



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

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nocortin-4 Receptor in Snakehead (Channa argus). 2021, 11, 481. https://doi.org/10.3390/ biom11030481

Citation: Wen, Z.-Y.; Liu, T.; Qin, C.-

Y.-X. MRAP2 Interaction with Mela-

J.; Zou, Y.-C.; Wang, J.; Li, R.; Tao,

Academic Editor: Paolo Annibale

Received: 26 January 2021 Accepted: 19 March 2021 Published: 23 March 2021

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Α					
		Extracellular Amino T	erminus		TMD1
	Zebrafish	MNTSHHHGLHHSFF	NHSQGALPVGK		ISTETFLILGLVSLL5/
	Swordtail	MNSTAQQGLIPCYL	NQSLRLGTLPE - K	DVSREEKDSSAGCYEQLP	ISTEVFLTLGIISLL 59
	Seabass	MNTTEAHGLIHGYH	INRSQTSGILPLNK	DLSAEEKDSSTGCYEQLL	SPEVFLTLGIVSLL 60
	Chicken	MNFTQHRGTLQPL	FWNHSN-GLHRGA	SEPSAKGHSSGGCYEQLF	SPEVFVTLGIISLL 59
	Rat	MNSTHHHGMYTSL	LWNRSSYGLHGNA	SESLGKGHPDGGCYEQLF	SPEVEVELGVISLL 60
	Human	* *	LWNKSSTRLHSNA	SESLORGISDOGCIEULF	/ SPEVFVILGVISLL 00
		• •	TMD2		
	Zebrafish	ENILVIAAIVKNK	NLHSPMYFFICSLA	VADLLVSVSNASETVVMAL	I TGGNL TNRES K 117
	Snakehead	ENILVVAAIVKNK	NLHSPMYFFICSLA	VADMLVSVSNASETIVIA	INGGTLTIPVTLIK 120
	Seabass	ENILVVAALIKNK	NLHSPMYFFICSLA	VADMLVSVSNASETIVIEL	
	Chicken	ENVLVIVAIAKNK	NLHSPMYFFICSLA	VADMLVSVSNGSETIVITI	LN - NTDTDAQSFT I 118
	Rat	ENILVIVAIAKNK	NLHSPMYFFICSLA	VADMLVSVSNGSETIVITI	LN-STDTDAQSFTV119
	Human	ENILVIVAIAKNK	NLHSPMYFFICSLA	VADMLVSVSNGSETIVITI	LN - STDTDAQSFTV 119
		TMD3			TMD4
	Zebrafish	NMDNVFDSMICSSI	LASIWSLLAIAVD	RYITIFYALRYHNIMTOR	RAGTIITCIWTFCTV 177
	Snakehead	SMDNVFDSMICSSI	LASICSLLAIAVD	RYITIFYALRYHNIVTLR	RAMLVICSIWTCCTV180
	Swordtail	SMDNVFDSMICSSI		RYITIFYALRYHNIVTIR	RALLVIASIWTCCIV 179
	Chicken	NIDNVIDSVICSSI		RYFTIFYALOYHNIMTVK	RVGVIITCIWAACTV178
	Rat	NIDNVIDSVICSSI	LASICSLLSIAVD	RYFTIFYALQYHNIMTVR	RVGIIISCIWAACTV179
	Human	NIDNVIDSVICSSI	LASICSLLSIAVD	RYFTIFYALQYHNIMTVK	RVGIIISCIWAACTV179
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	Zebrafish	SGVLFIVYSESTT		LMASLYVHMFLLARLHMK	RIAAL PGNGPIWQAA 237
	Snakehead	SGILFIIYSESTTV	LICLITMFFTMLV	LMASLYVHMFLLARLHMK	RIAAMQGNAPIQQRA 240
	Swordtail	SGILFIIYSESTM	LICLITMFFTMLV	LMASLYVHMFLLARQHMKF	R I GAL PGNAP I QQRA 239
	Seabass	SGILFIIYSESTT			RIAAL PGNAPIHQRA 240
	Rat	SGVLFIIYSDSSAV	VIICLISMFFTMLV	LMASLYVHMFLMARLHIK	RIAVLPGTGTIRQGT239
	Human	SGILFIIYSDSSAV	/ I I CL I TMF F TMLA	LMASLYVH <mark>MFLMARLHIK</mark> F	RIAVL PGTGA I RQGA 239
		:*:*:*:	* : * * * * : * * * * * * *	**********::** *:*	:*: ** * : TND7
	Zebrafish		VEVVCWAPEELHLL		
	Snakehead	NMKGAITLTILLG	FVVCWAPFFLHLI	LMITCPRNPYCTCFMSHF	MYLILIMCNSIIDP 300
	Swordtail	NMKGAITLTILLG	FVVCWAPFFLHLI	LMITCPRNPYCTCFMSHF	MYLILIMCNSIIDP 299
	Seabass	NMKGAITLTILIG	FVVCWAPFFLHLI		MYLILIMCNSVIDP 300
	Rat	NMKGAITLTILIG	FVVCWAPFFLHLL	FYISCPONPYCVCFMSHFI	NLYLILIMCNAVIDP 299
	Human	NMKGAITLTILIG	FVVCWAPFFLHLI	FYISCPONPYCVCFMSHF	NLYLILIMCNSIIDP 299
		* * * * * * * : * * * : * *	************	: *:** ****.****	* : * * * * * * * * : : * * *
	Zebrafieh				326
	Snakehead	IIYAFRSQEMRKT	KEIFCCS HSLL	SLCV CV	320
	Swordtail	IIYAFRSQEMRKT	KEIFCWC ISFL		324
	Seabass	IIYAFRSQEMRKT	KEIFCCS HALL	CV	327
	Chicken				331
	Human	LIYALRSQELRKT	KEIICCYPLGGLC	DLSSRY	332
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	н	uman MC4R	Rat MC4R	Zebrafish MC4R	Snakehead MC4R

