

杂色鲍*DAD1*的克隆及在发育、弧菌感染、高温和缺氧下的表达分析

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摘要: 抗细胞凋亡因子(*DAD1*)是细胞凋亡的负调控因子。在杂色鲍转录组测序的基础上, 通过RACE的方法, 获得了杂色鲍*DAD1*基因的全长cDNA, 命名为*HdDAD1*。该序列全长553 bp, 开放阅读框339 bp, 编码112个氨基酸。编码蛋白具有保守的DAD功能域和3段跨膜区。实时定量PCR结果表明, *HdDAD1*在杂色鲍所检测组织中均有表达, 消化道、鳃、血细胞、粘液腺和肾脏中表达量最高。同样, *HdDAD1*在发育各阶段均有表达, 幼虫阶段表达量最高, 原肠胚次之, 其他胚胎发育阶段和稚鲍表达量最低。*HdDAD1*能够响应细菌感染、高温和缺氧应激。在弧菌注射12 h后, 表达量显著上升。当水温由最适的25 °C上升到28 °C时, *HdDAD1*表达量升高; 在31 °C持续4和96 h时*HdDAD1*表达量显著上升。在低氧处理4和96 h时, *HdDAD1*的表达量也显著上升。

关键词: 杂色鲍; 抗细胞凋亡因子(*DAD1*); 基因表达; 发育; 应激

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杂色鲍(*Haliotis diversicolor*)自然分布于我国东南沿海, 是我国主要的养殖贝类之一。近年来因养殖规模工厂化和集约化程度的不断提高等原因导致杂色鲍暴发性病害频发^[1], 成为阻碍我国杂色鲍养殖发展的主要因素。目前杂色鲍的养殖方式以陆基水泥池和浅海多层箱笼为主。这2种养殖方式密度较大, 因而容易缺氧。在夏季养殖水体温度较高, 高温和缺氧是鲍大量死亡的主要因素^[2-3]。副溶血弧菌(*Vibrio parahaemolyticus*)是杂色鲍的主要病原菌之一, 常导致杂色鲍的大量死亡^[2, 4]。

抗细胞凋亡因子(defender against cell death 1, *DAD1*)是位于内质网膜的寡糖基转移酶(OST)的一个亚基, 参与了N-糖基化过程^[5-6]。其首次在肾源性细胞株(tsBN7)中被发现, 是细胞凋亡的一个负调控因子, 具有抑制凋亡的功能^[5]。*DAD1*基因具有较高的保守性, 广泛存在各物种中。该基因不仅具有抗细胞凋亡作用, 而且参与了

哺乳动物的炎症反应、器官发生以及自稳态维持等多种生理过程, 也参与了水生无脊椎动物的免疫反应^[7]。鉴于*DAD1*基因参与细胞凋亡和免疫反应过程, 本研究克隆鉴定了杂色鲍的*DAD1*基因, 并分析了在副溶血弧菌、高温、缺氧等条件应激下*DAD1*的表达水平, 希望为深入了解鲍的应激及免疫机制提供基础资料。

1 材料与amp;方法

1.1 实验材料

杂色鲍(壳长4.5~5.0 cm)购自厦门大嶝岛养殖场, 实验前暂养在溶氧充足的新鲜海水中, 投喂新鲜的江蓠(*Gracilaria Verrucosa*)和海带(*Laminaria japonica*)。每2天换1次水, 保持海水溶氧量大于5 mg/L, 水温控制在其最适生长温度23~25 °C。饲养2周后进行杂色鲍的育苗、成体取样及其细菌感染、高温缺氧应激实验。

