regulation process of the maternal immunity, to improve the disease resistance of fry under artificial insemination. Much efforts have been made to understanding the maternal immunity in turbot, for example, in the mucosal immune system of turbot, pIgR mediates the transportation and secretion of IgM to mucus [15]. Identifying the expression and distribution of pIgR in the ovary and embryo of turbot may help us to understand the underlying mechanism of maternofetal transmission of Igs, which are critical for protection of embryo in early development. Thus the present study aimed to investigate whether pIgR expressed in the ovary and embryo during the different stages of embryo development and the dynamic changes of pIgR in turbot. Our results could provide valuable information for elucidation of bio-function of pIgR in early phase of turbot embryo development.

2. Materials and methods

2.1. Sample preparation

Female Parent turbot (*S. maximus*) were obtained from Jiaonan Tongyong Aquatic Products Co., Ltd. (Qingdao, China). Twenty healthy female turbot (*S. maximus*, weight 4–6 kg) which showed gonad development were intramuscularly injected with labelled chip and kept with non-labelled female Parent turbot under standard laboratory conditions at 14 °C. Among labelled turbot, fifteen were designated as experimental group and five as control group. female Parent turbot were intraperitoneally injected with live attenuated *Vibrio anguillarum* vaccine at a dose of 1×10^4 cfu/g thrice at 14-day intervals. Twelve weeks after the first immunization when the gonad of the female Parent turbot were completely developed, mature eggs without fertilization and embryos after 8 h, 1 d, 2 d, 3 d, 4 d, and 5 d post in vitro fertilization were collected. The samples were rinsed with phosphate buffer saline (PBS) and stored in liquid nitrogen or fixed with 4% paraformaldehyde until further use. All animal experiments were conducted in accordance with the guidelines and approval of the Animal Research and Ethics Committees at the Ocean University of China.

2.2. Detection of antibody titer

Followed by centrifugation, 0.2 ml venous blood was collected from every turbot and allowed to stand at 4 °C for 3–5 h; the supernatant was collected and stored at −20 °C. Enzyme linked immunosorbent assay (ELISA) plate was coated with 50 μL of 10^7 cfu/mL inactivated *V. anguillarum* per well and incubated at 4 °C overnight. After three PBST (PBS containing 0.1% Tween 40, pH 7.4) washes, the wells were blocked with 100 μL of 5% non-fat milk (dissolved in PBS) at 37 °C for 1 h. Following three additional PBST washes, the wells were incubated with 50 μL of 2-fold serial diluted serum (starting at 1:100) at 37 °C for 30 min. After washing, the wells were incubated with 50 μL monoclonal antibody collected from ascitic fluid against IgM of turbot (diluted in 5% non-fat milk at 1:25000) at 37 °C for 30 min and then incubated with 50 μL horseradish peroxidase (HRP) labelled goat anti mouse secondary antibodies [(diluted in 5% non-fat milk at 1:2000); ZSGB-BIO, Beijing, China] at 37 °C for 30 min. Finally, the plate was visualized using tetramethylbenzidine kit (TianGen; Beijing, China) according to the manufacture's protocol. The optical density (OD) value was determined at 450 nm using a microplate reader (Bio-Rad ELX800; Bio-Rad, Inc., Hercules, CA, USA).

2.3. Construction of eukaryotic expression plasmid for pIgR

The primers for pIgR were designed according to the pIgR gene of turbot published in GenBank (KC142170). Total RNA were extracted from egg and embryos at different developmental stages using Trizol reagent (Invitrogen, Carlsbad, CA, USA). RNA samples were dissolved in RNase-Free H_2O (Takara, Shiga, Japan) and stored at −80 °C. cDNA was synthesized as following the instruction of PrimeScript™ II 1st Strand cDNA Synthesis Kit (Takara, Shiga, Japan). Briefly, 2 μg total RNA was used as template with oligo dt (18) as primers (Table 1). The reaction was performed at 42 °C for 1 h. The cDNA obtained was amplified using Ex Taq® HS (Takara, Shiga, Japan) according to the manufacturer's protocol. PCR products were inserted into pMD19-T Vector (Takara, Shiga, Japan) and the products were sequenced in Sango sequencer (Shanghai, China). pIgR gene was amplified from

