Functional characterization of melanocortin-3 receptor in rainbow trout (*Oncorhynchus mykiss*)

Hui-Xia Yu · Yang Li · Wei-Jia Song · Hui Wang · Hao-Lin Mo · Qiao Liu · Xin-Miao Zhang · Ze-Bin Jiang · Li-Xin Wang

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**Abstract** The melanocortin-3 receptor (MC3R) is an important regulator of energy homeostasis and inflammation in mammals. However, its function in teleost fish needs to be further explored. In this study, we characterized rainbow trout MC3R (rtMC3R), which encoded a putative protein of 331 amino acids. Phylogenetic and chromosomal synteny analyses showed that rtMC3R was closely related to bony fishes. Quantitative PCR (qPCR) revealed that the transcripts of rtMC3R were highly expressed in the brain and muscle. The cellular function of rtMC3R was further verified by the signal-pathway-specific luciferase reporter assays. Four agonists such as α-MSH, β-MSH, ACTH (1–24), and NDP-MSH can active rtMC3R, increasing the production of intracellular cAMP and upregulating MAPK/ERK signals. Moreover, we found that rtMC3R stimulated with α-MSH and NDP-MSH can significantly inhibit the NF-κB signaling pathway. This research will be helpful for further studies on the function of MC3R in rainbow trout, especially the role of energy metabolism and immune regulation.

**Keywords** Rainbow trout · Melanocortin-3 receptor · Molecular cloning · Tissue expression · Signaling

**Introduction**

Melanocortin receptors (MCRs) belong to the class A rhodopsin family of G protein-coupled receptors (GPCRs). To date, five members, namely, MC1R–MC5R, have been identified in mammals (Gantz and Fong. 2003). Although they all have 7 conserved transmembrane domains (TMDs) in their structure and can be activated by corresponding melanocortins, such as α-MSH, β-MSH, and adrenocorticotropic hormone ACTH among others, they exhibit differential tissue expression patterns and cellular functions (Novoselova et al. 2018). Among them, MC3R and MC4R are the subtypes mainly expressed in the central nervous system, where they play a crucial role in regulation of energy homeostasis. Studies have revealed that the mutations of *MC3R* and *MC4R* are closely related to obesity (Butler et al. 2000; Metherell et al. 2005), and knock-out of either of these receptors can result in obesity in mice (Patel et al. 2011). Notably, MC3R and MC4R employ different molecular mechanisms to regulate energy homeostasis. Functionally, MC4R mainly regulates food intake and energy
consumption (Balthasar et al. 2005), while MC3R is majorly involved in energy balance by controlling feed efficiency and circadian rhythm (Girardet and Butler. 2014). Additionally, recent reports have identified some anti-inflammatory effects associated with MC3R (Patel et al. 2011).

MC3R function depends on the stimulation of several endogenous ligands, namely, α-, β-, γ-MSH, and ACTH derived from the pro-opiomelanocortin (POMC). POMC activates the coupled Gα subunits, thereby triggering the downstream signal cascade (Cai and Hruby. 2016). Previous studies have shown that MC3R can be coupled with Gαs subunits to promote production of intracellular cyclic adenosine monophosphate (cAMP) and activation of protein kinase A (PKA) (Yang and Tao. 2017). After activating PKA, this receptor can protect IκB protein from phosphorylation, thereby inhibiting activation of the downstream NF-κB signaling pathways (Manna and Aggarwal. 1998). In addition, MC3R can also be coupled to Gαi protein to activate the MAPK/ERK signaling pathway in 293 T cells (Chai et al. 2007).

