# Topmouth culter (*Culter alburnus*) melanocortin-3 receptor: regulation of pharmacology by two isoforms of melanocortin receptor accessory protein 2



Ren-Lei Ji<sup>1</sup>, Lu Huang<sup>2</sup>, Yin Wang<sup>1</sup>, Si-Yu Fan<sup>2</sup>, Ting Liu<sup>1</sup>, Min Tao<sup>1,2</sup>, Shao-Jun Liu<sup>2</sup>, Ya-Xiong Tao<sup>1</sup> <sup>1</sup> Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, AL 36849, United States <sup>2</sup> State Key Laboratory of Developmental Biology of Freshwater Fish, College of Life Sciences, Hunan Normal University, Changsha, 410081, Hunan, PR China

## ABSTRACT

Melanocortin-3 receptor (MC3R) is a regulator in energy homeostasis, and interaction of MC3R and melanocortin receptor accessory protein 2 (MRAP2) plays a critical role in MC3R signaling of vertebrates. However, the physiological roles of MC3R in teleosts are not well understood. Herein we cloned topmouth culter *mc3r* with a 984 bp open reading frame encoding a protein of 327 amino acids. qRT-PCR indicated that *mc3r* had higher expression in the central nervous system. In the periphery, *mc3r* was expressed in liver, testis and head kidney in the male, whereas it was mainly present in skin and ovary in the female. Culter MC3R expressed in HEK293T cells could bind to five peptide agonists with a higher B<sub>max</sub> than human MC3R (hMC3R). In addition, caMC3R had higher affinity to ACTH and lower affinity to D-Trp<sup>8</sup>-γ-MSH compared to hMC3R. All agonists could stimulate caMC3R and increase dose dependently intracellular cAMP accumulation. Culter MC3R showed a higher constitutive activity in cAMP signaling, higher efficacies and  $R_{max}$  to  $\alpha$ -MSH, des- $\alpha$ -MSH, ACTH and D-Trp<sup>8</sup>- $\gamma$ -MSH. Culter MRAP2a remarkedly decreased cell surface expression but had no significant effect on total expression of caMC3R, whereas caMRAP2b had no effect on both cell surface and total expression of caMC3R. CaMRAP2a and caMRAP2b both remarkably decreased basal cAMP production. Furthermore, caMRAP2a significantly decreased B<sub>max</sub> and R<sub>max</sub>, while caMRAP2b did not affect ligand binding and agonist-stimulated cAMP. These results will promote further research on the physiological roles of MC3R in topmouth culter.

## INTRODUCTION

The MC3R plays a vital role in regulating energy homeostasis as well as several other physiological functions.

MC3R is Family A G protein-coupled receptors (GPCRs) and it primarily couples to G protein (Gs) to activate adenylyl cyclase, leading to increased intracellular cyclic adenosine mono phosphate (cAMP).

Previous studies indicated that teleost MC3Rs show different pharmacological characteristics and physiological functions with mammalian MC3Rs.

## MATERIALS AND METHODS

- 1. qRT-PCR was used to measure genes tissue distributions.
- 2. HEK293T cells were transiently transfected with hMC3R or caMC3R.
- 3. Ligand binding assays were performed using <sup>125</sup>I-NDP-MSH with or without different concentrations of unlabeled ligands.
- 4. Radioimmunoassay was used to detect cAMP levels.
- 5. Flow cytometry was used to determine receptors cell surface and total expression.