Table 1
Primers used in the present study.

primers		Sequence (5'-3')	products
pIgR-CDS	Forward Primer	ATGAGGGAGTTCTGCACCAGC	1005bp
	Reverse Primer	TCAGTGCATCTGAACCTCCTGG	
Q-pIgR	Forward Primer	TCTGCCGACGGACTCTGAT	186bp
	Reverse Primer	GACGCTGTTGTGGGTTGC	
β-actin	Forward Primer	TCCCTGTATGCCTCTGGTCGTA	190bp
	Reverse Primer	CAGTGGTGGTGAAGGAGTAGCC	
E-pIgR	Forward Primer	CGGAATTCGCCACCATGCCACAACTCCTCATACTCACTC	1054bp
	Reverse Primer	GCGTCGACTCACTTATCGTCGTCATCCTTGTAATC GTGCATCTGAACCTCCTGG	
Sequence primer	Forward Primer	GAGCGGATAACAATTTCACACAGG	–
	Reverse Primer	CGCCAGGGTTTTCCCAGTCACGAC	
Oligo primer		TTTTTTTTTTTTTTTTT	–
Probe primer	Dig Labeling	GACAACAUCAGCGCUCCAUAAACCGCCUAUCUCC	–

pMD19-T-pIgR using E-pIgR primers which produced fragment with a EcoR I site at 5′ end and a Sal I site and flag tag at 3′ end (Table 1). The PCR product was inserted into the EcoR I and Sal I sites of pIRES2-EGFP (Clontech, Madison, WI, USA) which resulted into the recombinant eukaryote expression plasmid pIRES2-EGFP-pIgR.

2.4. Immunofluorescence assay (IFA)

Human embryonic kidney (HEK) 293 T cells were maintained in DMEM (Gibco, Grand Island, USA) with 10% fetal bovine serum (FBS) (Gibco, Grand Island, USA). For transfection, 2×10^5 cells were seeded in 24-well plate. When cells were grown to 70% confluence, they were transfected with 500 ng pIRES2-EGFP-pIgR using lipofectamine 3000 (ThermoFisher, Pittsburgh, USA) according to the manufacturer's protocol. The cells were fixed with 4% paraformaldehyde for 48 h after transfection. Subsequently, the cells were blocked with 1% bovine serum albumin (BSA) at room temperature (RT) for 1 h and incubated with 1:800 diluted anti-flag monoclonal antibody (Abcam, Cambridge, MA, USA) at 4 °C overnight. Then the cells were incubated with 1:5000 diluted Alexa Flour 555 labelled goat anti-mouse secondary antibody (Abcam) at RT for 1 h and stained with Hochest (Beyotime, Zhejiang, China) for 15 min. Finally, the cells were mounted in 50% glycerol and signals were visualized under confocal microscope (LEICA TCS SP5, Germany).

2.5. Ligand binding assay

Human embryonic kidney (HEK) 293 T cells were transfected with pcDNA3.1 + pIgR, using lipofectamine 3000 (ThermoFisher, Pittsburgh, USA) according to the manufacturer's protocol. The cells were seeded in 24-well plate. Subsequently, the cells were incubated with anti-flag Rabbit polyclonal antibody and rat anti-turbot IgM monoclonal antibody at 4 °C overnight. And then incubated with Alexa Flour 488 labelled goat anti- Rabbit IgG antibody and Alexa Flour 594 labelled goat anti-rat IgG antibody at RT for 1 h. Finally, the cells were mounted in 50% glycerol and signals were visualized under confocal microscope (LEICA TCS SP5, Germany).