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1.2 杂色鲍各组织以及发育各时期样品采集

取10只健康杂色鲍, 解剖后分别采集肝胰腺、血细胞、鳃、肾、黏液腺、消化道和外套膜。所取的组织经液氮速冻后, 存于 -80°C 备用。

选取体质健壮, 足肌活动敏捷, 性腺外观饱满, 雌雄各30只为亲鲍。采用综合诱导法分别对雌雄亲鲍刺激。亲鲍分雌雄2组, 经0.5~1 h的阴干刺激后, 用紫外线照射海水(200~300 mW·h/L), 处理2~4 h, 然后经 $\pm 3^{\circ}\text{C}$ 左右的变温刺激, 可获得精子和卵子。随后进行人工授精, 精卵分别收集到一塑料容器中。受精时, 卵子密度为20~100个/mL, 精子密度为 5×10^5 个/mL, 并不断轻轻搅动水体, 加速精卵结合, 受精后及时清洗受精卵3~4次, 每次间隔30 min左右。受精完成后, 显微镜下及时观察卵裂状况。受精卵在水温($25 \pm 1^{\circ}\text{C}$), 盐度 32 ± 1 的新鲜海水中继续培养, 用普通光学显微镜观察。收集受精卵、2细胞期、4细胞期、桑葚胚期、囊胚期、原肠胚期、担轮幼虫、面盘幼虫、匍匐幼虫和变态后稚鲍, 将采集样品液氮速冻后, 存于 -80°C 待用。

1.3 副溶血弧菌细菌感染实验

根据Wang等^[8]和Ge等^[9]的方法进行。杂色鲍经2周驯养后, 向腹足肌肉注射50 μL 浓度为 1.1×10^8 cfu/mL的副溶血弧菌菌液, 对照组注射50 μL 灭菌新鲜海水。在注射后的0、3、6、12、24和48 h, 分别取实验组和对照各10只杂色鲍的血细胞, 离心(2000 r/min、 4°C 、10 min)后放入液氮速冻后, 存于 -80°C 待用。

1.4 杂色鲍高温应激处理和缺氧应激处理

高温应激处理和缺氧应激处理根据黄贻涛等^[10]的方法进行。高温应激实验组以杂色鲍最适生长温度($22 \sim 28^{\circ}\text{C}$)的中间温度 25°C 为基准, 以 $1^{\circ}\text{C}/\text{h}$ 升温至 31°C 作为高温应激处理, 当温度升至 28°C 时作为第1时相(0 h)取样, 升至 28.5°C 时作为第2时相(0.5 h)取样, 以 31°C 的持续高温应激分别将4、24、96和192 h时间点依次作为第3~6时相。对照组以杂色鲍最适生长温度($22 \sim 28^{\circ}\text{C}$)的中间温度 25°C 进行实验。取样方法同副溶血弧菌应激实验。

缺氧应激实验组的溶氧量设为2 mg/L, 对照组为空气曝气(溶氧量约为5.5 mg/L), 在处理之后的4、24、96和192 h取样。取样方法同副溶血弧菌应激实验。

1.5 总RNA提取和cDNA的合成

样品从 -80°C 转移至Trizol溶液(Roche公司)中, 按照说明书提取样品总RNA, 1.5%琼脂糖凝

胶电泳检测后, 微量紫外分光光度计测定浓度和纯度。

取1.5 μg 总RNA分别与1 μL oligo (dT) (10 $\mu\text{mol}/\text{L}$) 或1 μL 5'CDS Primer (10 $\mu\text{mol}/\text{L}$) 混合, 按照SMART PCR Synthesis Kit (Clontech 公司)的说明合成cDNA。

1.6 *HdDAD1*基因的克隆及序列分析

根据杂色鲍转录组筛选得到的*HdDAD1*片段, 用Primer3 (<http://primer3.ut.ee/>)设计的RACE引物扩增其全长cDNA, 并用head to toe PCR验证其开发阅读框的正确性。