Although MC3Rs have been extensively studied in mammals, only a handful of studies have reported their functions in bony fish. One possible reason is the lack of MC3R in some fishes, such as fugu and medaka, and stickleback (Klovins et al. 2004a; Logan et al. 2003; Selz et al. 2007). Notably, tissue expression of mc3r in fish has only been reported in spiny dogfish (Klovins et al. 2004b), whereas the receptor’s pharmacological functions, including ligand bind properties and downstream signal regulation, have only been studied in a few species of fish, such as red stingray (Takahashi et al. 2016) and channel catfish (Yang et al. 2019). The rainbow trout, a cold-water fish belonging to Salmonidae family, is one of the most important cultivated fish species in the world, with its annual production exceeding 800 thousand tons (FAO 2020). MC3R-mediated endocrine regulation of signal energy metabolism is a promising approach for improving the growth rate and feed efficiency of rainbow trout and may circumvent the time-consuming and labor-intensive problems associated with traditional selective breeding methods. In the present study, we cloned the coding sequence (CDS) of rtMC3R then analyzed its tissue expression patterns, as well as cellular functions, including regulation of the cAMP, MAPK/ERK and NF-κB signaling pathways.

Materials and methods

Chemicals, reagents, and primers

The ligands [Nle⁴, D-Phe⁷]-α-MSH (NDP-MSH), ACTH (1–24), α-MSH, and β-MSH used for pharmacological assays were purchased from GenScript Biotechnology (Nanjing, China). TNF-α was purchased from Sigma Chemical (St. Louis, MO, USA). Primers for conventional PCR and real-time quantitative PCR were designed through primer premier 5 software (PREMIER Biosoft International, Palo Alto, USA) (Table 1), and they were synthesized by Sangon Biotech (Shanghai, China). The restriction enzymes required for molecular cloning were obtained from TaKaRa Biotechnology (Dalian, China).

Total RNA extraction

Three experimental rainbow trouts (mean standard length ± SEM: 39 cm ± 2.5 cm) were purchased from the Shitou River Rainbow Trout Farm (Shaanxi, China). The fish were anesthetized with ether and then dissected. The tissues, including the kidney, spleen, muscle, stomach, brain, and intestine, were collected to extract total RNA by using the Trizol method.

Reverse transcription

A total of 5 μg of RNA and 1 μL oligo-deoxythymidine were premixed to a volume of 12 μL. After a short centrifugation, the mix was incubated at 65 °C for 4 min and cooled on ice for 2 min. Then 20 mM deoxynucleotide triphosphate (dNTP), 200 U of Moloney murine leukemia virus (M-MLV),

| Table 1 Primers for molecular cloning and quantitative PCR |
|-----------------------------------------------------------|
| Primer name     | Primer sequence |
|-----------------|-----------------|
| rtMC3R-F        | AAGGCAGTCGTTGTTGCTCT |
| rtMC3R-R        | GCAGCATGACACACCTCCTA |
| qPCR-F          | AAGAACCTCCACTCCACA |
| qPCR-R          | GACGATGAGACTATCCCA |
| β-actin-F       | GGAATTTGATTAGCA |
| β-actin-R       | GTTACATGTGACACAGCA |
| gapdh-F         | AAGCTGGTCGATGTTGATGAC |
| gapdh-R         | GTTCACATCGTAGTTCG |
reverse transcriptase (TaKaRa, Dalian, China) and 4 μL of 5× reaction buffer were added into the reaction mix in a total volume of 20 μL. The reverse transcription reaction was performed at 42 °C for 60 min, followed by a finally heating at 70 °C for 5 min. These reverse-transcribed cDNA samples were used for gene cloning and quantitative PCR of rainbow trout mc3r.

