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斑石鲷 *irf7* 基因克隆及其在病毒感染下的表达*

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摘要 为探究斑石鲷(*Oplegnathus punctatus*)干扰素调节因子 *irf7* 在虹彩病毒(Iridovirus)感染过程中的作用机制,本研究通过 PCR 扩增获得了斑石鲷 *irf7* 基因 CDS 区序列,对其序列特征进行分析,并在组织水平和细胞水平研究该基因在病毒感染中的表达模式。结果显示, *Opirf7* 基因 CDS 区全长为 1332 bp, 编码 443 个氨基酸的多肽,具有干扰素调节因子家族的保守结构域。荧光定量 PCR (qRT-PCR)结果显示, *Opirf7* 基因在健康个体的不同组织中均有表达,在肝脏中表达量最高,在皮肤和肠等免疫组织中的表达量也较高。对斑石鲷腹腔注射虹彩病毒诱导抗病毒免疫反应,在组织水平检测 *Opirf7* 对病毒感染的响应模式。与对照组(0 h)相比, *Opirf7* 在免疫组织中的表达水平有不同程度的升高。在细胞水平,建立 poly I: C 感染的斑石鲷脑细胞系模型,利用 qRT-PCR 检测 *Opirf7* 的表达变化。结果显示, poly I: C 刺激后,脑细胞系 *Opirf7* 的表达量显著升高。结果表明, *Opirf7* 在斑石鲷抗病毒的免疫应答过程中发挥重要作用。

关键词 斑石鲷; 干扰素调节因子 7; 抗病基因; 免疫反应

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干扰素调节因子(interferon regulatory factors, *irfs*)是一类转录因子家族,能够调控干扰素(interferon, IFN)的转录过程。在机体受到感染时, *irfs* 能结合 IFN 启动子并诱导调节 IFN 而得名(Veals *et al.*, 1992, Zhang *et al.*, 2012)。在哺乳动物 *irfs* 家族已发现 9 个 *irf* 成员(*irf1~irf9*)(Barnes *et al.*, 2002; Nguyen *et al.*, 1997),随后在鸟类和鱼类中鉴定出 *irf10* 基因。通过解析鱼类基因组发现,鱼类中存在 11 个 *irf* 家族成员,其中, *irf11* 为硬骨鱼类所特有(Stein *et al.*, 2007)。所有的 IRFs N 端都有一个由 115 个氨基酸组成的结构域,称为 DNA 结合域(DNA binding domain, DBD)。DBD 含有 5 个色氨酸重复序列,其中 3 个重复序列可识别干扰素刺激反应元件(IFN-sensitive response

elements, ISRE)正调控序列区域 DNA 的 GAAA 和 AANNNGAA 序列(Escalante *et al.*, 1998)。IRFs 通过 C 末端的 IAD 结构域(IRF-associated domain),与其他转录因子相互作用来激活信号通路,实现其转录调控作用(Taniguchi *et al.*, 2001)。IRFs 是模式识别受体信号转导、促炎症因子与趋化因子转录及激活免疫应答反应过程中的重要分子,不仅参与 IFN 的信号通路,还在防御病毒与应激反应的过程中具有重要作用(Honda *et al.*, 2006, Tanaka *et al.*, 2000)。干扰素调节因子 7 (*irf7*)是诱导 I 型干扰素表达的关键因子,于 1997 年首次报道(Zhang *et al.*, 1997),其最重要功能是诱导 IFN α/β 产生,从而诱导机体抗病毒功能(Fitzgerald *et al.*, 2003)。目前,已在斑马鱼(*Danio rerio*) (Xiang *et al.*,

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2010)、鳊鱼(*Siniperca chuatsi*) (Sun *et al*, 2007)、牙鲆(*Paralichthys olivaceus*) (Hu *et al*, 2010)等鱼类中被鉴定, 并揭示了鱼类 *irf7* 在先天免疫中的作用。

斑石鲷(*Oplegnathus punctatus*)具有外形美观、肉质细嫩、生长速度快、成活率高等优点, 深受消费者喜爱。2014年以来, 随着斑石鲷人工繁育和养殖取得成功, 斑石鲷已成为我国名贵海水养殖品种之一。但随着国内水产养殖业集约化水平不断提高, 病毒病成为制约斑石鲷健康养殖的主要问题。本研究通过克隆斑石鲷 *irf7* 基因, 分析在虹彩病毒(Iridovirus)刺激后各免疫组织的表达模式, 有助于研究斑石鲷抗病毒及细菌免疫应答中的功能, 旨在为阐述斑石鲷免疫机制提供理论基础。

1 材料与方法

1.1 实验材料

本实验所用斑石鲷购自山东莱州明波水产有限公司, 体重为(150±15) g, 健康无病。实验前在水箱中暂养1周, 水温维持在25℃左右。每日投喂饲料, 保持溶氧充足, 每日用清洁海水换水1次。

1.2 样品处理和采集

1.2.1 健康斑石鲷组织样品采集 随机选取4条健康斑石鲷, 经MS-222麻醉后抽取血液, 解剖后取肝、脾、肾、头肾、心脏、肠、鳃、脑、皮肤和肌肉等组织, 将所取组织迅速置于液氮中, 随后转移至-80℃冰箱中保存, 用于提取总RNA。

1.2.2 虹彩病毒感染斑石鲷及组织样品采集 选取15条健康斑石鲷用于斑石鲷虹彩病毒感染实验。本实验所用斑石鲷虹彩病毒由华南农业大学秦启伟教授提供。将斑石鲷虹彩病毒液用PBS稀释至 1×10^9 copies/mL。将斑石鲷麻醉后腹腔注射病毒液, 每尾注射100 μL。分别在0、1、4、7和10 d共5个时间点取样, 每个时间点取3条鱼, 每条鱼取肝脏、脾脏、肾脏3个组织。将组织立即放入液氮中冷冻, 并随后放入-80℃冰箱, 用于后续提取RNA。

