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淇河鲫肌肉生长抑制素基因的克隆与表达

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摘要: 肌肉生长抑制素(MSTN)是动物肌肉发育和生长过程中的负调控因子。为了明确淇河鲫MSTN基因序列及其在肌肉生长发育中的调控功能, 本研究利用RACE方法克隆淇河鲫MSTN全长cDNA序列, qRT-PCR分析其在不同组织和胚胎发育阶段的表达情况。结果显示, 淇河鲫MSTN cDNA序列全长2094 bp (no. KC851952.1), 包含86 bp 5'非编码区、880 bp 3'非编码区和1128 bp开放阅读框, 编码375个氨基酸残基, 前22个氨基酸残基为信号肽, 34~256位氨基酸残基为TGF- β 前肽域, 281~375是TGF- β 结构域, 具蛋白酶水解位点RIRR和C端生物活性区的9个保守半胱氨酸残基。蛋白同源分析发现淇河鲫MSTN与鲤形目鱼类相似性较高, 与哺乳动物和鸟类的相似性最低。系统进化分析显示, 淇河鲫MSTN与鲫、鲤、鳊等鲤科鱼类亲缘关系较近。实时定量PCR分析表明, 淇河鲫MSTN基因在各个组织中均有表达, 脑中表达量最高, 其次为肌肉和肝脏, 肠道表达量最低。淇河鲫胚胎发育的各阶段MSTN基因均有表达, 受精卵表达量最高, 其次为囊胚期, 神经胚期表达量最低。推测MSTN与淇河鲫肌肉发育生长具有一定的相关性, 可能参与“双背鲫”特征的形成。

关键词: 淇河鲫; 肌肉生长抑制素; 克隆; 组织表达

中图分类号: Q 785; S 917.4

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肌肉生长抑制素(myostatin, MSTN)首先从小鼠(*Mus musculus*)骨骼肌cDNA文库中克隆获得^[1], 具有转化生长因子- β (transforming growth factor β , TGF- β)家族共有的结构特点: N端前肽区; C端具保守的蛋白酶水解位点RXXR; C端活性区含多个保守Cys残基, 故其属TGF- β 超家族成员之一。MSTN基因在哺乳动物中分布较为保守, 主要存在于肌肉中, 抑制肌肉细胞增殖和分化, 是肌肉生长的负调控因子^[2]。在小鼠体内敲除MSTN基因可促进肌肉细胞增殖, 使骨骼肌增重2~3倍^[3]。Grobet等^[4]、McPherron等^[5]分别在研究比利时蓝牛和皮埃蒙特牛时发现, MSTN缺失与变异会造成双臀性状。因为MSTN基因在肌肉生长中的负

调控作用, 近年来受到研究者广泛关注。

迄今, 除哺乳类^[6]和鸟类^[7]外, 鱼类MSTN基因也被大量克隆, 包括鲤形目(Cypriniformes)的鲤(*Cyprinus carpio*)^[8]、斑马鱼(*Danio rerio*)^[9]、草鱼(*Ctenopharyngodon idella*)^[10]、鳊(*Elopichthys bambusa*)^[11]、泥鳅(*Misgurnus anguillicaudatus*)^[12], 鲑形目(Salmoniformes)的虹鳟(*Oncorhynchus mykiss*)^[13]、鲑(*Salmo salar*)^[14], 鲈形目(Perciformes)的鳊(*Siniperca chuatsi*)^[15]、莫桑比克罗非鱼(*Oreochromis mossambicus*)^[16]、金头鲷(*Sparus aurata*)^[17], 鲇形目(Siluriformes)的长鳍真鲷(*Ictalurus furcatus*)^[18], 鲽形目(Pleuronectiformes)的牙鲆(*Paralichthys olivaceus*)^[19]等, 为鱼类MSTN基

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因功能的深入研究奠定了基础。

淇河鲫(*Carassius auratus*)属鲤形目, 鲤科(Cyprinidae)、鲫属(*Carassius*), 是长期生态适应过程中形成的变异地方性种群之一, 为天然雌核发育三倍体鱼, 是河南省特有名贵优质鱼类^[20]。淇河鲫背部肌肉较厚, 故有“双背鲫”之称。*MSTN*是肌肉生长发育的重要调控因子之一, 研究其作用规律对了解淇河鲫肌肉生长发育调控机理有重要作用。本研究克隆了淇河鲫*MSTN*-1基因, 并分析其在8个不同组织以及胚胎和胚后发育时期的表达规律, 为进一步研究*MSTN*基因与淇河鲫肌肉生长发育机制提供基础数据, 也对淇河鲫的繁殖及育种工作起到积极作用。