**Molecular cloning of rainbow trout mc3r**

The coding sequence (CDS) of MC3R orthologue (MC3R-like) of rainbow trout was obtained from the National Center for Biological Information (NCBI, https://www.ncbi.nlm.nih.gov/) and then amplified by a PCR reaction. The reaction was conducted in a 10 μL system, which included 20 ng of cDNA template, 0.2 μM of upstream and downstream primers, and 5 μL of 2×Taq PCR Master Mix (Tiangen, Beijing, China). The amplification parameters were as follows: pre-denaturation at 94 °C for 5 min; denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s, extension at 72 °C for 1 min, 30 cycles; finally, extension at 72 °C for 10 min before the end of the reaction. Subsequently, 1.5% agarose gel electrophoresis was performed to verify the fragment size, and the PCR product with the expected size was isolated by a DNA gel extraction kit (Tiangen, Beijing, China). The purified DNA fragment was then sub-cloned into the pGEM-T easy vector (Madison, WI, USA) and transformed into E. coli (Escherichia coli). Clones containing insert fragments with expected size were sequenced.

**Homology, phylogenetic, and chromosome synteny analysis of rtMC3R**

The amino acid sequences of MC3R from different species were multiply aligned according to the sequence similarity by using Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/). The percentage of similarity was calculated with the same software either. The alignment was colored with DnaMAN 9.0 (LynnonBiosoft, CA, USA). The putative TMDs of rtMC3R were predicted through the online website TMHMM (http://www.cbs.dtu.dk/services/TMHMM/) and NCBI Conserved Domain Search (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi?). The phylogenetic tree of amino acid was constructed based on the Neighbor-joining (NJ) method (Saitou and Nei 1987) with Mega X. The strength of the branch relationship was assessed by 1000 bootstrap replications. The chromosome synteny was analyzed with Genomicus (http://www.genomicus.biologie.ens.fr/genomicus) and NCBI genomic browser (https://www.ncbi.nlm.nih.gov).

**Quantitative PCR for tissue distribution of rt-mc3r**

The expression of rt-mc3r in different tissues was measured by quantitative PCR (qPCR), with the geometric mean of the expression of β-actin and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) as the internal control (Bustin et al. 2009). The primers used are shown in Table 1. The qPCR was performed on an ECORT48 system in 10 μL reaction volume, containing 50 ng of cDNA template, 0.4 μm of forward and reverse primers, 5 μL of SYBR Green mix, and ddH2O as volume supplement. The operating parameter was set as follows: 95 °C for 30 s, followed by 30 cycles of 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 30 s. The PCR melting curves were depicted after amplification. All reactions were carried out in duplicate. The relative expression levels of different tissues were calculated based on the 2−ΔΔCT method (Schmittgen and Livak, 2008). The results were presented as mean ± SEM in arbitrary units (n = 6).

**Cell culture**

Human embryonic kidney (HEK) 293 T cells, obtained from American Type Culture Collection (ATCC, Manassas, VA, USA), were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS, Biological Industries, Kibbutz Beit Haemek, Israel) at 37 °C in 5% CO2-humidified atmosphere. The cells were maintained in a 25 cm²-cell-culture flask pre-coated with 0.1% gelatin at the density of 2×10⁵ cells/mL and passaged every 72 h.
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We first sub-cloned the coding sequence of rt-mc3r into pcDNA3.1 (+) and then co-transfected pcDNA3.1-rtMC3R (prt-MC3R) and each of three luciferase reporter vectors into HEK293T cells. The three luciferase reporter gene vectors, pGL4.29, pGL4.33 (Promega, Madison, WI, USA), and pNF-κB-Luc (Clontech, Palo Alto, CA, USA), contain cAMP response element (CRE), serum response element (SRE), and NF-κB (κB) response element in their promoter regions, respectively, which can monitor the activation of cAMP, MAPK/ERK, and NF-κB signaling pathways accordingly. The brief procedure of the test is as follows: the HEK293T cells were passaged to a 6-well plate and grew for 24 h before transfection. Then the cells were co-transfected using a mixture containing 1000 ng of luciferase reporter vector, 500 ng of receptor expression plasmid (or empty pcDNA3.1), 300 ng of pEGFP-N1 (as an internal control for transfection normalization), and 4 μL of PEI transfection reagent (Fushen Biotechnology, Shanghai, China). The transfected cells were cultured in the original medium for 24 h, and then pipetted down and transplanted in a 48-well plate to incubate at 37 °C for another 24 h before ligand treatment. The ligands, such as α-MSH, β-MSH, NDP-MSH, and ACTH (1–24) were diluted by serum-free DMEM medium to the working concentration and then added into the 48-well plate to treat cells for 6 h. After the treatment, cells were lysed with 1× passive lysis buffer (Beyotime Biotechnology, Shanghai, China), and then luciferase substrate was added for reaction. The luciferase activity was measured with an Infinite F200 microplate reader (Tecan, Männedorf, Switzerland). In addition, the density of cells used was a 48-well plate with 2×10^5 per well, and there were three additional 48-well plates technical replicates. All data were shown as mean±SEM.