1.2.3 poly I:C 刺激斑石鲷脑细胞系 将单层培养、生长状态良好的斑石鲷脑细胞系铺到12孔板上, 24 h后细胞覆盖率达到90%左右。将12孔板中的旧培养基吸弃, 使用无菌PBS (0.01 mmol/L, pH 7.2)洗涤3次。使用无菌PBS (0.01 mmol/L, pH 7.2)配制poly I:C (上海源叶生物科技有限公司)母液, 浓度为25 mg/ml。按照低、中、高浓度(50、100、200 μg/mL), 配制含有poly I:C的L-15培养基(北京索莱宝生物科技

有限公司), 加入培养板中, 每个浓度3个重复。对照组加入与高浓度组相同体积的PBS。在刺激6 h后, 吸出培养基, 加入Trizol (TaKaRa)收集细胞, 保存于-80℃冰箱待用。细胞实验重复3次。

1.3 总RNA提取与cDNA合成

使用Trizol对所有健康以及感染后样品提取总RNA, 提取后使用Pultron核酸浓度仪测定浓度及 $A_{260\text{ nm}}/A_{280\text{ nm}}$ 比值, 使用琼脂糖凝胶电泳检测所提RNA的质量。利用合格的RNA, 使用PrimeScript™ II 1st Strand cDNA Synthesis试剂盒(TaKaRa)合成cDNA。

1.4 *Opirf7* 基因 ORF 区验证

根据测序结果, 获得*Opirf7*基因的ORF区序列, 使用Primer 5.0软件设计引物*Opirf7*-F/*Opirf7*-R, 使用不同组织混合cDNA模板进行PCR扩增。总体积为15 μL: PCR Mix (TaKaRa) 7.5 μL, *Opirf7*-F 1 μL, *Opirf7*-R 1 μL, cDNA模板1 μL, ddH₂O 4.5 μL。PCR扩增程序: 95.8℃预变性5 min; 95℃变性30 s, 59℃退火30 s, 72℃延伸30 s, 35个循环; 72℃延伸7 min, 4℃保存。

将PCR扩增产物进行凝胶电泳检测, 条带大小与目的片段相一致。将正确目的片段切下, 使用胶回收试剂盒(诺唯赞)对目的片段进行回收纯化。将纯化后的产物连接到T载体中, 转化Trans1-T1感受态细胞(全式金)中, 冰浴30 min后42℃热激30 s, 置于冰上2 min后加入500 μL无抗LB培养基, 37℃摇床摇菌1 h, 取200 μL菌液于超净工作台中涂板, 培养过夜。次日, 挑取单克隆并进行菌液PCR验证。将条带大小正确的样品送青岛擎科生物技术有限公司测序。

1.5 生物信息学分析及构建系统发育树

使用DNASTAR软件的Editseq分析*Opirf7* ORF区, 预测氨基酸序列, 并推测分子量和等电点。使用网站(<http://www.cbs.dtu.dk/services/SignalP/>)预测其信号肽, 使用网站(<http://www.cbs.dtu.dk/services/TMHMM/>)进行跨膜结构分析。使用SMART(<http://smart.embl-heidelberg.de/>)进行结构域预测。通过DNAMAN 8软件进行氨基酸同源比对。检索不同生物*irf7*序列(表2), 导入MEGA X软件进行氨基酸多重比对, 采用邻接法(neighbor-joining, NJ)构建进化树。

1.6 *Opirf7* 实时荧光定量检测

使用实时荧光定量(qRT-PCR)检测斑石鲷健康个体、虹彩病毒感染及poly I:C感染细胞后*Opirf7*的表达模式。根据测序所获得的ORF序列设计*Opirf7*和内参

基因的引物(表 1)。实验步骤参照 TaKaRa 荧光定量试剂盒, 采用两步法, 反应程序: 95℃ 预变性 30 s, 95℃ 5 s, 58℃ 30 s, 共 40 个循环; 60℃ 30 s, 72℃ 30 s, 溶解曲线温度 55~95℃。以 β -actin 为内参进行 qRT-PCR 分析。利用 $2^{-\Delta\Delta Ct}$ 方法计算 *Opirf7* 基因的相对表达量。实验数据采用 SPSS 软件进行方差分析 (one-way ANOVA), 设定 P 值 < 0.05 时为差异显著。使用 GraphPad Prism 作图。

2 结果与分析

2.1 *Opirf7* ORF 区序列分析

以斑石鲷组织 cDNA 为模板, 获得斑石鲷 *Opirf7*

基因 ORF 区全长为 1332 bp, 编码 443 个氨基酸。通过氨基酸序列分析, 预测其分子量为 50.5 kDa, 等电点为 5.546 (图 1)。通过蛋白结构域预测, 发现其含有 IRF 家族保守结构域, 包括 DNA 结合域 (DNA binding domain, DBD)、IRF 相关结构域 (IRF-associated domain, IAD) 和 SRD 结构域 (Serine-rich domain, SRD) (图 2)。其中, DBD 位于多肽 N 末端, 由第 4~112 个氨基酸组成, 该区域包含 4 个色氨酸重复区域; IAD 位于多肽中间部分 (第 222~395 个氨基酸); 丝氨酸富含区位于多肽末端。