1 材料与方法

1.1 总RNA提取和第一链cDNA合成

淇河鲫3尾(平均每尾约重500 g), 采于河南淇县淇河鲫原种场。按TaRaKa RNAiso Plus (TaKaRa, 大连)试剂说明书对冻存于液氮的肌肉、鳃、脑、心脏、肝脏、肾脏、肠和脾脏8个组织提取总RNA。选择不同发育时期的淇河鲫胚胎(受精卵、囊胚期、原肠胚期、神经胚期、出膜小鱼)

置于液氮保存, 提取总RNA用于实验。ND-2000核酸蛋白仪测定RNA浓度与OD值, 琼脂糖凝胶电泳检测RNA完整性, 总RNA-80 °C保存。

1.2 淇河鲫*MSTN*基因全长的克隆

根据GenBank中已报道鱼类保守序列设计正反引物*MSTN*5和*MSTN*3(表1), 按TaKaRa RNA PCR Kit (AMV) Ver.3.0(TaKaRa, 大连)说明书进行淇河鲫肌肉总RNA反转录, 扩增*MSTN* cDNA部分序列。在已获得的部分序列基础上设计3'-RACE和5'-RACE的特异性引物(表1), 进行3'和5'末端扩增。

3'-RACE按照3'-Full RACE Core Set Ver2.0 (TaKaRa, 大连)说明书进行。5'-RACE过程: 用*MSTN* 5'-out合成cDNA第一链; 纯化后加Poly (A)尾巴, ddH₂O稀释后-20 °C保存; *MSTN* 5'-out、5'Olig(T)-Adaptor进行第一轮PCR扩增, 产物稀释50倍后, 5'RACE Adaptor、*MSTN* 5'-in进行巢式PCR扩增。PCR扩增产物回收纯化、连接转化挑选阳性克隆送于上海生工生物有限公司测序。

1.3 序列对比分析

测序结果与*MSTN* cDNA中间序列进行拼接, 拼接后全长序列用Blastn程序进行同源性比

表 1 实验中所用的引物
Tab. 1 Primers used in the study

引物 primer	序列(5'→3') sequence	用途 usage
<i>MSTN</i> 5	5'-AGATATAACGGCGCACCAGCAG-3'	中间片段克隆 partial sequence PCR
<i>MSTN</i> 3	5'-GAGCAATAATCCAGTCCCAGCC-3'	
<i>MSTN</i> 3'-in	5'-ACTTCGGCTGGGACTGGATTATTGC-3'	3'端扩增 3'- RACE PCR
<i>MSTN</i> 3'-out	5'-CTGGATTGGACTGCGACGAGAA-3'	
3'race inner	CGCGGATCCTCCACTAGTGATTTCACATATAGG	
3'race outer	TACCGTCGTTCCACTAGTGATTT	
<i>MSTN</i> 5'-in	5'-GCTGCTGGTGCGCCGTTATATCT-3'	5'端扩增 5'- RACE PCR
<i>MSTN</i> 5'-out	5'-GAATTTGGGACTTGATGGCATGCAG-3'	
5'Olig(T)-Adaptor	5'-GACTCGAGTCGACATCGATTTTTTTTTTTTTTTT-3'	
5'RACE Adaptor	5'-GACTCGAGTCGACATCGA-3'	
<i>MSTN</i> qF	5'-GCAGAGGAAAGCGAGCAGTG-3'	荧光定量 qRT-PCR
<i>MSTN</i> qR	5'-TTGGAGCCTGTTTGAGTCGG-3'	
<i>β-actin</i> F	5'-ACCATCTACCCCGGTATTGC-3'	内参基因 housekeeping gene
<i>β-actin</i> R	5'-TGGAAGGTGGACAGGGAAGC-3'	

对(<http://blast.ncbi.nlm.nih.gov/>)。ExPASy程序(<http://www.expasy.ch/tools/>)推算其开放阅读框和编码氨基酸序列情况。ClustalX2程序进行氨基酸同源性比对, SMART软件用于蛋白质结构域分析(<http://smart.embl-heidelberg.de/>)。MEGA6.0软件以邻接法构建系统进化树。

1.4 实时定量PCR分析淇河鲫MSTN的组织特异性表达

依据已克隆的淇河鲫MSTN全长序列设计荧光定量引物MSTNqF和MSTNqR(表1), 以 β -actin为内参基因(表1)。Prime ScriptTM RT Master Mix和SYBR Premix Ex TaqTM (TaKaRa, 大连)进行qRT-PCR, 反应体系(20 μ L): SYBR10 μ L, 上、下游引物各0.5 μ L, cDNA模板2 μ L, ddH₂O 7.2 μ L; 反应程序: 94 $^{\circ}$ C预变性2 min; 94 $^{\circ}$ C 10 s, 60 $^{\circ}$ C 15 s, 72 $^{\circ}$ C 20 s, 共40个循环。每个样本设置3个重复, 依据 $2^{-\Delta\Delta C_t}$ 法分析实验样本MSTN相对表达量, Excel、SPSS16.0软件One-Way ANOVA统计分析所得数据, 进行LSD和Duncan氏比较, 结果用平均值 $\pm$ 标准误(mean $\pm$ SE)表示。