2.6. Quantitative real-time reverse transcriptase PCR (qRT-PCR)

Total RNA of the embryos were extracted and reverse transcribed into cDNA using PrimeScript™RT Master Mix (Takara) in a total volume of 10 μL containing 0.7 μg total RNAs at 37 °C for 15 min. Quantitative PCR was performed using 2 μL cDNA products as template, with Q-pIgR primers or β-actin primers (Table 1) in LightCyler96 (Roche, Nutley, NJ, USA). In negative control group, DEPC water was set as template. Relative expression levels of pIgR mRNA was calculated with 2-ΔΔCt method.

2.7. In situ hybridization

Eggs at different stages fixed with 4% paraformaldehyde were embedded in paraffin and 5 μm thick serial sections prepared. The sections were dried in oven at 40 °C for 2 h; rehydrated with xylene and descending grades of ethanol (50%–100%); immersed in 0.2 M hydrochloric acid at RT for 20 min; and digested with 0.1 μg/ml protease K at 37 °C for 2 h. After washing, the slices were fixed with 4% paraformaldehyde at RT for 4 min; immersed in 0.25% acetic anhydride at RT for 15 min; dehydrated with increasing concentration of ethanol (50%–100%); and finally air dried. The slices were pre-hybridized using fresh prehybridization solution in a humid box at 40 °C for 2 h. Hybridization was performed using digoxigenin-labelled probes transcribed from cDNA amplified from pIgR with digoxigenin-labelled probe primer (Table 1). Slices were incubated with hybridization solution containing probe and denatured at 95 °C for 10 min; cooled down on ice; immersed in 100% ethanol for 1 min; and dried at RT. Then the slices were incubated with hybridization solution containing probe; blocked with liquid paraffin and placed in a humid box at 40 °C for 16 h for hybridization. After washing with 2 × SSC, 1 × SSC, and 0.5 × SSC for 1 h, respectively, the slices were incubated with 1:2000 diluted HRP labelled anti-digoxigenin antibodies at RT for 1 h and visualized using DAB kit.

2.8. Statistical analysis

Data are expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS 16.0 (IL, USA). Between-group differences were assessed using Analysis of Variance with Turkey post-hoc analysis. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Immune response to vaccination in female parent turbot

Specific antibodies were detected in female Parent turbots at week 4 post-immunization with a live attenuated *V. anguillarum* vaccine. The initial titer was 1:3200 and it gradually increased over time. The peak titer was 1:10240 at week 10 and was maintained at this high level for 2 weeks. The results showed that strong humoral immune response was stimulated with high level specific antibodies.

3.2. Expression analysis of pIgR in turbot embryos during development

The open reading frame (ORF) of pIgR gene in turbo was 1005 bp and it encodes a protein of 334 amino acids with molecular mass 37.8 kDa. The mRNA of pIgR gene was detected in ovary, unfertilized egg, and embryos at different developmental stages (from day 1 to day 5). This indicated that the transcription of pIgR occurred during the hatching period. Furthermore, mRNA expression levels of pIgR were quantified using qRT-PCR. As shown in Fig. 1b, mRNA levels of pIgR in embryo increased during the hatching period. There were almost the same levels for the first 4 days (0–3 d) of hatching and increased significantly at 4th and 5th day. Although no significant difference was observed in the first 4 days ($P > 0.05$), the pIgR mRNA levels at 5th day were significantly greater than that in the first 4 days ($^*P < 0.05$). Additionally, results showed vaccination had no influence on the mRNA levels of pIgR in embryos at different stages of development. In the Fig. 1c, no significant difference was observed in the pIgR mRNA levels of embryos between immunized and non-immunized female Parent turbot ($P > 0.05$).

3.3. Immunofluorescence assay analysis of turbot pIgR

Under subcellular localization of turbot pIgR, a general diffusion of pIgR was detected in HEK293T cells transfected with recombinant eukaryote expression plasmid containing pIgR gene of turbot. The Alexa Flour 555 labelled pIgR was detected to be located in the cellular membrane by using IFA (Fig. 2), with two side-by-side Ig-like domains exposed to extracellular sites, and glycosylation sites concentrated within second Ig-like, which may be related with the protein transport function of pIgR.