采用NCBI的Vecscreen去掉测序载体序列, 用bl2seq拼接序列, 经BLASTX和ORF Finder确定其开放阅读框(ORF)后, 用EMBOSS (<http://imed.med.ucm.es/EMBOSS/>)的Prettyseq程序进行核酸序列和蛋白序列翻译。使用ExPASy (<http://www.expasy.org/>)预测其编码蛋白等电点及分子量, 使用NetPhos 2.0 Server (<http://www.cbs.dtu.dk/services/NetPhos/>)预测磷酸化位点, 使用NetNGlyc 1.0 Server (<http://www.cbs.dtu.dk/services/NetNGlyc/>)预测糖基化位点, TMHMM2.0 Server (<http://www.cbs.dtu.dk/services/TMHMM-2.0/>)预测蛋白跨膜结构域, 利用BioEdit软件进行序列的多重比对, 利用MEGA 4.0软件中邻接法构建系统进化树。

1.7 实时定量PCR

利用表1的定量引物, 以oligo (dT) 逆转录合成cDNA为模板, 按照SYBR Green Realtime PCR Master Mix (TOYOBO公司)的说明进行实时定量PCR。发育阶段以*YB1*为内参基因, 其他以 β -actin为内参基因。检测扩增产物熔解曲线和扩增曲线并测序, 以验证扩增特异性。每个时相分析10个样品, 计算每个样品的 $2^{-\Delta\Delta\text{Ct}}$ 为RQ值, 基因的表达水平表示为RQ平均值 \pm 标准误。组织表达和发育阶段用ANOVA进行数据方差分析, Duncan多重比较分析各组织和各阶段表达的差异性, 其他应激处理进行*t*检验分析, 显著性差异表示为 $P < 0.05$ 。

2 结果

2.1 *HdDAD1*基因全长克隆及序列分析

HdDAD1 cDNA全长为553 bp, 包括71 bp的5'非编码区(UTR), 140 bp 3'UTR和342 bp的开放阅读框(ORF), 编码113个氨基酸。预测编码蛋白的分子量为12.68 ku, 等电点为7.72, 具有4个磷

表 1 所用引物及其序列

Tab. 1 Primer sequences used in the experiments

| 引物名称 primer name | 引物序列(5'→3') primer sequences | 目的 purpose |
|---------------------|--|---------------|
| 5-outer | GGCTCGTTCAGGGCTGATTC | RACE 扩增 |
| 5-inner | CCCGGCAAATCGTACCAATG | RACE 扩增 |
| UPM | Long: CTAATACGACTCACTATAGGGCAAGCAGTGGTAACAACGCAGAGT Short: CTAATACGACTCACTATAGGGC | RACE 扩增 |
| NUP | AAGCAGTGGTAACAACGCAGAGT | RACE 扩增 |
| 5-CDS | (T) 25VN | 逆转录 |
| SMART II | AAGCAGTGGTATCAACGCAGAGTACGCGGG | 逆转录 |
| head | CCGGGTGGATACATCAAAAT | 验证ORF |
| toe | TGTGACGGACTCAATCTTGC | 验证ORF |
| HdDAD1-F | GGGAACCTTCCCATTCAACT | 定量PCR |
| HdDAD1-R | GCAAGATTGAGTCCGTCACA | 定量PCR |

酸化位点, 无糖基化位点, 有1个DAD结构域(4~112 aa), 3个跨膜区域分别为30~48、53~73和92~112 aa (图1)。

2.2 HdDAD1多重比对及进化树构建

多重比对表明HdDAD1与其他物种的DAD1具有较高的保守性, 与黑腹果蝇(*Drosophila melanogaster*)、紫球海胆(*Strongylocentrotus purpuratus*)和人(*Homo sapiens*)的DAD1一致性分别为75%、

73%和71%。DAD1的3个跨膜区同样具有很高的保守性(图2)。

采用DAD1序列为GenBank上已注册的DAD1及其同源物的氨基酸全长序列, 拟南芥(*Arabidopsis thaliana*)作为外群(outgroup)构建NJ进化树。结果显示HdDAD1与加利福尼亚海兔(*Aplysia californica*)聚为一小支, 再与贝类聚成一支, 然后与佛罗里达文昌鱼(*Branchiostoma floridae*)、沙蚕(*Capitella teleta*)和脊椎动物形成的大支聚在一起(图3)。