2.2 氨基酸多序列比对及系统进化树分析

经 BLAST 比对发现, 斑石鲷 *irf7* 氨基酸序列与

表 1 本研究中所使用的引物

Tab.1 Primers used in this study

引物 Primer	序列 Sequence (5'~3')	目的 Purpose
<i>Opirf7</i> -ORF-F	ATGTTCCCTCCCAGCCTTCCC	ORF verification
<i>Opirf7</i> -ORF-R	TCAGATCGGGTGTAGAAAAGGCGC	ORF verification
<i>Opirf7</i> -qRT-F	TTTGCCAGCACCAGTGACCC	qPCR
<i>Opirf7</i> -qRT-R	GACCACCTTGACAACGATAA	qPCR
β -actin-F	GCTGTGCTGTCCCTGT	qPCR
β -actin-R	GAGTAGCCACGCTCTGTC	qPCR

1	ATGTTCCCTCCCAGCCTTCCCAGCCTCAGTTTGCCAGCTGGCTCATAGAGCAGGTGGAGACCGGCCAGTACACG
1	M F P P S L P K P Q F A S W L I E Q V E T G Q Y T
76	GGCCTATGCTATGTGGCCAAAACAAGTTCAGAGTCCCTTGGGAAGCACAACCTCCAGGAAGGATGCAACCGCAGG
26	G L C Y V G Q N K F R V P W K H N S R K D C N D E
151	GACAGTAAAATATCCGGGCATGGCAGTAGCCAGTGGAAAAATCAACGAGTTCGCCAACGCACAGGCCAGGTGG
51	D S K I F R A W A V A S G K I N E F P N D K A R W
226	AAAACCAACTCCGATGTGCTCTGAACAACCTCTCTGAGCGCTTCAAGATGATGACGGATAATCCAAAAATTCT
76	K T N F R C A L N N L S E R F K M M T D N S K N S
301	GACGACCCCATAAAATCTACGAGATTATCAACACAGAATAACAATGAAACCTGCCAAGCAAGACTCCCG
101	D D P H K I Y E I I N T E Y N Y E T L P T Q D S Q
376	GAGGATCTGACATGATCCAGATATCTACAGCTCCCCATAGAGTGCTACCCCTGAGGAAATGAGCTCAACCTA
126	E D S D M I P D I Y S S F I E C Y P A G N E L N L
451	CTACACAACCTTCATGGCCTTGGATCTTGGGAACCATCCATCAGAGGAGCAACCATGGGCAGAGAACTACGGTCAG
151	L H N F M A L D L G N H P S E E Q P W A E N Y G Q
526	CCAAATCCAGCTGTGCTCGGAAGCTATTCTGTAGCAGCCGAGAACCCCCACACCTGTTACCAAATCTGCTGCTC
176	P N P A V L G S Y S V A A E N P P H L L P N L L L
601	CCAGATCAGCCGTAAGTACTACGAGTAAATCCTCCACCGGTCCTCAGTTCACCTCTGCAGCCAAAGTATATGACCTG
201	P D Q P Y Y E V N P P P V L S S P L Q P S I Y D L
676	GAGATCTCCACTATAGGAAGAAGAAATGCTAAGACCAGGTGACCCAGCCCGCTCTCCAGTCCACTAC
226	E I S I H Y R K K E M L K T R L T T A R L Q L H Y
751	CAGCATGAGGCCCTGAGCTCAATGCCGATATCTCTGCTTCCCCTCCACTGATGGTCTGCTGGACCACAACAG
251	Q H E A P E L N A D H L C F P S T D G L L D H K Q
856	ATTGATTTCAACAACCGCATCCTTAACAGCATCCAGAATGGTCTGCTCCTGGAGGTACAGGAGACTGGTATCTAT
276	I D F T N R I L N S I Q N G L L L E V Q E T G I Y
901	GCCTGGAGGCAGGACAGGTGCCACGTGTTTGCCAGCACCAGTGACCCAGTGTGGCTCACCCAGACCAAGAAAG
301	A W R Q D R C H V F A S T S D P S V A H P D P R K
976	CTGCCCGAAGACCATGGTAGAGTCTCCTCAGCTTTGAGAAGTATGTGAACGAACTGAACAGTTTAAAGAGAAC
326	L P Q N T M V E L L S F E K Y V N E L K Q F K E N
1051	AATGGCGGCTCCTGACTACACCATCAACATGTGCTTTGGGGAGAAGTTTCTGATGGGAAACCTTTGGAGAG
351	N G G S P D Y T I N M C F G E K F P D G K P L E K
1126	AAGCTTATCGTTGTCAAGGTGGTCCCTCTGATCTGTCGACACTTTCATGAGATGGCCAGATGGAGGGGGCTCG
376	K L I V V K V V P L I C R H F H E M A Q M E G A S
1201	TCTCTTACAGTCCCAACGTCAGCCTGCAGATCTCACACAACAGCCTCTATGATCTCATTAACTCTGCTTTAGT
401	S L H S A N V S L Q I S H N S L Y D L I N S V F S
1276	CTGCCAAGTGTGAAGAGTCAGCAGGGGTGCTGCGCCTTTTCTACACCCGATCTGA
426	L P T V E E S A R A A A P F L H P I *

图 1 *Opirf7* 核苷酸序列及推导的氨基酸序列

Fig.1 *Opirf7* nucleotide sequence and deduced amino acid sequence

氨基酸序列在核苷酸序列下方, 起始密码子“ATG”用红色标明, 终止密码子“TAG”用星号标明。

DBD 结构域用灰色阴影标识, IAD 结构域用黄色阴影标识。

The amino acid sequence is below the nucleotide sequence, the start codon “ATG” is marked with red, and the stop codon “TAG” is marked with an asterisk. The IRF domain is indicated by gray shading, and the IRF3 domain is indicated by yellow shading.