The results of IgM and receptor after incubation were observed under confocal laser microscope (Fig. 3). The nucleus emitted blue fluorescence under ultraviolet laser, the cell membrane showed red cell fluorescence under green laser, showed green fluorescence under blue laser, and green fluorescence and red fluorescence were overlapped. There was no fluorescence in the negative control group and the empty vector group, which indicated that turbot immunoglobulin IgM could bind to its receptors.

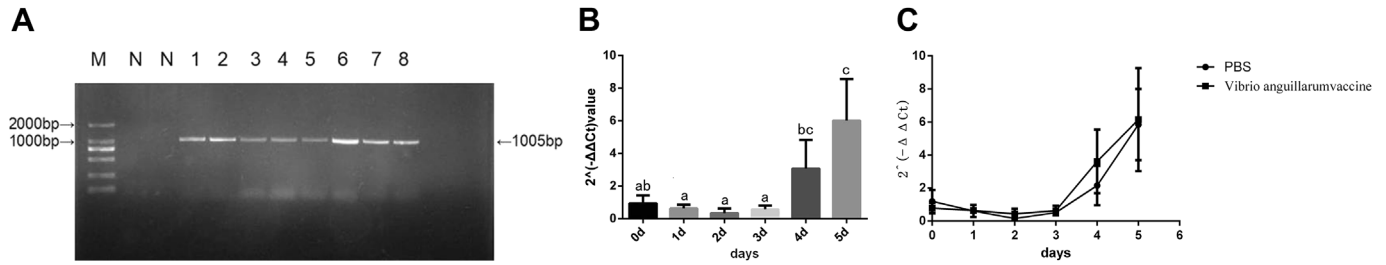

Fig. 1. Cloning and expression of pIgR gene from egg and embryos of turbot *(Scophthalmus maximus)* during different developmental stages with or without parent immunization.

Female Parent turbot were immunized with or without a live attenuated *Vibrio anguillarum* vaccine. The ovary, unfertilized egg and 1st-5th day embryos were collected and expression of pIgR was detected. (A) RT-PCR products of pIgR gene from ovary, unfertilized egg, 0–5 d embryos. M: marker DL2000;N, negative control;1:0d embryos;2:1d embryos; 3:2d embryos;4:3d embryos; 5:4d embryos;6:5d embryos; 7,8: ovary (B) mRNA expression levels of pIgR in 0–5 d embryos. (C) mRNA expression levels of pIgR in 0–5 d embryos from parents with or without immunization. Values with different letters in the same column (a-c) are significantly different ($P < 0.05$) from each other.

pIgR, Polymeric immunoglobulin receptor.

3.4. Localization of pIgR in turbot embryos during development

Next, the localization of pIgR mRNA in the embryos of turbot was detected using in situ hybridization. Since the level of mRNA of pIgR in the 3rd-day embryos was almost as the same level as that in 2nd-and 3rd-day, hence only 1st day embryo were subjected to ISH. In Fig. 4, the unfertilized egg lacked expression of pIgR mRNA in both egg plasma membrane and ooplasm. In the 1st-day embryo, which was at the late blastocyst and gastrula stage, pIgR mRNA positive cells were observed evenly distributed in the whole blastoderm (Fig. 4b), extended along the blastoderm and enveloped the yolk (Fig. 4c). In the 4th-day embryo, undergoing organogenesis where digestive tract, liver, and neuro system were getting formed, the distribution of pIgR mRNA positive cells showed a certain taxis. They were distributed evenly around each somite, which presented as V-shape and pointed to the head of embryo body. mRNA of pIgR were also distributed evenly around primary digestive tract and pronephric tubule tract as well as surrounding head (Fig. 4d). In the 5th day embryo, which was at incubation period when Rathke's pouch degenerates, and optic capsule and notochord are in the late stages of formation, pIgR mRNA positive cells were found to be distributed mainly at the joint of abdomen and vitelline membrane (Fig. 4d). Moreover, the expression levels were also higher than that in the early embryos, which may be involved with exchange and transportation of substances and antibodies between yolk and embryo.