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1 GGGCTGAGGACGGAAGAGCACTTGCCACGTTTCGTGAACATTGGTACGATTGCCGGGTG 60
61 GATACATCAAAtgccagagaattaacgtcagtcgttgcaagttttatgacgaatata 120
1 M P E K L T S V V A K F Y D E Y I 17
121 tccagtcaacgccaaaagacttaaaattgtagatgcttacctagtgtatgtgtcttga 180
18 Q S T P K R L K I V D A Y L V Y V F L T 37
181 cgggagatttccagtttgatattgttggtttagtgggaaccttccattcaactcatttc 240
38 G V F Q F G Y C C L V G T F P F N S F L 57
241 tctctggatttattcaactgttggttcattgttttgggagtgctctgagccttcaag 300
58 S G F I S T V G S F V L G V C L R L Q V 77
301 tgaatccacagaataaaaacgacttgtcggaaatcagccctgaacgagccttgcctgact 360
78 N P Q N K N D F V G I S P E R A F A D F 97
361 ttgtgttctgctcagtcattctacacttagttgttataaacttctgggtTGATGGACAG 420
98 V F A H V I L H L V V I N F L G* 113
421 CAGACACTGGGGCAAGATTGAGTCCGTCACAAGGGGAGGTGGGGACAGGGGGGATAT 480
481 TCATGCATATACAACACATTATCAATAAATGTTAATTTAACAGCAAAAAAAAAAAAAA 540
541 AAAAAAAAAAAAAA 553
    
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图 1 HdDAD1基因cDNA及推导的氨基酸序列

终止密码子和加尾信号用粗体表示, *表示蛋白序列终止, 方框表示磷酸化位点, DAD结构域(4~113 aa)用加粗斜体, 下划线部分为跨膜区域

Fig. 1 The cDNA and deduced amino acid sequence of HdDAD1 gene from *H. diversicolor*

The stop codon (tga) and polyadenylation signal sequence (AATAAA) are characterized in bold, * means the end of protein sequence, potential phosphorylation site is in box, DAD domain is both in bold and italic, transmembrane span is labeled with underline