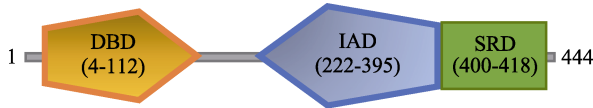


图 2 斑石鲷 Opif7 蛋白结构域预测

Fig.2 Protein domain prediction of Opif7 in *O. punctatus*

其他硬骨鱼类的 *irf7* 具有较高同源性。斑石鲷 *irf7* 与尖吻鲈(*Lates calcarifer*)的相似性为 82.92%，与大黄鱼(*Larimichthys crocea*)、牙鲆、半滑舌鲷(*Cynoglossus semilaevis*)的相似性分别为 81.99%、79.95%、73.74%，与哺乳动物小鼠(*Mus musculus*)和人(*Homo sapiens*)

的相似性较低，分别为 31.00%和 30.00%(图 3)。

通过 MEGA X 软件采用 Neighbor-joining 法构建了 Opif7 的系统进化树(图 4)。发育分析显示，斑石鲷 Opif7 与其他硬骨鱼类的 *irf7* 聚为一支，鸡(*Gallus gallus*)、小鼠猕猴(*Macaca mulatta*)和人类的 *irf7* 聚为另一大支。斑石鲷 Opif7 在进化上与大黄鱼、鲑鱼(*Micththys miuy*)的亲缘关系较近，而与哺乳类、爬行类、鸟类等动物的 *irf7* 亲缘关系较远。