2 结果

2.1 淇河鲫MSTN基因cDNA全长和氨基酸序列分析

所克隆序列经测序、拼接, 结果显示淇河鲫MSTN基因cDNA全长2094 bp。其中5'非编码区长86 bp, 3'非编码区长880 bp, 编码区长1128 bp, 包含AATAA典型转录终止子加A信号, 共翻译375个氨基酸(图1)。经ExPASy程序分析显示预测MSTN氨基酸序列前22个氨基酸残基组成信号肽, 切割位点在Gly22与Asp23之间; 存在一个蛋白水解位点RIRR (263~266), C端生物活性区含9个保守的半胱氨酸残基, 分别位于第272、281、282、309、313、339、340、372和374位氨基酸(图1), 蛋白等电点为6.14, 分子量为41.89 ku。SMART分析氨基酸序列显示, 淇河鲫MSTN含有2个典型的TGF- β 蛋白结构域: TGF- β 前肽域(34~256)和TGF- β 结构域(281~375)(图1)。

2.2 淇河鲫MSTN同源性比对及蛋白系统进化树构建

利用Clustalx软件将淇河鲫MSTN氨基酸序列与鲤形目、鲑形目、鲈形目、鲇形目、鲾形

目及哺乳动物的MSTN氨基酸进行比对(表2)。结果显示, 淇河鲫MSTN信号肽剪切位点与已知的其他鱼类MSTN蛋白基本一致, 与哺乳动物相差较远(第18~19位)。与已报道的多数动物MSTN相似, 淇河鲫MSTN在TGF- β 前肽域和TGF- β 结构域之间存在保守的RXRR蛋白水解位点(图2)。Blast比对结果显示, 淇河鲫MSTN与鲫相似度最高(98%), 与鲤形目MSTN-1序列相似度均在94%以上, 与MSTN-2相似度为62%~68%。相较于硬骨鱼类不同物种间MSTN-1较高的一致性(73%~98%), 其与哺乳动物人、小鼠等的相似度较低, 为60%~67%。利用MEGA6.0软件构建蛋白系统进化树(图3), 结果显示, 哺乳动物与鱼类形成两个独立的进化分支, 淇河鲫MSTN首先与鲫聚为一支, 随后是其他鲤科鱼类, 与Clustalx结果一致。硬骨鱼类相比鸟类和哺乳动物多一次全基因组复制, 可能具有两个旁系同源基因, 为进一步分析淇河鲫MSTN同源性, 本研究选取多个鱼类MSTN家族成员进行氨基酸序列比对, 系统分析发现, 淇河鲫MSTN与鲤MSTN-1b紧密聚为一支, 因此可推测所克隆的MSTN可能为MSTN-1型。

2.3 淇河鲫MSTN的组织 and 时空特异性表达

qRT-PCR检测结果显示, 淇河鲫MSTN mRNA几乎在所有组织中都有表达, 其中脑中表达量最高($P<0.05$), 其次为肌肉、肝脏、鳃和肾脏, 肠道中表达量最低(图4)。淇河鲫胚胎和胚后发育各个阶段均检测到MSTN mRNA的表达, 受精卵和囊胚期表达量较高, 其次为原肠胚期, 神经胚期和出膜期MSTN表达量较低, 随着胚胎发育的进行, 20 dph (day post hatching, 出膜后天数)、40 dph MSTN表达量分别是神经胚期的8.50倍和96倍, 成鱼背部肌肉中MSTN表达量最高, 约为神经胚期的315倍(图5)。

3 讨论

本研究利用RACE方法首次克隆得到淇河鲫MSTN基因全长序列, 其编码的蛋白质N末端具有分泌必需的信号肽序列、蛋白水解位点RIRR和9个保守半胱氨酸残基, 与其他物种MSTN具有相同特征。同源性比对结果显示, 淇河鲫MSTN信号肽剪切及蛋白水解位点同鲤形目种类相同, 切割位点均在Gly22和Asp23之间, 类胰蛋白