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.

	ТМО
Human	MSAQRLISNRTSQQSASNSDYTWEYEYYEIG - PVSFEGLKAHKYSIVIGFWVGLAVFVIF 59
Chicken	MSALRLISNRTSQQALSNSDYTWEYEYYEYG - PVSFEGLKAHKYSIVIGFWVGLAVFVIF 59
Snakehead	MSEF HNRSQTSARRSDYVWQYEYYD - EEPVSFEGLKAHRYS I VIGFWVGLAVFVIF 55
Grouper	MSDF HNRSQTSARRSDYVWQYEYYDDEEPVSFEGLKAHRYS I VIGFWVGLAVFVIF 56
Fugu	MSAR GNRSQSSARRGDY I WQYEYYDYDEPVSFEGL RAHRYS I VI GFWVGL AV FVI F 56
Zebrafish 2a	MPRE OI SNSTSVPNHNYEWSYEYYDDEEPVSEEGI KAHRYSI VIGEWVGI AVEVI 56
Zebrafish 2h	MS EXS. NRSOAGADYEWHYEYYEDEEPVSEEGI RANRYSI VIGEWVGI AVEVIE 53
Lebranon_Lb	
Human	MEEVITII TVTCADUODNAESSEVBEDMNSEVSDECODIE DDVVESD OCNEESDSIE 446
Chieken	METVLILLIKIGAFHQUNAESSERKERMINSEVADEGREE- PDRVESK-QUNEESKSLFIG
Chicken	MFFVLTLLTKTGAPTQENTESSEKKFKMNSFVADFGFLE-SEKVFSK-QTAEESKSLFTG
Snakenead	MFFVLTLLTKTGAPHQENPDSADKRHRPDSCLVDTDGLQD-ENDKAFSR-PLLAGSHSYL113
Grouper	MFFVLTLLTKTGAPHQENPDSAEKRHRPGSCLVDTGSPQD - ENDKAFSR - PLLAESRSYF 114
Fugu	MFFVLALLSKTRAPRQENPESADKHHRPDGYPVDIDSLQD-EKAPSFTH-PLLSESRAYS114
Zebrafish_2a	MFFVLTLLTKTGAPHPEAAEPYEKRMRLTSCADGLGRQRETDGRTGLSR - PLLEESRSLF 115
Zebrafish_2b	MFFVLTLLTKTGAPHPEMCDASMKPHVLIGCELEVGGSLAFSLPPLPDQSRSLF107
	****:**:** **::::: * :: *::
Human	HCYINEVERLDRAKACHQTTALDSDVQLQEAI RS
Chicken	HFCINEVEHLDKAQQSQKGPDLESNIHFQEVSRS
Snakehead	HFYVNKEDQGQGKQKTEDMNGGKHPGAWAHPGACSGARGIGSSGMG-DMEEEAEETGGNQ172
Grouper	HFYINEEDQGQGKQKPGDERVGKHCGARGQQGTRGISSSGMMDEMEEDAEEARGHQ170
Fugu	HFCIQN-SQDSGKKTSEDSRFGEQNLAPQVSCHHLGGGSSPRRDTVEVDLEGVVSKQ170
Zebrafish 2a	HCYINEEEREGGRAATDAGALTHGRSGIGNS RG QVEEVGL 155
Zebrafish 2b	HFY1HKEERVKTHKDAVIGR-GMHCGRGNAE137
	* • • • •
	••••
Human	SCOPEEL NRIMKEDIENEVNTON, YEGEDDII I SEPPIVIETKPI SOTSHK 202
Chicken	SGTI FENNCIAKYN I NNEWNTEONSSI GEGNI I I ODDRVI ESKTAMOSSHR 203
Snakehead	DI KGLIEDS, TDESALI SHENI NEVNI ENSSIEGED, I VEDSV. MI EDSNO, - HC 227
Groupor	
Grouper	PLNGLIEEGKIDRECAFSOFNIFNIFNIFNIFNIERSSILGEDELLTEFSA-ILERQOSQUANG223
rugu Zaharafiah Oa	PLAGRAGDVIAEDDVVFFIQFNIPNFVSLEQSSELGEEELLYEPSA-VLEKREAHC223
Zebrafish_2a	VVQNMVLESRAEREAALLAHFNIPNFVNSELNSALGDEDLLLGDPP-IIME203
Zebrafish_2b	······RADEDEHFMSSFNIPNFVNSEQSSSLGHDDFLLSEPP-IIIDGQSDELKIA18/
Human	DLD 205
Chicken	ILD 206
Snakehead	DLH 230
Grouper	DIR 232
Fugu	DIH 228
Zebrafish_2a	205
Zebrafish_2b	EPAHLCYDIIRH 199