2.3 Opif7 基因在正常斑石鲷各组织中的表达

Opif7 在健康斑石鲷个体 11 个组织中均有表达。

<i>Oplegnathus punctatus</i>MFPSPKPFQFASWLIIEQVETGVYVIGCYVGVGN..KFRVPWKHNSRDKDNEDESKITFRAWAVASGKI.....NEFFPN	70
<i>Cynoglossus semilaevis</i>MOSPPKPFQFASWLIIEQVETGVYVIGCYVGVGN..KFRVPWKHNSRDKDNEDESKITFRAWAVASGKI.....NEFFPN	68
<i>Larimichthys crocea</i>MOSPPKPFQFASWLIIEQVETGVYVIGCYVGVGN..KFRVPWKHNSRDKDNEDESKITFRAWAVASGKI.....NEFFPN	68
<i>Micththys miuy</i>MOSPPKPFQFASWLIIEQVETGVYVIGCYVGVGN..KFRVPWKHNSRDKDNEDESKITFRAWAVASGKI.....NEFFPN	68
<i>Lates calcarifer</i>MOSPPKPFQFASWLIIEQVETGVYVIGCYVGVGN..KFRVPWKHNSRDKDNEDESKITFRAWAVASGKI.....NEFFPN	68
<i>Paralichthys olivaceus</i>MOSPPKPFQFASWLIIEQVETGVYVIGCYVGVGN..KFRVPWKHNSRDKDNEDESKITFRAWAVASGKI.....NEFFPN	68
<i>Takifugu rubripes</i>MLRFSPKPFQFASWLIIEQVETGVYVIGCYVGVGN..KFRVPWKHNSRDKDNEDESKITFRAWAVASGKI.....NDFEEN	68
<i>Danio rerio</i>MQSTNAKPFQFASWLIIEQVETGVYVIGCYVGVGN..KFRVPWKHNSRDKDNEDESKITFRAWAVASGKI.....NEFFPN	69
<i>Gallus gallus</i>MAALDSEGDQAKRLREGGWLLNVAVSSGLYVIGCYVGVGN..KFRVPWKHNSRDKDNEDESKITFRAWAVASGKI.....EGNAE	76
<i>Macaca mulatta</i>MVFPERPAASPDSPRPGTRRAAPRVLEGGWLLNVAVSSGLYVIGCYVGVGN..KFRVPWKHNSRDKDNEDESKITFRAWAVASGKI.....GAWPPSSRGGG.PPFAETA	100
<i>Homo sapiens</i>MALAPERAAERVLEGGWLLNVAVSSGLYVIGCYVGVGN..KFRVPWKHNSRDKDNEDESKITFRAWAVASGKI.....GAWPPSSRGGG.PPFAETA	86
<i>Mus musculus</i>MAEVRVQVQVLEGGWLLNVAVSSGLYVIGCYVGVGN..KFRVPWKHNSRDKDNEDESKITFRAWAVASGKI.....GAWPPSSRGGG.PPFAETA	83
Consensus	m s p k p q f a s w l i e q v e t g y g l y c y v d n f r v p w k h n s r d k d e d k i f r a w a v a s g k i n e f f p n	
<i>Oplegnathus punctatus</i>	DKA...RWKTNFRGALNNLSVREKMMIDNSKNSDDHKKIYETINTE.YNYETL..PTQ...DSQEDSDMI.PDIYSSIECYFAGNELNLLHFMALDL	159
<i>Cynoglossus semilaevis</i>	DKA...RWKTNFRGALNNLSVREKVEDNSKNSDDHKKIYETINAN.GROEVL..PNE...KAFEDLMAT.LDIYSSIECYFAGNELNLLHFMALDL	157
<i>Larimichthys crocea</i>	DKA...RWKTNFRGALNNLSVREKMKIDNSKNSDDHKKIYETINTE.YNYES...Q...AKQEDTNMI.PDIYNSPTIYIPGTEHNLLNLMALDL	154
<i>Micththys miuy</i>	DKA...RWKTNFRGALNNLSVREKMKIDNSKNSDDHKKIYETINTE.HNYES...Q...AKQEDTNMI.PDIYNSPTIYIPGTEHNLLNLMALDL	154
<i>Lates calcarifer</i>	DKA...RWKTNFRGALNNLSVREKMKIDNSKNSDDHKKIYETINTE.HNYENQ..PTQ...DSQEDSFMT.PNIYSSPTIYIPGTEHNLLNLMALDL	157
<i>Paralichthys olivaceus</i>	DKA...RWKTNFRGALNNLSVREKMKIDNSKNSDDHKKIYETINTE.HROENCSMPKN...DSQEDL.MT.PEIMYSSPTIYIPGTEHNLLNLMALDL	158
<i>Takifugu rubripes</i>	DKA...RWKTNFRGALNNLSVREKVEDNSKNSDDHKKIYETINTE.CKVENL..PTQ...SSQEDSDMD.IYSP.PVKHFTLQSELNHNDFALDL	155
<i>Danio rerio</i>	DKA...RWKTNFRGALNNLSVREKMKIDNSKNSDDHKKIYETINTE.HNYES...Q...AKQEDTNMI.PDIYNSPTIYIPGTEHNLLNLMALDL	154
<i>Gallus gallus</i>	DPA...RWKTNFRGALNNLSVREKMKIDNSKNSDDHKKIYETINTE.HNYENQ..PTQ...DSQEDSFMT.PNIYSSPTIYIPGTEHNLLNLMALDL	157
<i>Macaca mulatta</i>	ERA...GKWTNFRGALNNLSVREKMKIDNSKNSDDHKKIYETINTE.HROENCSMPKN...DSQEDL.MT.PEIMYSSPTIYIPGTEHNLLNLMALDL	158
<i>Homo sapiens</i>	ERA...GKWTNFRGALNNLSVREKMKIDNSKNSDDHKKIYETINTE.HROENCSMPKN...DSQEDL.MT.PEIMYSSPTIYIPGTEHNLLNLMALDL	158
<i>Mus musculus</i>	ERA...GKWTNFRGALNNLSVREKMKIDNSKNSDDHKKIYETINTE.HROENCSMPKN...DSQEDL.MT.PEIMYSSPTIYIPGTEHNLLNLMALDL	158
Consensus	ERERRRWKTNFRGALNNLSVREKMKIDNSKNSDDHKKIYETINTE.HROENCSMPKN...DSQEDL.MT.PEIMYSSPTIYIPGTEHNLLNLMALDL	178
Consensus	dka rwktnfrcalnnlslsrfkm dnsknsddphkiiyei nte ynyetl q dsqedsdmi pdiyssieicyfagne lllhfmaldl	
<i>Oplegnathus punctatus</i>	GNHPSEEQPWAENYQ.QPNPAVL.....GSYVSAEENPFPHEENL	198
<i>Cynoglossus semilaevis</i>	ENQPAEFPWAENYQ.QPNPAVL.....GSYVSAEENPFPHEENL	196
<i>Larimichthys crocea</i>	GNHPSEEQPWAENYQ.QPNPAVL.....GSYVSAEENPFPHEENL	196
<i>Micththys miuy</i>	GNHPSEEQPWAENYQ.QPNPAVL.....GSYVSAEENPFPHEENL	196
<i>Lates calcarifer</i>	GNHPSEEQPWAENYQ.QPNPAVL.....GSYVSAEENPFPHEENL	196
<i>Paralichthys olivaceus</i>	GNHPSEEQPWAENYQ.QPNPAVL.....GSYVSAEENPFPHEENL	196
<i>Takifugu rubripes</i>	GNHPSEEQPWAENYQ.QPNPAVL.....GSYVSAEENPFPHEENL	196
<i>Danio rerio</i>	GNHPSEEQPWAENYQ.QPNPAVL.....GSYVSAEENPFPHEENL	196
<i>Gallus gallus</i>	GNHPSEEQPWAENYQ.QPNPAVL.....GSYVSAEENPFPHEENL	196
<i>Macaca mulatta</i>	GNHPSEEQPWAENYQ.QPNPAVL.....GSYVSAEENPFPHEENL	196
<i>Homo sapiens</i>	GNHPSEEQPWAENYQ.QPNPAVL.....GSYVSAEENPFPHEENL	196
<i>Mus musculus</i>	GNHPSEEQPWAENYQ.QPNPAVL.....GSYVSAEENPFPHEENL	196
Consensus	GNHPSEEQPWAENYQ.QPNPAVL.....GSYVSAEENPFPHEENL	196
<i>Oplegnathus punctatus</i>	LLPFGKPLDKKLLVVKVVFLLCRHFHMAQMCACASSLHGANVSOISHSN.LYDLSNSVFSLEPVEESGAAAPFLHP	442
<i>Cynoglossus semilaevis</i>	LLPFGKPLDKKLLVVKVVFLLCRHFHMAQMCACASSLHGANVSOISHSN.LYDLSNSVFSLEPVEESGAAAPFLHP	442
<i>Larimichthys crocea</i>	LLPFGKPLDKKLLVVKVVFLLCRHFHMAQMCACASSLHGANVSOISHSN.LYDLSNSVFSLEPVEESGAAAPFLHP	442
<i>Micththys miuy</i>	LLPFGKPLDKKLLVVKVVFLLCRHFHMAQMCACASSLHGANVSOISHSN.LYDLSNSVFSLEPVEESGAAAPFLHP	442
<i>Lates calcarifer</i>	LLPFGKPLDKKLLVVKVVFLLCRHFHMAQMCACASSLHGANVSOISHSN.LYDLSNSVFSLEPVEESGAAAPFLHP	442
<i>Paralichthys olivaceus</i>	LLPFGKPLDKKLLVVKVVFLLCRHFHMAQMCACASSLHGANVSOISHSN.LYDLSNSVFSLEPVEESGAAAPFLHP	442
<i>Takifugu rubripes</i>	LLPFGKPLDKKLLVVKVVFLLCRHFHMAQMCACASSLHGANVSOISHSN.LYDLSNSVFSLEPVEESGAAAPFLHP	442
<i>Danio rerio</i>	LLPFGKPLDKKLLVVKVVFLLCRHFHMAQMCACASSLHGANVSOISHSN.LYDLSNSVFSLEPVEESGAAAPFLHP	442
<i>Gallus gallus</i>	LLPFGKPLDKKLLVVKVVFLLCRHFHMAQMCACASSLHGANVSOISHSN.LYDLSNSVFSLEPVEESGAAAPFLHP	442
<i>Macaca mulatta</i>	LLPFGKPLDKKLLVVKVVFLLCRHFHMAQMCACASSLHGANVSOISHSN.LYDLSNSVFSLEPVEESGAAAPFLHP	442
<i>Homo sapiens</i>	LLPFGKPLDKKLLVVKVVFLLCRHFHMAQMCACASSLHGANVSOISHSN.LYDLSNSVFSLEPVEESGAAAPFLHP	442
<i>Mus musculus</i>	LLPFGKPLDKKLLVVKVVFLLCRHFHMAQMCACASSLHGANVSOISHSN.LYDLSNSVFSLEPVEESGAAAPFLHP	442
Consensus	LLPFGKPLDKKLLVVKVVFLLCRHFHMAQMCACASSLHGANVSOISHSN.LYDLSNSVFSLEPVEESGAAAPFLHP	442

