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Review Article

Melanocortin-4 Receptor in Fish: A Review

Abstract

The melanocortin-4 receptor (MC4R) belongs to the melanocortin system, has been well investigated in mammals. Which plays important roles in several physiological processes, such as regulating energy homeostasis, cachexia, cardiovascular function, glucose and lipid homeostasis, reproduction and sexual function, drug abuse, pain perception, brain inflammation, and anxiety. Although there are some studies have performed, its role still remain mystery in fish. This review will summarize the studies on the fish MC4R from its cloning and tissue distribution to its physiological roles. Which should be useful for further studies in fish and might provide a new insight in aquaculture industry.

acids. Alignment of MC4R with other MCRs showed that it has the highest homology with the MC3R, with 58% identity and 76% similarity. By fluorescent in situ hybridization, the MC4R gene was localized to chromosome 18q21.3 [9,10].

Since the cloning of hMC4R, the MC4R has been cloned from mouse, rat, hamster, guinea pig, dog, cat, fox, pig, sheep, cow, and several primates including marmoset, cynomolgus macaque, vervet monkey, and orangutan. It has also been cloned from several nonmammalian species including fish, chicken, and pigeon. The amino acid sequences between the different species are highly conserved [11,12].

It has been proved that MC4R is involved in regulating energy homeostasis in mammals [7]. Knockout of the MC4R resulted in increased food intake and decreased energy expenditure therefore obesity in mouse [13]. A similar phenotype is also observed in mice ubiquitously overexpressing Agouti or AgRP genes [14,15]. The frameshift mutations found in MC4R lead to a dominant form of obesity in human [16,17]. Furthermore, central administration of the MC4R agonist α -MSH has been shown to inhibit appetite and increase basal metabolic rate [18]. Conversely, MC4R antagonism by AgRP results in hyperphagia and decreased metabolic activity.

Recent years, despite the MC4R has been cloned from several fishes, the physiological functions are still very limited to us. Herein we summarize the studies of MC4R in fish, including the molecular cloning, tissue distribution and physiology, which should be useful for us to understand the MC4R evolutionary history in vertebrate, as well as improving the aquaculture industry.

Introduction

The melanocortin system is an ancient system conserved from teleosts to mammals. It consists of four agonists, two antagonists, and five receptors. The four agonists including α , β , and γ melanocyte-stimulating hormone (MSH), and adrenocorticotropin (ACTH), are derived from tissue-specific posttranslational processing of the prohormone proopiomelanocortin (POMC) [1-3]. The two endogenous antagonists are consist of agouti and agouti related peptide (AgRP), Agouti is an antagonist for MC1R whereas AgRP for MC3R and MC4R [2,4]. The five melanocortin receptors are called melanocortin receptors 1-5 (MC1R-MC5R) based on the sequence of their cloning. They are all members of Family A G protein coupled receptors (GPCRs) consisting of seven transmembrane domains (TMs) connected by alternating intracellular and extracellular loops, with the N-terminus extracellular, and the C-terminus intracellular [5-7].

MC4R is a member of family A GPCRs with seven TMs connected by alternating extracellular loops (ELs) and intracellular loops. The molecular processes involved in the binding of the MSH peptides to the MC4R were extensively studied. Most notably, the acidic residues Asp^{122/126} (TM3) and the basic residue His²⁶⁴ (TM4) are essential for ligand binding [8].

The human MC4R is an intron less gene with an open reading frame of 999 bp that encodes a protein of 332 amino

Molecular cloning of the fish MC4R

The MC4R was first cloned from zebrafish in 2002 [19], since then, more and more MC4R were cloned from different fishes. To date, there are more than twenty fish MC4R can be found in the National Center for Biotechnology Information database (NCBI: <https://www.ncbi.nlm.nih.gov/>), including Osteichthyes fishes, Chondrichthyes fishes and Cyclostomata fishes (Table 1). The Osteichthyes fishes are the primary investigated object involved in five orders including Perciformes (*Dicentrarchus labrax*, *Scatophagus argus*, *Astatotilapia burtoni* and *Channa argus*), Pleuronectiformes (*Verasper moseri* and *Paralichthys olivaceus*), Tetraodontiforms (*Takifugu rubripes*, *Takifugu chinensis* and *Tetraodon nigroviridis*), Salmoniforms (*Oncorhynchus mykiss*) and Cypriniforms (*Danio rerio*, *Cyprinus carpio*, *Carassius auratus*, *Ctenopharyngodon idella*, *Luciobrama microcephalus*, *Schizothorax prenanti* and *Xiphophorus maculatus*). The Chondrichthyes fishes involved in three Orders including Squaliformes (*Squalus acanthias*), Heterodontiformes (*Heterodontus francisci*) and Myliobatiformes (*Dasyatis akajei*). The Cyclostomata fish only including one kind of Petromyzoniformes fish (*Lampetra fluviatilis*).

Tissue distribution of the fish MC4R

In mammals, over the past two decades, since the initial cloning [9,36], significant attention has been paid to the melanocortin-4 receptor (MC4R), due to its central expression and roles in regulation of energy homeostasis and body

weight. Simultaneously, it also expressed in some peripheral tissues such as heart, lung, kidney medulla, renal nerve, ureter, intercostal muscle and skull bone [37]. The extensive expression of mammalian MC4R suggested it could be involved in many physiological processes.

In tetrapods, five MCRs (MC1R–MC5R) have been identified. Of the five MCRs, only MC3R and MC4R are significantly expressed within the central nervous system, hence these two MCRs are also called neural MCRs [38,39]. MC4R is also expressed in some peripheral tissues such as enteroendocrine L cells [40].