## RESULTS

1	ATG	AAC	AAC	TCG	TAC
1	М	Ν	Ν	S	Υ
61	сст	тст	AAT	GGC	AGT
21	Р	s	Ν	G	S
121	CAG	GCA	GAG	GTT	ТТТ
41	Q	Α	Е	V	F
181	TCG	GCT	GTG	GTC	AAA
61	S	Α	V	V	Κ
241	GCT	GCT	GCG	GAC	ATG
81	Α	Α	Α	D	Μ
301	СТА	AAC	AGT	CGC	ATT
101	L	Ν	s	R	Т
361	GAC	TCA	ATG	ATC	TGC
121	D	S	Μ	1	С
421	GAC	CGG	TAC	GTC	ACG
141	D	R	Υ	V	Т
481	GCG	CTG	GTC	GCA	ATC
161	Α	L	V	Α	1
541	GTG	TAC	тст	GAG	AGC
181	V	Y	S	Е	S
601	GTT	стс	ATG	GCA	ACT
201	V	L	М	Α	Т
661	ATC	GCA	GCA	TTA	CCC
221	Т	Α	Α	L	Ρ
721	TGC	ATG	AAG	GGA	GCC
241	С	М	Κ	G	Α
781	ccc	TTT	ттс	стс	CAC
261	Р	F	F	L	Η
841	TAC	ATG	тсс	CAC	TTC
281	Y	М	S	н	F
901	стс	ATC	TAC	GCC	TGC
301	L		Υ	Α	С
961	TTT	GGC	TGC	CAA	CCT
321	F	G	С	Q	Ρ





```
CTG CAA TTC ATT AAA GGA CAG AAA CCT GCT AAC AGC ACA TCT TTG 60
LQFIKGQKPANSTSL20
ACT GTG GAT CCT CCA GCA GGG GCG CTG TGC GAG CAG GTC CAG ATC 120
 TVDPPAGALCEQVQI40
CTC ACC TTG GGT ATT GTG AGT CTT CTG GAG AAC ATA CTC GTC ATC 180
            IVSLL
 AAC AAA AAC CTT CAC TCT CCA ATG TAC TTT TTC CTG TGC AGC CTG 240
          L H S P M Y F F L C S L 80
TTG GTA AGT GTA TCG AAC TCT CTG GAG ACC ATT GTC ATT GCA GTA 300
          V S N S L E T I V I A V 100
TTG GTG GCC AGT GAT TAT TTT GTA CGT TTG ATG GAC AAT GTG TTT 360
         S D Y F V R L M D N V F 120
ATT TCT CTT GTG GCG TCC ATC TGC AAC CTT CTG GCC ATT GCC GTC 420
            ASICNLLAI
                                       A V 140
G ATT TTC TAC GCC TTA CGC TAC CAC AGT ATA GTG ACT GTA CGT AGA 480
         ALRYHSIVTV RR160
GCT GCG ATC TGG CTG GTG TGT GTG GTT TGT GGG ATC GTC TTT ATA 540
AAG ACC GTG ATC GTG TGT CTA ATC ACA ATG TTC TTT GCC ATG CTG 600
         IVCLITMFFAML200
CTC TAC GTA CAC ATG TTT CTT CTC GCC AGA CTT CAT GTC CAG AGA 660
            MFLLAR
CCA GCA GCA GCT GCC GCT GGC AAC CCG GCC CCA CGT CGA CAC AGC 720
         A A A G N P A P R R H S 240
GTG ACC ATC TCC ATC CTC CTC GGA GTG TTT GTG TGT TGC TGG GCG 780
            ILLGV
CTC ATT CTG CTG GTG TCG TGT CCG CAC CAT CCG CTG TGC CTC TGC 840
         L V S C P H H P L C L C 280
ACC ACG TAC CTG GTC CTC ATT ATG TGC AAC TCT GTG ATT GAC CCC 900
TTYLVLIMCNSVIDP300
CGC AGC CTG GAA ATG AGG AAG ACT TTT AAG GAG ATA CTC TGC TGT 960
RSLEMRK TFK EILCC320
TCA CTT TAG
S L *
```

Fig. 1. Nucleotide and deduced amino acid sequences of caMC3R













### Fig. 8. The effect of caMRAP2s on caMC3R in cAMP signaling

## CONCLUSIONS

- 1. Culter *mc3r* was primarily expressed in the central nervous system.
- 2. Culter MC3R had high constitutive activity.
- 3. Culter MRAP2a and MRAP2b had different effects on cell surface expression, ligand binding and cAMP production of caMC3R.