图 3 斑石鲷与其他物种 irf7 氨基酸序列多重比对

Fig.3 Multiple alignment of the deduced amino acids of irf7 among *O. punctatus* and other different species

物种间氨基酸相似度 100%、75%以上和 50%以上分别用黑色、玫红色和蓝色阴影表示。

The color scheme for amino acid similarity among species: black, 100%; rock red, >75%; blue, >50%.

表2 本研究用于序列比对和进化树分析的 *irf7* 蛋白信息Tab.2 The species information used in the *irf7* for sequence alignment and phylogenetic tree analysis in this study

物种 Species	GenBank 登录号 GenBank access No.	相似性 Similarity /%
人 <i>Homo sapiens</i>	NP_001563.2	30.00
猕猴 <i>Macaca mulatta</i>	XP_028688388.1	32.71
小鼠 <i>Mus musculus</i>	NP_058546.1	31.00
鸡 <i>Gallus gallus</i>	QDO67136.1	47.17
半滑舌鳎 <i>Cynoglossus semilaevis</i>	NP_001281157.1	73.74
牙鲆 <i>Paralichthys olivaceus</i>	ACY69214.1	79.95
大黄鱼 <i>Larimichthys crocea</i>	NP_001290279.1	81.99
斑马鱼 <i>Danio rerio</i>	AAH65902.1	46.77
尖吻鲈 <i>Lates calcarifer</i>	XP_018519912.1	82.92
鲮鱼 <i>Miichthys miiuy</i>	AUD11407.1	81.91
红鳍东方鲀 <i>Takifugu rubripes</i>	XP_003967453.2	73.00

其中,在肝脏中表达量最高,在皮肤、肌肉、脑、肠等组织中表达量较低,在头肾中表达量最低(图5)。

2.4 虹彩病毒刺激后 *Opirf7* 基因的表达变化

虹彩病毒刺激斑石鲷后, *Opirf7* 的表达量在脾脏、肾脏中显著上调。脾脏中, *Opirf7* 的表达量在第4天升高至对照组的15倍,在第7天表达量最高,约为对照组的37倍,在第10天表达量降低。在肾脏中,第4天升高2.5倍,第7天时达到表达峰值,为对照组的4倍。在肝脏组织中, *Opirf7* 表达量稍有变化,但差异并不显著。

2.5 poly I:C 刺激斑石鲷脑细胞系 *Opirf7* 的表达

在病毒类似物 poly I:C 感染的斑石鲷脑细胞系中, *irf7* 的表达呈现先升高后降低的趋势,在 50 $\mu\text{g/mL}$ poly I:C (低浓度)刺激时表达量显著升高(13~14倍),在 100 $\mu\text{g/mL}$ poly I:C (中浓度)刺激时表达量也显著升高(14~17倍),而在 200 $\mu\text{g/mL}$ poly I:C (高浓度)刺激时, *irf7* 的表达量仍显著高于对照组(8~9倍),推测 200 $\mu\text{g/mL}$ 浓度较高,对细胞造成一定的损伤,因而表达水平有所下降。

3 讨论

本研究通过 PCR 技术获得了斑石鲷 *Opirf7* 基因 CDS 区序列,并对其序列进行分析。预测其编码蛋白的分子量为 50.5 kDa,无信号肽及跨膜区。氨基酸序列比对结果发现,斑石鲷 *Opirf7* 基因编码的多肽具有典型的 DNA 结合域(DBD)、干扰素相关区(IAD)和丝氨酸富含区(SRD)。在哺乳动物中,所有 IRF 家族成员中的 DBD 通常包含一个 5-色氨酸重复序列,而在硬骨鱼类的 DBD 则只有 4 个间隔的色氨酸重复序列。