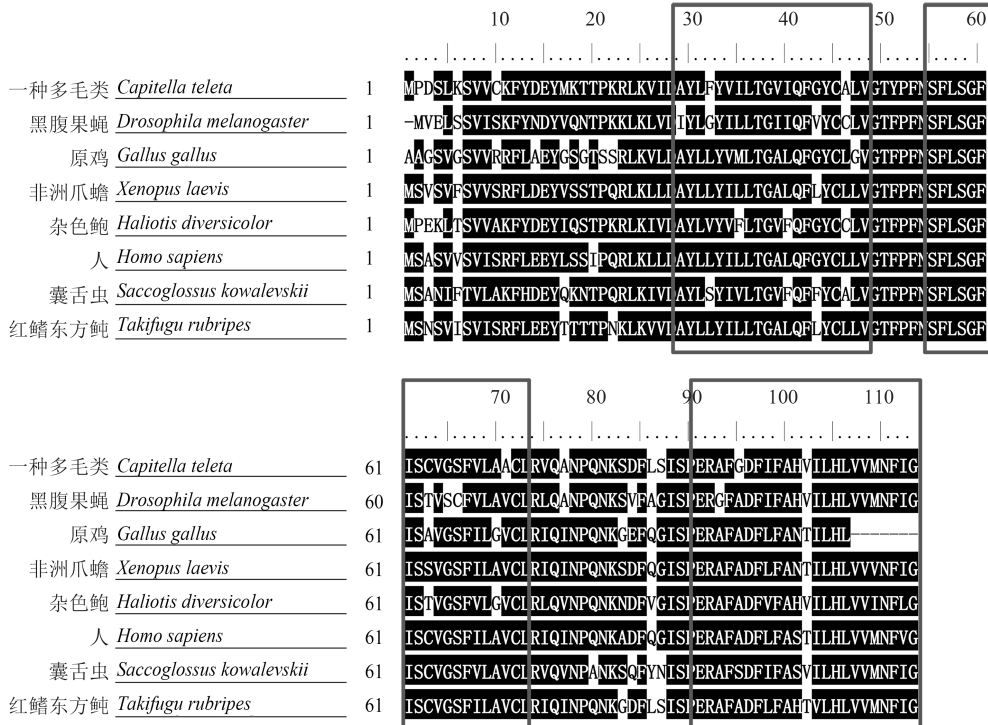


图 2 杂色鲍和其他物种的DAD1氨基酸序列比较分析

方框为跨膜区

Fig. 2 Amino acid sequences alignment of DAD1 from *H. diversicolor* and other species

The transmembrane spans are marked with filament box

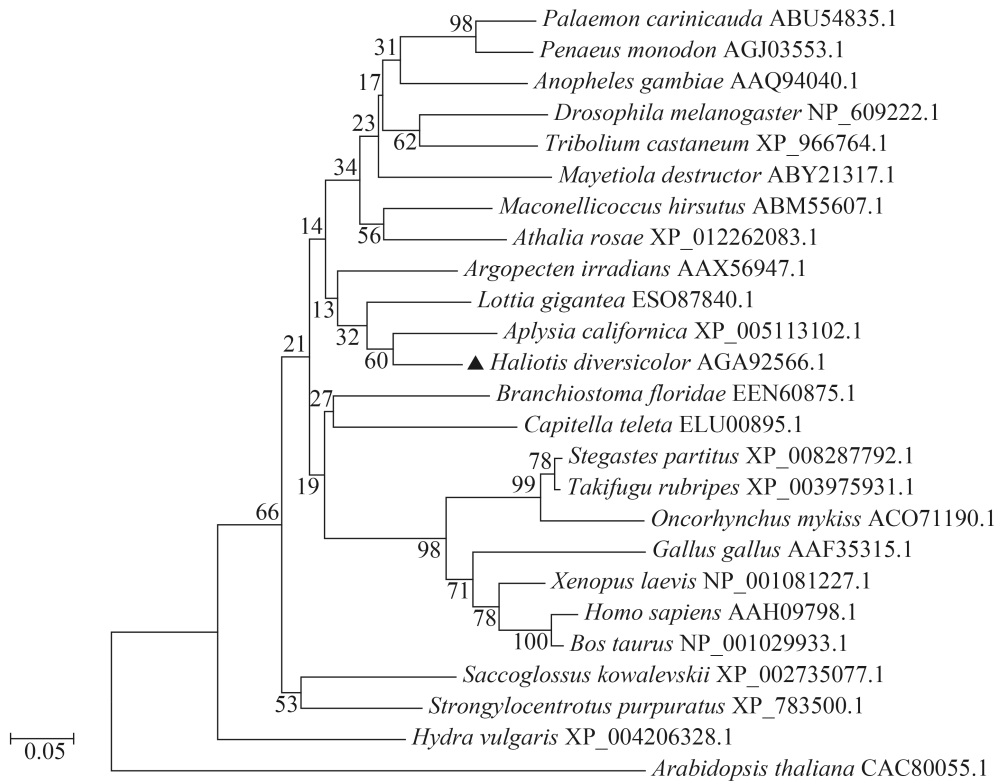


图 3 DAD1氨基酸序列的NJ系统进化树

Fig. 3 NJ phylogenetic tree based on DAD1 amino acid sequences by MEGA 4.0

2.3 *HdDAD1*的组织表达

*HdDAD1*基因在所有检测的组织中都表达(图4)。在外套膜中的表达量最低,显著低于其他6个组织($P<0.05$)。消化道、鳃、血细胞、黏液腺和肾脏之间没有显著性差异($P>0.05$),属于表达量最高的组织。肝胰腺中的表达量显著低于表达量最高的消化道等5个组织($P<0.05$),而显著高于外套膜($P<0.05$)。