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.

Primers	Primer sequence (5'- 3')	
mc4r-F1	GTTCCTGCTCGCTGTTAA	
mc4r-R1	CACGTTGTCCATGCTTTT	
mc4r-F2	CGTGTTTGACTCTATGAT	
mc4r-R2	TTTTACTTGGAGATTGTA	
mrap2-F1	TGTTTTCAGATAGGCTTCG	
mrap2-R1	ATCACCCATCCCAGAGG	
mrap2-F2	TTCTTTGTTCTCACGCTG	
mrap2-R2	TCTGTGCTTTATCTGTTCC	
mc4r-qF	TGCCAGTGAACAAGGACC	
mc4r-qR	AGCAGCGACAACCAAGAT	
mrap2-qF	TGTTCTCACGCTGCTCA	
mrap2-qR	GCTTTGTCGTTTTCATCT	
Tuba1-qF	AGCCTGATGGTCAAATGC	
Tuba1-qR	TTCCAATGGTGTAGTGCC	

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

Number	Species	Protein ID
1	Xiphophorus nigrensis	ADO60278
2	Xiphophorus maculatus	NP_001303841
3	Xiphophorus multilineatus	ADO60279
4	Haplochromis burtoni	NP_001274332
5	Oreochromis niloticus	ENSONIP0000025763
6	Channa argus	AMM02541
7	Larimichthys crocea	XP_019120241
8	Dicentrarchus labrax	CBN82190
9	Takifugu porphyreus	BAB71733
10	Takifugu rubripes	AAO65551
11	Takifugu radiatus	BAB71732
12	Tetraodon nigroviridis	AAQ55178
13	Clupea harengus	XP_012679593
14	Cyprinus carpio	CBX89936
15	Ctenopharyngodon idella	AOZ60534
16	Squaliobarbus curriculus	ADV40875
17	Xenocypris argentea	ADV40878
18	Danio rerio	NP_775385
19	Hypophthalmichthys molitrix	ADV40873
20	Hypophthalmichthys nobilis	ADV40874
21	Astyanax mexicanus	ENSAMXP0000027055
22	Sus scrofa	ABD28176
23	Homo sapiens	NP_005903
24	Canis lupus familiaris	EDL09662
25	Mus musculus	EDL09662
26	Ovis aries	ACC77651
27	Bos taurus	ACR43465
28	Ornithorhynchus anatinus	XP_001505445
29	Gallus gallus	AAT73771
30	Anolis carolinensis	XP_003226797

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

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