Statistical analysis

The tissue expression of rt-mc3r uses the 2^{−ΔCT} method to calculate the expression of each tissue relative to the Internal control gene (Schmittgen and Livak, 2008) and then uses the one-way ANOVA method to test the equality and Turkey’s HSD method was applied for post comparison.

The luciferase activities of the transfected cells treated with different concentrations of ligand were converted into a relative multiple of the treatment group relative to the control group (DMEM serum-free medium). Data analysis was processed by Graphpad Prism 8 software (Graphpad Software, San Diego, CA, USA), and we carried out nonlinear regression analysis, followed by a dose–response-stimulation model. The luciferase activities of the transfected cells treated with TNF-α and MC3R agonist (α-MSH or NDP-MSH) were converted into a change fold of the treatment group relative to the internal control (EGFP). Data were analyzed by the one-way ANOVA method and Turkey’s HSD post comparison. For verification results, each test was repeated 3 times.

Results

Nucleotide and deduced amino acid sequences of rtMC3R

As shown in Fig. 1, the obtained rainbow trout mc3r (accession number: MW884245) contains an open reading frame of 996 bp encoding a putative protein of 331 amino acids. Similar to human MC3R (hMC3R), the receptor we studied had 7 putative hydrophobic transmembrane domains (TMDs). Three conserved domains PMY, DRY, and DPVIY in rtMC3R can be found in TMD2, TMD3, and TMD7, respectively. Residues important for ligand binding in hMC3R were also found in rtMC3R, such as E99 (E131 in hMC3R) in TM2, D122 (D154 in hMC3R), and D126 (D158 in hMC3R) in TM3, F261 (F295 in hMC3R), and H264 (H298 in hMC3R) in TM6. Besides, the ORF exhibited 3 N-glycosylation sites (NNT, NET, and NIT) in the extracellular N-terminus and 3 phosphorylation sites in the C-terminus through prediction.

Alignment of the amino acid sequence of rtMC3R with other species showed that rtMC3R with that of other fish had high homology (Oncorhynchus tshawytscha: 99.7%, Danio rerio: 73.85%), whereas the homology of rtMC3R to mammal MC3Rs was relatively low (Sus scrofa: 70.67%, Chelonia mydas: 70.65%). As shown in Fig. 2, rtMC3R was 31 residues shorter at the N-terminus and 3 residues longer at the C-terminus when compared with human MC3R. The amino acid sequence of rtMC3R was
related to salmonoids MC3R. The clade of bony fishes and was more evolutionarily consistent in TMDs and intracellular loops (ICLs) to that of other species while less consistent in N-terminus and extracellular loops (ECLs).

Phylogenetic and chromosome synteny analyses of rtMC3R

We performed phylogenetic analysis on the amino acid sequences of MC3R from different species to assess the evolutionary relationship between the putative rtMC3R and other vertebrate MC3Rs. An NJ tree based on amino acid sequences of MC3Rs is as shown in Fig. 3. The rtMC3R was nested into the clade of bony fishes and was more evolutionarily related to salmonoids MC3R.