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1      tctacttgcgcgtgcgtggtgacgttcatttccatagcaaatcagagcaaacatcgcaaacatcccttagctcg
76     ctttggaaacATGCATTTTACACAGGTTTAAATTTCTCTAAGTGTATTAATTGCATGCGGTTCACTGGGTGATGGA
1      M H F T Q V L I S L S V L I A C G S V G H G
151    GATATAACGGCGCACCGAGCCTTCCACAGCGCGAGGAAAGCGAGCAGTGTCCACATGTGAGTTTCAGACAA
23     D I T A H Q Q P S T A A E E S E Q C S T C E F R Q
226    CACAGCAAGCTGATGAGACTGCATGCCATCAAGTCCCAAATTCTTAGCAAACTCCGACTCAAAACAGGCTCCAAAC
48     H S K L M R L H A I K S Q I L S K L R L K Q A P N
301    ATCAGCCGGGACGTGGTCAAGCAGCTGTTACCCAAAGCACCGCCTTTGCGAGCAACTTCTGGATCAGTACGATGTT
73     I S R D V V K Q L L P K A P P L Q Q L L D Q Y D V
376    CTGGGGGATGACAGTAAGGATGGAGCTGTGAAGACGATGATGAACATATCACCACAGAGACCATTATGACCATG
98     L G D D S K D G A V E D D D E H I T T E T I M T M
451    GCCACAGAGCCTGACCCCATCGTTCAAGTAGATCGGAAACCGACGTGTTGTTTTTCTCCTTCAGTCCGAAGATC
123    A T E P D P I V Q V D R K P T C C F F S F S P K I
526    CAAGCGAACCGGATCGTAAGAGCGCAGCTGTGGGTTCATCTGGGACCCGCGGAAGAGCGACCCGCTCTCTTA
148    Q A N R I V R A Q L W V H L G P A E E A T T V F L
601    CAGATATCACGGCTGACGCCGTCGCGGACGGAGGACACATACGAATACGATCTTTGAAGATCGATGTGAAC
173    Q I S R L T P V A D G G R H I R I R S L K I D V N
676    GCAGGAGTGTGCTTGGCAGAGTATAGACGTAAACAGGTGCTGACGGTGTGGTTAAGACAACCGGGGACCAAC
198    A G V M S W Q S I D V K Q V L T V W L R Q P G T N
751    TGGGGCATTGAGATAAACCGGTATGACGCGAAGGAAACGACTTGGCCGTCACCTCAGCTGAGCCGAGAGGAT
223    W G I E I N A Y D A K G N D L A V T S A E P G E D
826    GGACTGCTCCCTTCACGGAGGTGAAAATTTAGAGGGCCAAAGCGAATCCGAGGGGACTCTGGATTGGACTGC
248    G L L P F T E V K I S E G P K R I R R D S G L D C
901    GACGAGAATTCATCAGAGTCTCGATGCTGCCGGTACCCTCTCACTGTGGACTTCGAGGACTTCGGCTGGGACTGG
273    D E N S S E S R C C R Y P L T V D F E D F G W D W
976    ATTATTGCTCAAAACGCTATAAGGCAATTACTGTTGCGGAGAATGCGACTACATGCACCTGCAGAAATATCCC
298    I I A P K R Y K A N Y C S G E C D Y M H L Q K Y P
1051   CACACCCATCTGGTGAACAGGCCAATCCACGAGGCACCGCGGGCCCTGCTGCACCCCAACCAAGATGTCTCCC
323    H T H L V N K A N P R G T A G P C C T P T K M S P
1126   ATCAACATGCTTTACTTCAACGGCAAGAGCAGATCATCTACGGCAAGATCCCTCAATGGTAGTAGACCGCTGT
348    I N M L Y F N G K E Q I I Y G K I P S M V V D R C
1201   GGCTGCTCGTGAaccagtgccagcaggactgatcgctctcacagaccggacatctgatcacaccatccacc
373    G C S *
1276   atccactatcagtgcttccgcgaagacactgtgcaatagaaggacgtcactcactctctgggcaccogcttcatt
1351   tgactatgtttttttgtcatttttctcctaatacagtatctctgccacaggagtcacacgtttacgtggatgtacta
1426   aaggaaagtcatactatggctggactttgggaatggacacttttgaaatggagacattctctgctttatttccatg
1501   ttttcaacttgcagaatactctcatttaggatacacatgtattatgcagccactccaaaatacagtcatttaggt
1576   ctttgcgttaacagctgagctgttttaagaagagatccaagagaaacagagagggactcttgaacactgaacag
1651   agcttgaatacagttatctgacgcaaaacttccacatacactgcaataaacacactgatcaaatggtaacgtgag
1726   ctttgcgtccactactcactgtcacaacagccctacacacttgaaattatttgagatcgacattaggggacagaa
1801   ggacttgaagcaaggcactgtgaaggcggtccacacatcgaaatgtgttcgagacagagacgtcggaactgtga
1876   gaatgaatgttaatacagctaacactctgtcttcaaacacacagtttgactatggcagacccaatagaaga
1951   attggttgcataaaatgttgaataaactgtatttgatattttgctaattgtattgtatacttgccattgtttcc
2026   attaacagttgccttttttaacaacgggttagtacatgtatatgaacacgaatatgcaaaaaaaaaa