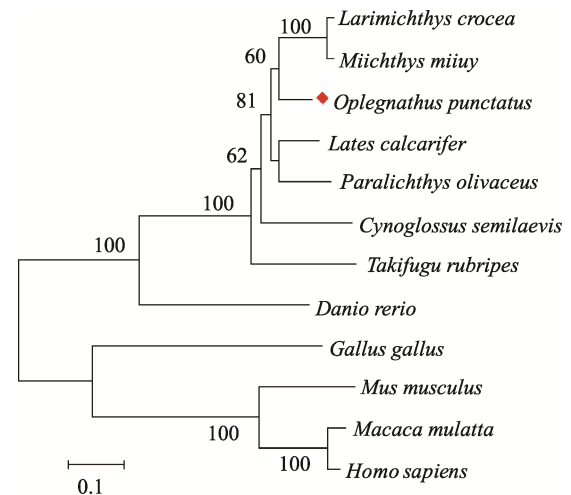


图4 斑石鲷 *Opirf7* 与其他物种系统进化分析
Fig.4 Phylogenetic analysis of *Opirf7* and other species

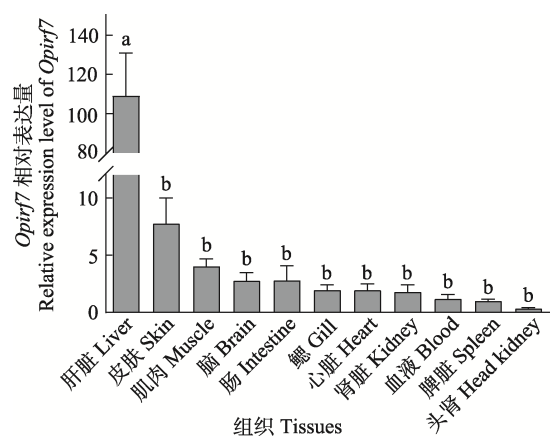
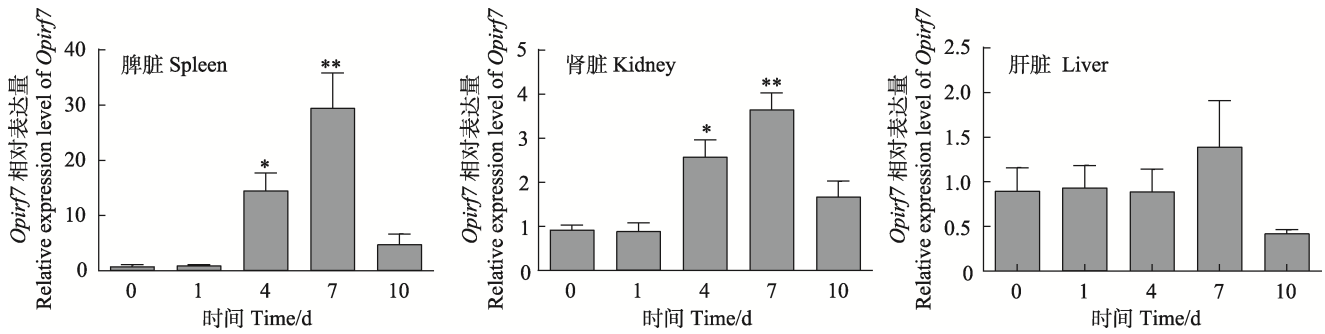
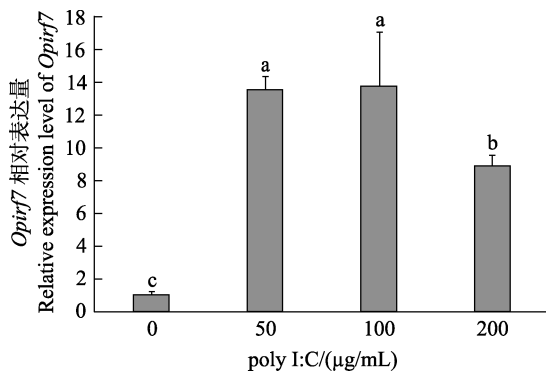


图5 *Opirf7* 基因在健康斑石鲷组织中的相对表达量
Fig.5 The relative expression of the *Opirf7* gene in different tissues of *O. punctatus*

不同字母表示差异显著($P < 0.05$)。
Different letters indicate the significant difference ($P < 0.05$).

图 6 斑石鲷感染虹彩病毒后 *Opirf7* 基因在免疫组织中的实时定量表达分析Fig.6 Real-time quantitative expression analysis of *Opirf7* gene in immune tissues of *O. punctatus* infected with Iridovirus* 表示差异显著($P<0.05$), ** 表示差异极显著($P<0.01$)。* indicates significant difference ($P<0.05$), ** indicates highly significant difference ($P<0.01$).图 7 斑石鲷脑细胞系感染 poly I:C 后 *Opirf7* 基因表达分析Fig.7 Real-time quantitative expression analysis of *Opirf7* gene in brain cell line infected with poly I: C

这些间隔的色氨酸重复序列通过形成螺旋-转角-螺旋结构与靶基因启动子中的 IFN 刺激反应元件(ISRE)和 IRF 调节元件(IRFE)共有序列结合(Escalante *et al.*, 1998; Paun *et al.*, 2007)。IAD 是除 IRF-1 和 IRF-2 以外的所有 IRF 家族成员均具有的结构域。在哺乳动物中, IAD 可通过形成 IRF 同源/异源二聚体与其他转录因子结合以激活干扰素(Eroshkin *et al.*, 1999)。SRD 仅存在于 IRF-3/5/7 中(Holland *et al.*, 2009; Xu *et al.*, 2010), 与病毒诱导的磷酸化有关。通过氨基酸多重序列比对发现, 斑石鲷 *Opirf7* 与哺乳类、鸟类及其他硬骨鱼类 *irf7* 的结构域特征高度保守。*Opirf7* 与硬骨鱼类相似性较高, 与尖吻鲈的相似性为 82.92%, 而与哺乳类、鸟类相似性较低。系统进化结果表明, 斑石鲷与硬骨鱼类的 *irf7* 聚为一大支, 而与鸟类、哺乳类进化关系较远。