4. Discussion

The immunity of embryos is feeble during the early stages of life, especially in the pathogen-rich aquatic environment. The transmission of maternal Igs confers protection against pathogens [19] in these early development stages. The transferred Igs may protect eggs, embryos, and juvenile fishes before the development of their own functional immune system [2–5]. Previously, pIgR has been reported to play vital roles in transporting immunoglobulins in teleost mucosal surfaces, which are the first line of host immune defense [20,21], but the detailed role of pIgR for transmission of maternal Igs are still limited. In this study, we investigated the expression patterns, subcellular localization of pIgR in early development stages of embryos, as well as its binding capacity to immunoglobulins for the first time in turbot.

Previously, experimental results indicated that after the parent is immunized, the Igs are enriched in parent and absorbed by oocyte. These Igs then enter the circulatory system through yolk sac of the offspring and protect them against pathogens [22,23]. There are similar reports of maternal complement components C3 being transported from mother to offspring, although the function of C3 on egg and offspring remains unclear [24]. Here, the antibody titer was increased significantly in female Parent turbot following immunization with a live attenuated *V. anguillarum* vaccine. However, there was no significant difference of the expression levels of pIgR between the embryos from the immunized and non-immunized female Parent turbot. Mostly, the high level of antibody in vaccinated broodstock could incorporated into vitellogenic oocytes to transfer to larvae, which could then have more disease resistance than that from unvaccinated broodstock. For example, the larvae from Sea bream broodstock immunized with photobacteriosis vaccine showed lower mortality under *Photobacterium damsela* subsp. Piscicida infection compared to control group [25]. Similar transfer patterns of antibodies were also found in tilapia [4] and gilthead sea bream [26]. In contrast, vaccination of some species could not result in high levels of antibody in the offspring [27]. In current study, during the development of embryo in turbot, although the expression of pIgR was gradually increased over time, parent immunization showed no effect on the expression levels of pIgR. However, our previous study showed that immunization of parent fish can improve the IgM levels in offsprings [28]. Thus, the relationship between immunization and the expression levels of IgM and pIgR needs to be further investigated. Indeed, the maternal transfer of antibody could provide significant protection of the offspring, further studies are needed to determine the most effective vaccination protocols for teleost

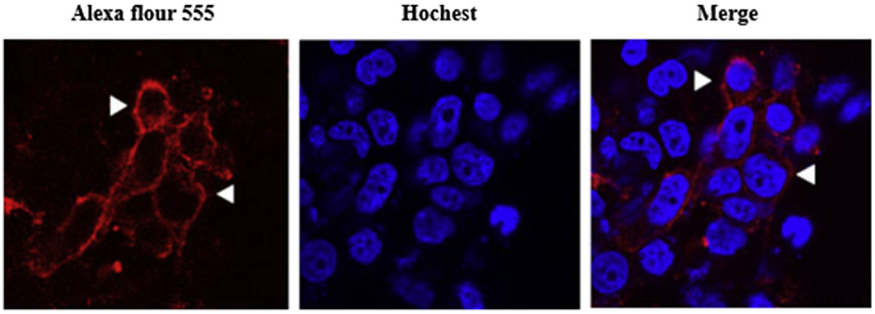

Fig. 2. Localization of pIgR in HEK293T cells.

HEK293T cells were transfected with pIgR recombinant expression plasmid. Post 48 h, the expression and localization of pIgR in the transfected cells was detected using IFA and visulized under confocal microscope.

White triangles indicate the expression and membrane localization of pIgR.

pIgR, Polymeric immunoglobulin receptor; HEK293T, Human embryonic kidney.