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图1 淇河鲫*MSTN*基因序列和氨基酸序列

红色下划线部分序列为信号肽序列；黑色下划线部分序列为蛋白水解位点；红色边框字母C为保守的半胱氨酸残基；蓝色和绿色下划线部分序列分别为TGF- β 前肽域和TGF- β 结构域；*为终止密码子

Fig. 1 Nucleotide sequence and putative amino acid sequence of *MSTN* in *C. auratus*

The letters with red and black underline are the sequences of signal peptide and conservative hydrolytic site, respectively; the letters Cs with red box are 9 conservative cysteine residues; the blue and green underlined letters respectively were the conserved domain of TGF- β propeptide and TGF- β ; asterisk indicated the stop codon (TGA)

酶作用位点为“RIRR”，相较于鲑形目、鲈形目种属差异的多样性，鲤形目*MSTN*蛋白水解位点较保守。*MSTN*与TGF- β 超家族其他成员一样，先合成较长的前体蛋白，切除信号肽后，在RXRR位点水解形成潜在相关肽和成熟肽，随后形成肌抑素复合物调控肌肉生长^[21]。鲤形目信号肽剪切和蛋白水解位点的高度保守性，从侧面反映

*MSTN*在鲤形目肌肉发育调控中的进化限制和重要作用。进化树分析结果显示淇河鲫*MSTN*蛋白序列同鲫、鲤、鳊等鲤形目鱼类*MSTN*同源性最高，亲缘关系最近，而与哺乳类和鸟类同源性较低，亲缘关系较远。这一结果与淇河鲫属于鲤科鱼类的分类地位相一致。鱼类中存在*MSTN*-1、*MSTN*-2两个基因亚型，虹鳟^[13]、鲑^[14]、斑马鱼^[22]

表 2 淇河鲫MSTN氨基酸序列比对和
系统进化树所用物种信息

Tab. 2 Species data for multiple alignment and phylogenetic analysis for MSTN of *C. auratus*

序号 no.	物种 species	登录号 accession no.	序号 no.	物种 species	登录号 accession no.
1	淇河鲫 <i>C. auratus</i> mstn	AGR44669.1	30	花鲈 <i>Lateolabrax japonicus</i> mstn	AAX82169.1
2	鲫 <i>C. auratus</i> mstn	AGK84718.1	31	长鳍真鲷 <i>I. furcatus</i> mstn	AAS48403.1
3	露斯塔野鲮 <i>Labeo rohita</i> mstn	AKF02410.1	32	阿马逊花鲈 <i>Poecilia formosa</i> mstn1	ENSPFOP00000012179
4	鲤 <i>C. carpio</i> mstn1a	ACY01745.1	33	阿马逊花鲈 <i>P. formosa</i> mstn2	ENSPFOP00000005059
5	鲤 <i>C. carpio</i> mstn1b	ACY01746.1	34	大西洋鲑 <i>Gadus morhua</i> mstn1	ENSGMOP00000013522
6	鲤 <i>C. carpio</i> mstn2a	ACY01747.1	35	墨西哥丽脂鲤 <i>Astyanax mexicanus</i> mstn1	XP_007253308
7	鲤 <i>C. carpio</i> mstn2b	ACY01748.1	36	墨西哥丽脂鲤 <i>A. mexicanus</i> mstn2	ENSAMXP00000014198
8	岩原鲤 <i>Procypris rabaudi</i> mstn	ADD71155.1	37	青鳉 <i>Oryzias laticeps</i> mstn1	NP_001188428.1