To determine whether rt-mc3r was orthologous to the genes in other vertebrates, synteny analysis was performed in Atlantic salmon, channel catfish, zebrafish, humans, horses, and mice. The results were shown in Fig. 4; the adjacent genes of the rt-mc3r, including tcf15, slc13a3, and srxn1, were consistent with these genes in Atlantic salmon, channel catfish, and zebrafish. However, no conserved synteny was observed in adjacent genes of MC3R between rainbow trout and mammals.

Fig. 1 Nucleotide sequence of rt-mc3r and its deduced amino acid sequence. The position information of nucleotides and amino acids was marked on both sides. Dark gray shading indicates 7 transmembrane structure regions, and three conserved motifs were highlighted with black solid boxes (PMY, DRY, DPVIY). The glycosylation site at the N-terminus was shown in the dashed box. Amino acid residues important for ligand binding were filled with light gray. The phosphorylation site at the C-terminus was highlighted with black circles. The initiation codon (ATG) and a termination codon (TAG) were underlined.

Tissue expression of rainbow trout mc3r

The tissue expression of rainbow trout mc3r was analyzed by qPCR. As shown in Fig. 5, the mRNA was predominantly expressed in the brain and muscle, followed by the liver, intestine, gonad, and stomach. The lowest expression was found in the spleen and kidney.

The functional characteristics of rtMC3R in HEK293T cells

To determine whether rtMC3R can function as a GPCR, rtMC3R was transiently expressed in HEK293T cells and treated with different concentrations of α-MSH, β-MSH, ACTH (1–24), and NDP-MSH. A luciferase reporter vector with a CRE in the promoter (pGL4.29) was used to monitor receptor-mediated cAMP accumulation and pGL4.33 (with SRE in promoter) was used to monitor MAPK/ERK activation. As shown in Figs. 6 and 7, all four agonists activated cAMP and MAPK/ERK signaling pathways via rtMC3R in a dose–response manner, while the luciferase activity of cells transfected with the empty vector pcDNA3.1 (+) did not appear obvious changes.
These four agonists had different potencies for the above two pathways (Table 2). For the cAMP signaling pathway, ACTH (1–24) had the strongest potency (EC\textsubscript{50} = 0.63 μM × 10^{-8} M), followed by NDP-MSH, α-MSH, and β-MSH. In terms of the concentration for 50% of maximal effect (EC\textsubscript{50}) of the MAPK/ERK activation, the order of efficacy was NDP-MSH, β-MSH, α-MSH, and ACTH (1–24).

To determine whether rtMC3R was involved in regulating the NK-κB signaling pathway, we co-transfected HEK293T cells with prtMC3R and the luciferase reporter genes pNFκB-Luc which had NF-κB enhancer elements upstream of the promoter. Results showed that (Fig. 8), cells transfected with prtMC3R could significantly inhibit TNF-α induced NF-κB elevation, whereas cells transfected with pcDNA3.1 (+) empty vector have no similar results. In addition, NDP-MSH showed a better inhibitory effect than α-MSH.

**Discussion**

Results of multiple sequence alignments and several structural features confirmed that the cDNA we cloned was indeed rt-mc3r (Fig. 1 and Fig. 2). The rtMC3R is highly conserved (> 90% identity) with other bony fishes in amino acid sequence. Structurally, rtMC3R, which is similar to mammalian MC3Rs, exhibited classic GPCR characteristics, including the 7-TM domain and the conserved motifs PMY, DRY, and DPxxY (Huang and Tao, 2014; Yang et al., 2015). Notably, the NPxxY motif...
DPVIY is only found in rainbow trout and Chinook salmon, while DPLIY exists in humans (Homo sapiens), mouse (Mus musculus), western clawed frog (Xenopus tropicalis), and zebrafish (Danio rerio), indicating a unique evolutionary status of salmonids. Moreover, analysis of rtMC3R revealed several residues that were important for ligand recognition in both rtMC3R and hMC3R, including