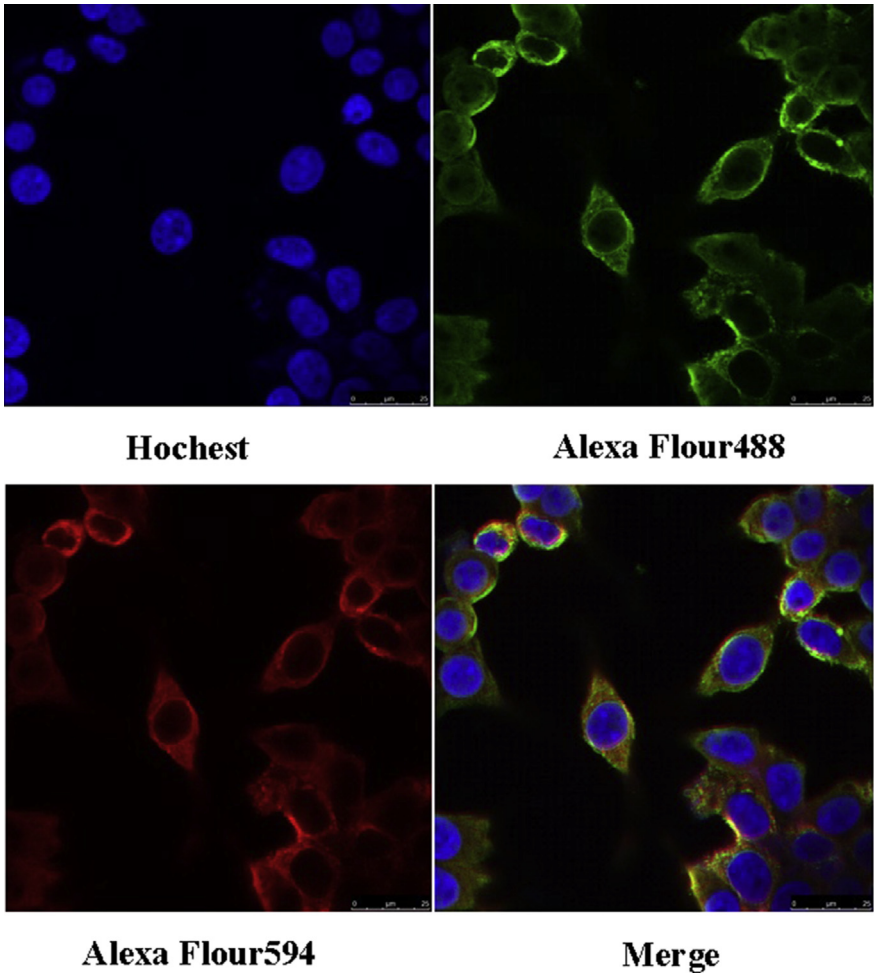

Fig. 3. The binding of pIgR protein to IgM.
HEK293T cells were transfected with pIgR recombinant expression plasmid. Post 48 h, The binding of pIgR protein to IgM was detected using IFA and visulized under confocal microscope.
pIgR, Polymeric immunoglobulin receptor; HEK293T, Human embryonic kidney.

broodstock.

PIgR is conserved among all vertebrates [29]. The domain I of human pIgR was shown to be responsible for binding with IgA and IgM [26]. When pIgR cDNA was transfected and stably expressed in Chinese hamster ovary (CHO) cells, pIgR was found to be located on the membrane surface, and specifically interacted with pIgA [27]. FcRY is needed for the transmission of chicken IgY from maternal serum to oocytes [28]. The lack of pIgR results in a lack of secretion of dIgA into the mucosa, and a buildup of serum IgA [30]. Sea bass pIgR can interact with Vg and result in similar down-stream immune responses [31]. In the present study, we found that pIgR was first located in the whole blastoderm and it enveloped the yolk sac, then it was observed around entoderm including primary digestive tract and pronephric tubule tract. Finally, it was located at the joint of abdomen and vitelline membrane. From the results of in situ hybridization, expression of pIgR was detected in oocyte and embryos, which suggested that the maternal Igs were transported by pIgR in turbot fish. Cytokines, hormones, hosts, microbes and environmental factors can stimulate some specific signaling pathways to modulate the expression of pIgR [32]. Upregulation of pIgR enhanced the transportation of IgA by mucosal epithelial cells. A single pIgR allows transport of a single IgA in transgenic mice, thus indicating that the number of pIgR restricts the transportation of IgA [33]. The extracellular domain of fish pIgR is presumed to have the ability to bind IgM. The expression of pIgR and IgM was detected in the mucosa associated tissues of many teleost fishes, suggesting that pIgR in teleost fish has a similar role in the mucosal immune system as in mammals.