9	鳊 <i>E. bambusa</i> mstn	ADK94456.1	38	花斑剑尾鱼 <i>Xiphophorus maculatus</i> mstn1	ENSXMAP00000006421
10	厚颌鲂 <i>Megalobrama pellegrini</i> mstn	ADD71156.1	39	花斑剑尾鱼 <i>X. maculatus</i> mstn2	ENSXMAP00000001644
11	稀有鮡鲫 <i>Gobiocypris rarus</i> mstn	ACK76230.1	40	眼斑雀鲷 <i>Lepisosteus oculatus</i> mstn1	ENSLOC00000010736
12	斑马鱼 <i>D. rerio</i> mstn1	AAP85526.1	41	三刺鱼 <i>Gasterosteus aculeatus</i> mstn1	ENSGACP00000003445
13	斑马鱼 <i>D. rerio</i> mstn2	AAT77805.1	42	三刺鱼 <i>G. aculeatus</i> mstn2	ENSGACP00000020558
14	草鱼 <i>C. idella</i> mstn1	AJF48833.1	43	暗绿鲀 <i>Tetraodon nigroviridis</i> mstn1	ENSTNIP00000018789
15	虹鳉 <i>O. mykiss</i> mstn1a	AAZ85121.1	44	暗绿鲀 <i>T. nigroviridis</i> mstn2	ENSTNIP00000020296
16	虹鳉 <i>O. mykiss</i> mstn1b	ABA42586.1	45	尼罗罗非鱼 <i>Oreochromis niloticus</i> mstn1	ENSONIP00000009034
17	虹鳉 <i>O. mykiss</i> mstn2a	ABA42587.1	46	尼罗罗非鱼 <i>O. niloticus</i> mstn2	ENSONIP00000015357
18	虹鳉 <i>O. mykiss</i> mstn2b (partial)	ABB72461.1	47	茴鱼 <i>Thymallus thymallus</i> mstn2a	AFK82287.1
19	条纹狼鲈 <i>Morone saxatilis</i> mstn	AF290910_1	48	北极茴鱼 <i>T. arcticus</i> mstn2a	AFK82286.1
20	牙鲆 <i>P. olivaceus</i> mstn	ABK91834.1	49	海湾扇贝 <i>Argopecten irradians</i>	AAT36326.1
21	斑鳊 <i>Siniperca scherzeri</i> mstn	AEW22728.1	50	野猪 <i>Sus scrofa</i> mstn	AEP13978.1
22	莫桑比克罗非鱼 <i>O. mossambicus</i> mstn	AAK28706.1	51	人 <i>Homo sapiens</i> mstn	AAH74757.2
23	金眼狼鲈 <i>M. chrysops</i> mstn	AF197194_1	52	狒狒 <i>Papio hamadryas</i> mstn	AAB86686.1
24	金头鲷 <i>Sparus aurata</i> mstn	AAK53544.1	53	牛 <i>Bos taurus</i> mstn	AFN44374.1
25	鲑 <i>S. salar</i> mstn1a	ABN72586.1	54	牦牛 <i>B. grunniens</i> mstn	AEO20321.1
26	鲑 <i>S. salar</i> mstn1b	CAC59700.1	55	褐鼠 <i>Rattus norvegicus</i> mstn	AAB86691.1
27	鲑 <i>S. salar</i> mstn2a	AFK82284.1	56	绵羊 <i>Ovis aries</i> mstn	CAQ52586.1
28	红鳍东方鲀 <i>Takifugu rubripes</i> mstn1	NP_001027843.1	57	山羊 <i>Capra aegagrus hircus</i> mstn	AEG76961.1
29	红鳍东方鲀 <i>T. rubripes</i> mstn2	NP_001027844.1	58	小鼠 <i>Mus musculus</i> mstn	AAI05675.1