在鱼类中, *irf7* 基因在各物种中表达量最高的组织并不完全一致。在尖吻鲈中, 相对表达量较高组织为鳃和后肠(Krishnan *et al.*, 2019); 在斜带石斑鱼 (*Epinephelus coioides*)的脾和肠中表达量较高(Cui *et al.*,

2011); 在赤眼鲟(*Squaliobarbus curriculus*)的脾中表达量最高, 而在肝脏中表达量最低(Zhao *et al.*, 2017)。本研究中, 对健康斑石鲷组织中 *Opirf7* 的相对表达量分析发现, *Opirf7* 在肝脏中的表达量远高于其他组织, 说明肝脏可能在抗病毒免疫防御中也发挥一定的作用。

研究表明, *irf7* 在硬骨鱼类中参与细菌、病毒感染后的免疫应答。欧洲鳗鲡(*Anguilla anguilla*)感染迟缓爱德华氏菌(*Edwardsiella tarda*)后, 其免疫组织中的 *irf7* 表达量显著上调; 迟缓爱德华氏菌或细胞肿大虹彩病毒(Megalocytivirus)刺激都能导致半滑舌鲷各免疫组织 *irf7* 表达量显著上调(Zhang *et al.*, 2015)。在本研究中, 虹彩病毒感染斑石鲷后, 脾脏、肾脏组织中 *Opirf7* 的表达量显著上调, 总体趋势表现为先升高至峰值, 随后逐渐恢复至正常水平。在细胞水平中也发现了相似的表达趋势, 说明 *Opirf7* 对病毒刺激的反应非常迅速, 有可能参与斑石鲷抗病毒感染机制。

综上所述, 本研究通过对 *Opirf7* 基因克隆、序列特征及表达模式分析, 初步表明 *Opirf7* 参与斑石鲷免疫应答过程, 为进一步研究 *Opirf7* 在斑石鲷抗病免疫中的作用机制奠定了基础。

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Cloning and Expression of *irf7* Gene in Spotted Knifejaw (*Oplegnathus punctatus*) Under Virus Infection

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Abstract Interferon regulatory factor (*irf7*) is an immune regulatory factor that plays an important role in the antiviral process. To explore the role of *irf7* in *Oplegnathus punctatus* (Temminck & Schlegel, 1844) under viral infection, we cloned the coding DNA sequence (CDS) region of *irf7* through PCR and analyzed the expression patterns at both tissue and cell levels. The results showed that the CDS region of *Opirf7* was 1 332 bp and encoded a peptide with 443 amino acids. The predicted molecular weight was 50.5 kDa and the theoretical isoelectric point was 5.546. Protein structure analysis showed that *Opirf7* has three conserved domains: the DNA binding domain (DBD), IRF-associated domain (IAD), and serine-rich domain (SRD). Amino acid similarity analysis showed that *Opirf7* had the highest similarity to the IRF7 of *Lates calcarifer*, which was 82.92%. The similarity of *Opirf7* with the IRF7 of *Larimichthys crocea*, *Paralichthys olivaceus*, and *Cynoglossus semilaevis* were 81.99%, 79.95%, and 73.74%, respectively. Phylogenetic analysis showed that *Opirf7* and other fish *irf7* genes were clustered into one branch, and *irf7*s from *Gallus gallus*, *Mus musculus*, *Macaca mulatta*, and *Homo sapiens* were clustered into another. Tissues from healthy *O. punctatus* were collected, including the liver, spleen, kidney, head kidney, intestine, gill, skin, muscle, brain, heart, and blood. After RNA extraction and cDNA synthesis, real-time PCR (qPCR) was performed to detect the expression level of *Opirf7* using the comparative CT method ($2^{-\Delta\Delta CT}$ method). The results of qPCR showed that *Opirf7* was expressed in different tissues of healthy individuals and its expression was highest in the liver, followed by the skin and intestines. The lowest expression was observed in the head kidney. In this study, the expression profiles of *Opirf7* before and after viral infection were determined at the tissue and cell levels. For the *in vivo* challenge study, fish were intraperitoneally injected with spotted knifejaw iridovirus, and the expression level of *Opirf7* was tested in the spleen, kidney, and liver. Compared with the control group at 0 h, the expression level of *Opirf7* was 15-fold in the spleen and 3-fold in the kidney 4 days after infection, and the expression peak was at 7 days after infection. However, the expression of *Opirf7* was not significantly altered in the liver. A poly I:C-infected *O. punctatus* brain cell model was established, and the expression profiles of *Opirf7* mRNA were detected before and after infection. The expression of *Opirf7* mRNA in the low and medium concentration groups (50 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$, respectively) increased by 13 to 17 times, and the expression level of *Opirf7* mRNA in the high concentration group (200 $\mu\text{g/mL}$) increased by approximately 8 times. It was speculated that the high concentration of 200 $\mu\text{g/mL}$ caused some damage to the cells and that the expression level in the high concentration group was lower than that in the low and medium groups. In this study, the full-length open reading frame sequence of *Opirf7* was cloned and characterized for the first time. The deduced amino acid sequence displayed a structure similar to those of other vertebrates. Further functional analysis showed that *Opirf7* has a significant response to viral infection at both tissue and cell levels. This study demonstrated that the *Opirf7* gene might play an important role in the antiviral response of *O. punctatus* and provide a potential molecular marker for antiviral breeding of *O. punctatus*.

Key words Spotted knifejaw (*Oplegnathus punctatus*); Interferon regulatory factor-7; Anti-virus gene; Immune response

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