

Article

MRAP2 Interaction with Melanocortin-4 Receptor in Snakehead (*Channa argus*)

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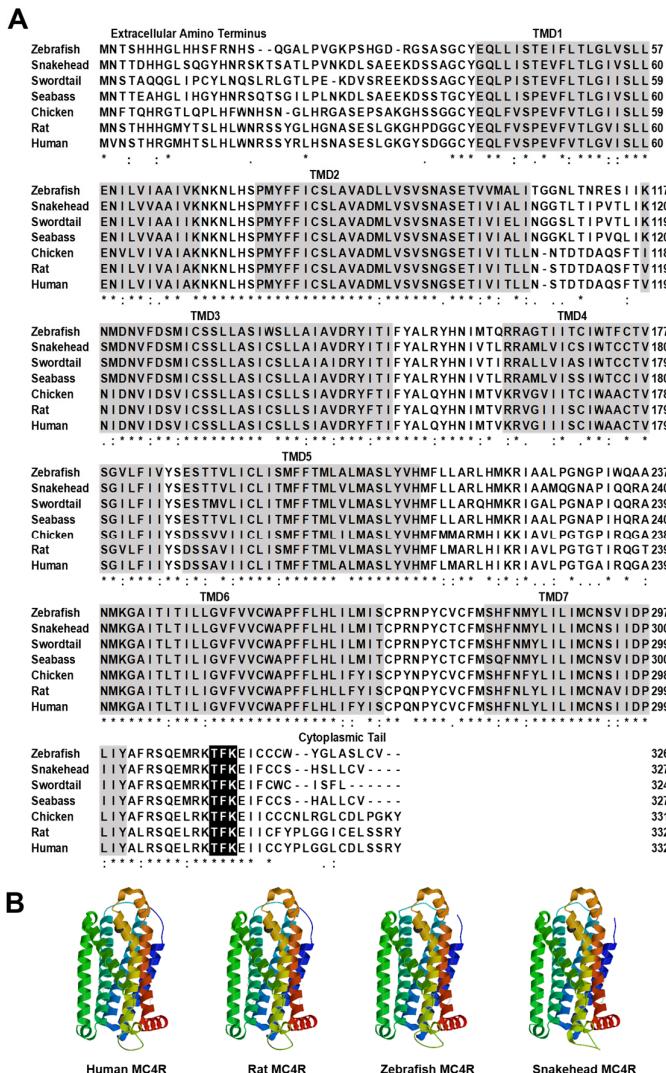


Figure S1. Comparison of amino acid sequences between caMC4R and MC4Rs from other species (A) and the putative three-dimensional structure of snakehead MC4R and those of relative model species (B). Labeled as follows: trans-membrane domains were shown in shaded boxes and named as TMD 1-7, amino and carboxyl termini were represented as extracellular amino terminus and cytoplasmic tail, respectively. Predicted phosphorylation sites were shown by dark shadow. Asterisk (*) indicated the same amino acids. Cellular was shown by a green frame box, followed with an extracellular N-terminus and intracellular C-terminus, respectively. Seven transmembrane domains were shown in the figure. Amino acid was represented by a circular round.

Figure S2. Multiple alignment of snakehead MRAP2 with that of other species. The transmembrane domain (TMD) was boxed. The above solid line showed the conserved motif (LKAHRYS) required for the formation of antiparallel homodimer. The above dashed line denoted the conserved motif (NIPNFVN) in C-terminus. Asterisk (*) indicated the same amino acids.

Table S1. PCR primers used for cloning and gene expression studies.