等物种中均发现了两种形式的MSTN。所克隆的淇河鲫MSTN系统进化分析结果显示其与鲤MSTN1-b型相近, 并与其他鱼类MSTN-1聚为一大支, 因此推测其可能为淇河鲫MSTN-1型。实验未能扩增出MSTN-2型基因序列, 淇河鲫是否存在MSTN-2

还有待进一步研究。

针对哺乳动物的相关研究发现, MSTN主要在骨骼肌中高表达。由于鱼类MSTN存在多个旁系同源基因, 不同基因亚型的表达水平和分布都存在差异。MSTN-2型通常仅在脑和肌肉中表

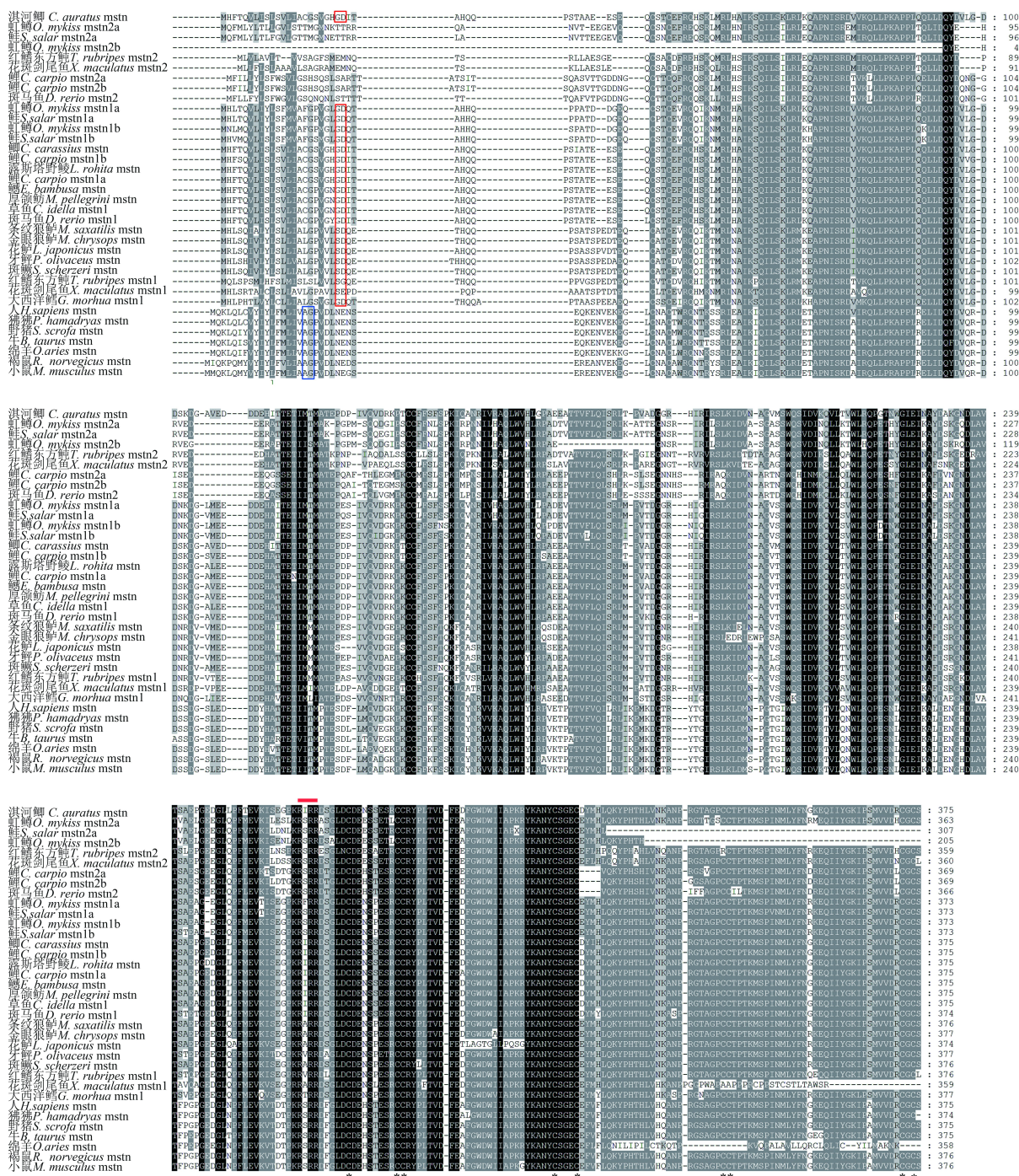


图2 淇河鲫MSTN与其他动物源性分子的氨基酸比对

ClustalX进行多序列比对，分别用黑色底纹和灰色底纹表示100%和80%的氨基酸残基相似度。9个保守的半胱氨酸残基用“*”表示。红色上划线为RXRR蛋白水解位点，蓝色实线框为哺乳动物MSTN信号肽剪切位点，红色实线框为鱼类MSTN信号肽剪切位点。

Fig. 2 Amino acid sequence alignment of MSTN in *C. auratus* with other animals

The program Clustal X was used to align the MSTN sequences among 25 species. The 100% and 80% identity among these sequences were respectively highlighted by black and grey shade. 9 conservative cysteine residues were identified by asterisk. The red unlined letters was RXRR conservative hydrolytic site, the letters in blue and red boxes respectively were the signal peptide cleavage sites in mammals and fishes

达^[23], MSTN-1型分布较广泛, 除肌肉外, 脑、眼、肝脏、脾脏、鳃、肠、肾、性腺等多个组织中均有表达^[8-19]。本实验中, 淇河鲫MSTN组织

表达分布同罗非鱼、斑马鱼、虹鳟、金头鲷、鲤和鳊相似, 多个组织中均检测出*MSTN*, 其中脑中表达量最高, 其次为肌肉。尽管鱼类种类



图 3 淇河鲫与其他物种MSTN的系统进化树

线的长度与遗传距离成正比, 淇河鲫用“●”表示

Fig. 3 Phylogenetic tree based on the amino acid sequences of MSTN among species

The length of the phylogenetic tree identified the genetic distance, and the MSTN of *C. auratus* was marked by the black spot

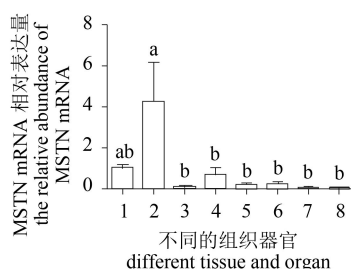


图 4 淇河鲫MSTN组织表达分布

1. 肌肉; 2. 脑; 3. 心脏; 4. 肝脏; 5. 肾脏; 6. 鳃; 7. 肠; 8. 脾脏; 不同字母表示差异显著 ($P<0.05$)

Fig. 4 Tissues expression of MSTN mRNA in *C. auratus*

1. muscle; 2. brain 3. heart; 4. liver; 5. kidney; 6. gill; 7. gut; 8. spleen; different letter superscripts indicate significant differences ($P<0.05$)