In fish, the MC4R is extensively expressed in a variety of tissues. Cerda-Reverter et al. reported that MC4R was expressed in gill, spleen, retina, and ovaries in goldfish [27], Kobayashi et al. and Ringholm et al. reported that the flounder MC4R was mainly expressed in liver, ovary, and testis [19,22]. Li et al., reported that the spotted scats MC4R was found to be expressed in the brain, pituitary, and gonads in both male and female [21]. Further research showed that common carp MC4R was highly expressed in brain, testis, and eye, followed by expression in the pituitary and heart [26]. Recent study on grass carp showed that MC4R was found to be highly expressed in the brain and eye, but expressed at low levels in the muscle, heart, intestine, liver, gill, spleen, and kidney [28]. The extensive expression pattern of MC4R in fish suggested it might act as different physiological functions in different tissues.

Physiology of the fish MC4R

Regulating food intake and energy homeostasis: The roles of mammalian MC4R in regulating energy homeostasis were well studied [7]. MC4R knockout mice exhibit maturity-onset obesity, hyperphagia, increased linear growth, hyperinsulinemia, hyperglycemia, and delayed meal termination and reduced sensitivity to cholecystokinin [13]. Conversely, MC4R over expressed mice found to be obese, similar to mice over-expressing AgRP [41]. In addition, Human genetic studies demonstrated that mutations in MC4R are the most common form of monogenic obesity, characterized by its early-onset and severity [2,16,17,39]. These studies suggest that MC4R plays a key role in regulating energy homeostasis and body weight in mammals including human.

Recent studies showed that the mechanism of regulation of energy homeostasis by the MC4R is also operational in lower vertebrates including fish. In goldfish, ICV injection of NDP-MSH or MTII inhibits food intake, whereas the MC4R-specific antagonist HS024 increases food intake [27,42]. These experiments suggested that the MC4R is exerting a tonic inhibitory effect on food intake. Similar results were obtained in rainbow trout, ICV injection of MTII decreases food intake, whereas ICV injection of HS024 and the MC3/4R antagonist SHU9119 increases food intake in rainbow trout [43]. Moreover, nonsynonymous mutations in cavefish MC4R are identified to contribute to enhanced appetite, growth, and starvation resistance [44]. Taking together, these studies indicating the piscine also involved in regulating food intake and energy homeostasis.

Table 1: Melanocortin-4 receptor identified from the main fish species.

Class	Order	Species	References
Osteichthyes	Perciformes	<i>Dicentrarchus labrax</i>	Sánchez et al. [20]
		<i>Scatophagus argus</i>	Li et al. [21]
		<i>Astatotilapia burtoni</i>	Unpublished
		<i>Channa argus</i>	Unpublished
	Pleuronectiformes	<i>Verasper moseri</i>	Kobayashi et al. [22]
		<i>Paralichthys olivaceus</i>	Lee et al. [23]
	Tetraodontiforms	<i>Takifugu rubripes</i>	Klovins et al. [24]
		<i>Takifugu chinensis</i>	Unpublished
		<i>Tetraodon nigroviridis</i>	Unpublished
	Salmoniforms	<i>Oncorhynchus mykiss</i>	Haitina et al. [25]
	Cypriniforms	<i>Danio rerio</i>	Ringholm et al. [19]
		<i>Cyprinus carpio</i>	Wan et al. [26]
		<i>Carassius auratus</i>	Cerda-Reverter et al. [27]
<i>Ctenopharyngodon idella</i>		Li et al. [28]	
<i>Luciobrama macrocephalus</i>		Tao et al. [29]	
		<i>Schizothorax prenanti</i>	Wei et al. [30]
		<i>Xiphophorus maculatus</i>	Volff et al. [31]
Chondrichthyes	Squaliformes	<i>Squalus acanthias</i>	Ringholm et al. [32]
	Heterodontiformes	<i>Heterodontus francisci</i>	Baron et al. [33]
	Myliobatiformes	<i>Dasyatis akajei</i>	Takahashi et al. [34]
Cyclostomata	Petromyzoniformes	<i>Lampetra fluviatilis</i>	Haitina et al. [35]

Modulating reproductive function: Several recent studies suggested that the MC4R is also involved in modulating reproductive function through affecting the secretion of reproductive hormones, and consequent sexual maturation in fish [7]. Moreover, the MC4R was found to be highly expressed in gonads of several fish, such as goldfish [42], Ya-fish [30], and snakeskin gourami [45]. In addition, some previous studies revealed that MC4R copies located on the sex chromosomes participate in the functional modulation of onset of sexual maturity in both male and female swordtails and platyfish, suggesting the potential participation of MC4R in the regulation of reproduction [31,46]. Hence, understanding how MC4R mediates the reproductive function in economically important species is important for artificial breeding.

Other functions of the MC4R: The functions of fish MC4R are very limited, it might be involved in some physiological functions such as mediating body color, modulating blood glucose homeostasis and other miscellaneous functions. However, these hypotheses have not yet established, and it need to be further studied.

Conclusions and Future Directions

Tremendous progress has been made on the MC4R since 1993 when it was cloned. As an important transmembrane protein, it's involved in many physiological processes not only in higher vertebrate but also in lower vertebrate. Although it has well studied in mammals, there are still exist many mysteries need to be resolved especially in fish. In future, the MC4R should be well known by people following extensive research carrying out, and it might be as a useful tool for improving aquaculture industry.

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