Primers	Primer sequence (5'- 3')
mc4r-F1	GTTCCCTGCTCGCTGTTAA
mc4r-R1	CACGTTGCCATGCTTT
mc4r-F2	CGTGGTTGACTCTATGAT
mc4r-R2	TTTTACTTGGAGATTGTA
mrap2-F1	TGTTTCAGATAGGCTCG
mrap2-R1	ATCACCCATCCCAGAGG
mrap2-F2	TTCTTGTTCTCACGCTG
mrap2-R2	TCTGTGCTTATCTGTTCC
mc4r-qF	TGCCAGTGAACAAGGACC
mc4r-qR	AGCAGCGACAACCAAGAT
mrap2-qF	TGTTCTCACGCTGCTCA
mrap2-qR	GCTTGTGTTTCATCT
Tub α 1-qF	AGCCTGATGGTCAAATGC
Tub α 1-qR	TTCCAATGGTGTAGTGCC

Table S2. Listing of MC4R sequences used in this study. Protein IDs are given to allow access to the protein sequence on Ensembl or GenBank website.

Number	Species	Protein ID
1	<i>Xiphophorus nigrensis</i>	ADO60278
2	<i>Xiphophorus maculatus</i>	NP_001303841
3	<i>Xiphophorus multilineatus</i>	ADO60279
4	<i>Haplochromis burtoni</i>	NP_001274332
5	<i>Oreochromis niloticus</i>	ENSONIP00000025763
6	<i>Channa argus</i>	AMM02541
7	<i>Larimichthys crocea</i>	XP_019120241
8	<i>Dicentrarchus labrax</i>	CBN82190
9	<i>Takifugu porphyreus</i>	BAB71733
10	<i>Takifugu rubripes</i>	AAO65551
11	<i>Takifugu radiatus</i>	BAB71732
12	<i>Tetraodon nigroviridis</i>	AAQ55178
13	<i>Clupea harengus</i>	XP_012679593
14	<i>Cyprinus carpio</i>	CBX89936
15	<i>Ctenopharyngodon idella</i>	AOZ60534
16	<i>Squaliobarbus curriculus</i>	ADV40875
17	<i>Xenocypris argentea</i>	ADV40878
18	<i>Danio rerio</i>	NP_775385
19	<i>Hypophthalmichthys molitrix</i>	ADV40873
20	<i>Hypophthalmichthys nobilis</i>	ADV40874
21	<i>Astyanax mexicanus</i>	ENSAMXP00000027055
22	<i>Sus scrofa</i>	ABD28176
23	<i>Homo sapiens</i>	NP_005903
24	<i>Canis lupus familiaris</i>	EDL09662
25	<i>Mus musculus</i>	EDL09662
26	<i>Ovis aries</i>	ACC77651
27	<i>Bos taurus</i>	ACR43465
28	<i>Ornithorhynchus anatinus</i>	XP_001505445
29	<i>Gallus gallus</i>	AAT73771
30	<i>Anolis carolinensis</i>	XP_003226797

Table S3. Listing of MRAP2 sequences used in this study. Protein IDs are given to allow access to the protein sequence on Ensembl or GenBank website.

Number	Species	Protein ID
1	<i>Homo sapiens</i>	NP_001333471.1
2	<i>Mus musculus</i>	NP_001346884.1
3	<i>Ornithorhynchus anatinus</i>	XP_028903035.1
4	<i>Gallus gallus</i>	NP_001307836.1
5	<i>Anolis carolinensis</i>	XP_008119910.1
6	<i>Xenopus tropicalis</i>	XP_002933963.1
7	<i>Channa argus</i>	Present study
8	<i>Callorhinichthys milii</i>	XP_007906624.1
9	<i>Danio rerio</i>	XP_001342923.4
10	<i>Danio rerio</i>	XP_005168578.1
11	<i>Epinephelus coioides</i>	MK425026.1
12	<i>Esox lucius</i>	XP_010888023.1
13	<i>Larimichthys crocea</i>	XP_027140224.1
14	<i>Oreochromis niloticus</i>	XP_003458293.2
15	<i>Oryzias latipes</i>	XP_004083625.1
16	<i>Xiphophorus couchianus</i>	XP_027897067.1
17	<i>Xiphophorus maculatus</i>	XP_005813802.1