The function of proteins is closely related to their location in the cell, as well as its own domain, the binding sites of protein interactions, phosphorylation sites, glycosylation sites, and other molecular structures. The turbot pIgR structure includes the domain containing two immunoglobulin, 19 phosphorylation sites, 6–8 potential glycosylation sites, and has a transmembrane region, which is in agreement with its transporter function. Additionally, through the establishment of eukaryotic expression system, and using indirect immunofluorescence assay showed that the pIgR was expressed in the cell membrane, and proved that turbot serum IgM could bind to pIgR expression vector. Similarly, pIgR-expressing HEK 293 T cells were found to be interacted with the IgG and IgM, and also showed the enhanced cell phagocytosis [31]. Although the detailed mechanisms of pIgR binding to different antibody need to be further examined, the binding capacity of the turbot pIgR to antibody were well demonstrated in current study.

5. Conclusion

We investigated the expression and localization of pIgR in the ovary and in embryos of turbot. The distribution profiles of pIgR may provide some information for study about the function of pIgR on maternal Igs transportation. In the following research, function of pIgR will be further explored to understand the process of maternal Igs transportation.

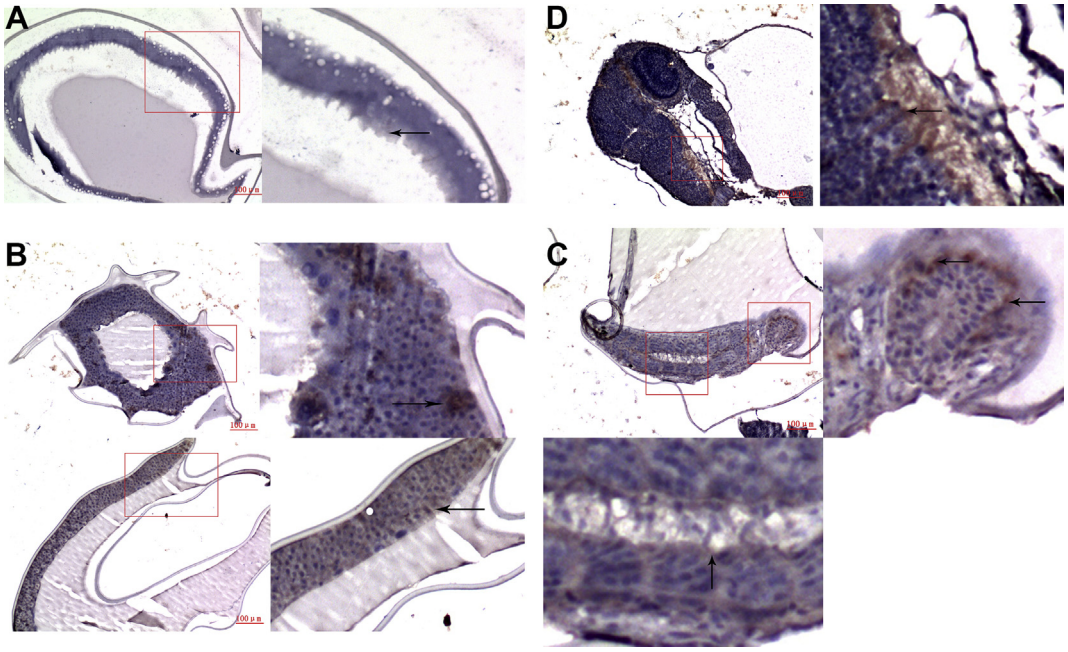

Fig. 4. Distribution of pIgR mRNA during development of turbot embryos.
The expression and distribution of pIgR mRNA in 0–5th day embryos using in situ hybridization. (A) Unfertilized egg. (B) 1st day embryo. (C) 4th-day embryo. (D) 5thDay embryo.
pIgR, Polymeric immunoglobulin receptor.

Acknowledgments

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