![Phylogenetic tree of MC3Rs](image)

Fig. 3 Phylogenetic tree of MC3Rs. The tree was constructed using the Neighbor-Joining (NJ) method. The number on the node represents the guide value (expressed as a percentage) obtained for 1000 repetitions. The diamond indicates rtMC3R. MC3Rs: Oncorhynchus tschawytscha (XP_024229914.1), Oncorhynchus kisutch (XP_020360426.1), Schleipapges femoros (XP_018615783.1), Chelonia mydas (XP_007059824.1), Mus pahari (XP_021049931.1), Taricha granulosa (AAX18229.1), Canis lupus familiaris (NP_001128596.1), Cricetulus griseus (XP_003501490.1), Tyto alba alba (XP_009967958.1), Aperyx rovi (XP_025911964.1), Cyanistes caeruleus (XP_023795719.1), Latimeria chalumnae (XP_005990660.1), Chelonia mydas (XP_007059824.1), Homo sapiens (AKI72214.1), Mus musculus (NP_032587.1), Sus scrofa (AFK25142.1), Danio rerio (AA62747.1), Xenopus tropicalis (XP_002935436.1)

![Chromosome synteny analysis of MC3Rs](image)

Fig. 4 Chromosome synteny analysis of MC3Rs in different species. The synteny genes were represented by boxes and connected by black lines. The MC3R genes were indicated by black-filled box. The conserved synteny of fish were represented by dark gray boxes, while in other species, they were filled with white. Unknown genes were highlighted in light gray boxes.

Rainbow trout
Chr, 9
Atlantic salmon
Chr, ssa15
Channel catfish
Chr, 5
Zebra fish
Chr, 8
African ostrich
Chr, AL206811.1
Human
Chr, 20
Horse
Chr, 22
Mouse
Chr, 2
E99, D122, D126, F261, and H264, indicating that rtMC3R might have similar ligand-binding sites to hMC3R (Yang and Harmon, 2017). Results from phylogenetic and synteny analyses further supported the conservation of MC3Rs in bony fishes from the evolutionary perspective.

In mammals, MC3R is mainly expressed in the central nervous system (CNS), peripheral tissues, and immune cells, including the brain, intestine, and placenta, among others (Gantz et al. 1993). Functionally, it plays a crucial role in the regulation of energy metabolism, cardiovascular function, and inflammation (Getting. 2006). In the present study, results from real-time qPCR showed that mc3r was predominantly expressed in the brain of the rainbow trout, which was consistent with previous studies in mammals (Gantz and Fong. 2003) and some cartilaginous fishes, such as spiny dogfish (Squalus acanthias) (Klovins et al., 2004b) and stingray (Dasyatis akajei) (Takahashi et al. 2016). These results suggest that rtMC3R may be involved in the CNS-regulated energy homeostasis. The mRNA was also highly expressed in the muscle, which was consistent with the results obtained in Megalobrama amblycephala (Liao et al. 2019). Moreover, we also detected its expression in the stomach, liver, gonads, intestines, and kidney. Since the liver, kidneys, and intestines are crucial immune organs in fish, we inferred that rtMC3R may also be playing
a role in regulation of inflammatory response, just like in mammals.