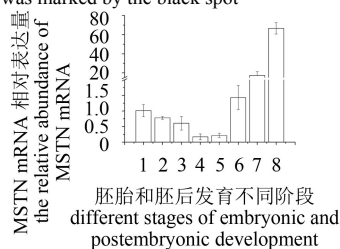


图 5 淇河鲫MSTN胚胎和胚后发育时期表达分布

1. 受精卵; 2. 囊胚期; 3. 原肠胚期; 4. 神经胚期; 5. 出膜期; 6. 出膜后20 d; 7. 出膜后45 d; 8. 成鱼背部肌肉

Fig. 5 The relative expression of MSTN mRNA in different stages of embryonic and postembryonic development

1. fertilized eggs; 2. blastula stage; 3. gastrula stage; 4. neurula stage; 5. hatching stage; 6. 20 dph; 7. 45 dph; 8. back muscle of adult fish

不同导致*MSTN*表达分布存在差异,但肌肉中都有表达,进一步表明其在鱼类肌肉生长发育中的重要调控作用。*MSTN*基因在不同鱼类脑组织中均被证实表达量较高,仅次于或者高于肌肉,表明脑也是*MSTN*的重要表达场所,该基因可能参与成鱼神经系统和免疫系统的发育^[23]。淇河鲫胚胎发育过程中,*MSTN*-1在整个发育阶段均有表达,类似的结果在对斑马鱼^[24]、草鱼及青海湖裸鲤(*Gymnocypris przewalskii*)^[25]研究中也发现。*MSTN*在不同物种胚胎发育各阶段的表达趋势也存在较大差异,草鱼*MSTN*-1随着胚胎发育的进行,表达量逐步升高^[26]。青海湖裸鲤*MSTN*-1表现为先升后降的趋势^[25]。淇河鲫*MSTN*-1胚胎发育阶段的表达趋势同斑马鱼相似,在囊胚期和原肠胚期表达水平较高,神经胚期显著降低,出膜期时有所上升,这可能与神经胚期肌节形成具有一定相关性^[24]。此外,随着胚后发育的进行,*MSTN*-1表达量逐渐升高,在成鱼背部肌肉中达到峰值,这可能与*MSTN*在个体发育不同阶段所起的调控作用相关。尽管本实验中*MSTN*-1与淇河鲫肌肉发育存在一定的相关性,但其对淇河鲫肌肉发育和形成的调控作用还有待进一步研究。

*MSTN*对肌肉生长与发育的负调控作用已被广泛证实。通过转RNAi和*MSTN*重组前肽产物可抑制*MSTN*表达,促进鱼类肌肉增长和体质量增加^[21]。因此,淇河鲫*MSTN*-1基因的克隆及序列分析为探索淇河鲫“双背”脊与*MSTN*的关系、*MSTN*表达调控机理等方面奠定分子基础,也为淇河鲫的生产和育种应用研究创造条件。

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Molecular cloning and expression analysis of myostatin gene in *Carassius auratus* in Qihe River

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Abstract: Myostatin (*MSTN*) is a negative regulatory factor in animal muscle development and growth. In order to identify the sequence information and the function of *Carassius auratus* *MSTN* in the process of muscle development, rapid amplification of cDNA ends (RACE) and real time PCR were used to clone the full length cDNA sequence and analyse the expression level of *MSTN* in embryonic development stages and different tissues. The results showed that the full length cDNA of *MSTN* was 2094 bp (no. KC851952.1), including 86bp at 5'-UTR, 880 bp at 3'-UTR, and a 1128 bp open reading frame encoded a peptide of 375 amino acids. The putative peptide contained a 22 amino acids signal peptide, a TGF- β propeptide domain (34-256), a TGF- β domain (281-375), a conserved RIRR proteolytic processing site and 9 conserved cysteine residues in the C-terminal of the protein. Multiple sequence comparison indicated that the *MSTN* of *Carassius auratus* has high similarity with cypriniformes species, while has low similarity with mammalian and bird. The phylogenetic analysis also showed that the crucian carp *MSTN* has close relationship with cypriniformes species like *Carassius carassius*, *Cyprinus carpio* and *Labeo rohita*. Real time PCR results showed that *MSTN* mRNA was checked in eight different tissues of crucian carp, among which the maximum level was detected in brain, followed by muscle and liver, the minimum level was displayed in intestine. In addition, the *MSTN* was also confirmed to express at every stage of embryogenesis, in which the highest level was found at fertilized eggs, followed by blastula and the lowest level was detected at neurula. Thus, the *MSTN* gene was speculated to have relation with crucian carp muscle development and growth, and might play an important role in the "double dorsal muscle" formation.

Key words: *Carassius auratus*; myostatin; cloning; tissue expression pattern

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