Previous studies have shown that mammalian MC3R is coupled to Gαs protein and can activate the cAMP and MAPK/ERK signaling pathways (Chai et al. 2007; Lee et al. 2001). To determine whether rtMC3R has similar functions, we used the luciferase reporter system to detect activity of the aforementioned pathways after treatment with four different agonists. Results showed that ACTH (1–24) had the highest potency in activating the cAMP signaling pathway, followed by NDP-MSH, α-MSH, and β-MSH. The high activation efficacy of ACTH (1–24) in this study is similar to that reported in channel catfish (Yang et al. 2019) but is different from that of pigs (Fan et al. 2008). ACTH (1–24) is considered a "primitive" ligand and exhibits high and low affinity for MCRs in fishes (Klovins et al. 2004b; Li et al. 2016) and mammals (Lisak and Benjamins 2017), respectively. Our results provide further evidence to support the concept that ACTH may be the “original” ligand for the ancestral melanocortin receptors (Dores et al. 2014; Dores and Baron 2011). MAPK/ERK signals play a key role in regulation of energy homeostasis (Yang and Tao 2017). Results of the present study demonstrated that the synthetic ligand NDP-MSH has a higher activation potency for the MAPK/ERK signaling pathway, than the endogenous ligands, which is similar to that observed in mammals (Fan et al. 2008). The differences in activation efficacies among the four ligands on cAMP and MAPK/ERK signals suggest that structural differences between fish and mammalian MC3R may bring about

### Table 2: The signaling properties of rtMC3R in response to ligand stimulation

| Ligand      | cAMP response | ERK1/2 response |
|-------------|---------------|-----------------|
|             | EC₅₀ (μm)     | EC₅₀ (μm)       |
| α-MSH       | 4.33 ± 1.52   | 0.50 ± 3.19     |
| β-MSH       | 9.31 ± 1.57   | 0.48 ± 2.33     |
| ACTH (1–24) | 0.63 ± 1.01   | 0.83 ± 3.71     |
| NDP-MSH     | 2.93 ± 6.47   | 0.09 ± 1.09     |

Values were expressed as the mean ± SEM of at least three independent experiments. Abbreviations: α-MSH α-melanocyte-stimulating hormone, β-MSH β-melanocyte-stimulating hormone, ACTH adrenocorticotropic hormone, NDP-MSH [Nle⁴, D-Phe⁷]-MSH. EC₅₀ were calculated from fitting curves based on triplicate measurements within 1 experiment.
The NF-κB signaling system is composed of NF-κB dimer, IκB modulator, and IKK. Upon activation and reception of specific signals, this system triggers occurrence of some physiological or immune processes, such as inflammation, immunity, and oxidative stress, among others (Mitchell et al. 2016). Previous studies have reported that ligand-mediated activation of the human MC3R activates PKA by elevating cAMP levels, and protects the IκB protein from phosphorylation, thereby inhibiting NF-κB nuclear translocation (Manna and Aggarwal. 1998).

In this study, we added a premix of NDP-MSH or α-MSH and TNF-α to cells transfected with rtMC3R and found that activated rtMC3R inhibited the NF-κB signal (Fig. 8). These results were consistent with those observed in the human central and peripheral nervous systems (Ichiyama et al. 1999; Teare et al. 2004), affirming that rtMC3R also has an anti-inflammatory function following its activation.

In conclusion, we cloned the evolutionarily highly conserved mc3r from rainbow trout and investigated its tissue expression patterns as well as signaling properties. We found that this gene was highly expressed in the brain and muscles, and also abundant in other peripheral tissues. Results from functional analyses in cells indicated that endogenous and synthetic ligands can activate the cAMP and MAPK/ERK signaling pathways, through rtMC3R, albeit at different potencies. Furthermore, rtMC3R regulated the inflammation-related NF-κB signaling pathway. Future studies are expected to elucidate the function of rtMC3R in the muscle and peripheral tissues of rainbow trout.

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Author contribution Li-Xin Wang conceived and designed this study; Hui-Xia Yu and Yang Li cloned rainbow trout mc3r, performed the cellular experiment, and prepared the manuscript; Wei-Jia Song, Hui Wang, and Hao-Lin Mo collected the samples; Qiao Liu, Xin-Miao Zhang, and Ze-Bin Jiang extracted RNA and examined the tissue distribution of rtMC3R.

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Data availability The data and materials that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval The Faculty Animal Policy and Welfare Committee of Northwest A&F University under contract (NWFU-314020038) approved this experiment, and the experimental process complied with protocols of international guidelines for the ethical use of animals in research.
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