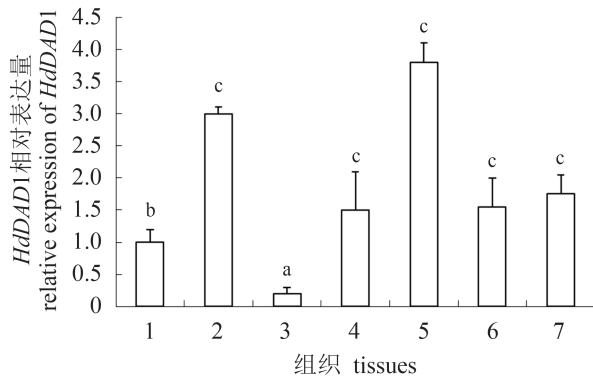


图4 *HdDAD1*基因的各组织相对表达量

1.肝胰腺; 2.鳃; 3.外套膜; 4.肾; 5.消化道; 6.黏液腺; 7.血细胞; 不同字母表示有显著差异($P<0.05$),下同

Fig. 4 The expression level of *HdDAD1* in different tissues

1. hepatopancreas; 2. gill; 3. mantle; 4. kidney; 5. digestive tract; 6. mucus gland; 7. hemocyte; different letters mean significant difference ($P<0.05$), the same below

2.4 *HdDAD1*在发育各阶段的表达

*HdDAD1*在胚胎发育阶段表达量显著低于幼虫阶段($P<0.05$) (图5)。原肠胚期是胚胎时期表达量最高的阶段,显著高于胚胎发育其他时期($P<0.05$)。杂色鲍的幼虫阶段表达量最高,显著高于稚鲍和除原肠胚期外的胚胎阶段($P<0.05$)。

2.5 *HdDAD1*在副溶血弧菌感染、高温和缺氧处理后的表达

副溶血弧菌注射感染后12 h,实验组的表达量显著高于对照组($P<0.05$) (图6)。其他取样时相对对照组和实验组之间没有显著性差异($P>0.05$)。

水温由25升至28℃时,高温处理组*HdDAD1*的表达量显著高于对照组($P<0.05$) (图7)。水温升至31℃并持续4和96 h时,高温处理组的表达量也显著高于对照组($P<0.05$)。其他取样时相处理组和对照组之间没有显著性差异($P>0.05$)。

缺氧处理4和96 h后,缺氧组*HdDAD1*的表达量显著上升($P<0.05$),其他取样时相*HdDAD1*

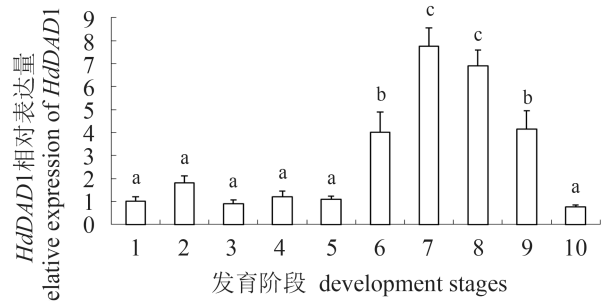


图5 *HdDAD1*基因在各发育阶段的表达量

1. 受精卵; 2. 2细胞期; 3. 4细胞期; 4. 桑葚胚期; 5. 囊胚期; 6. 原肠胚期; 7. 担轮幼虫; 8. 面盘幼虫; 9. 匍匐幼虫; 10. 稚鲍

Fig. 5 The expression level of *HdDAD1* in different developmental stages

1. fertilized egg; 2. 2 cleavage; 3. 4 cleavages; 4. morula; 5. blastula; 6. gastrula; 7. trochophore; 8. veliger; 9. creeping larva; 10. juvenile abalone

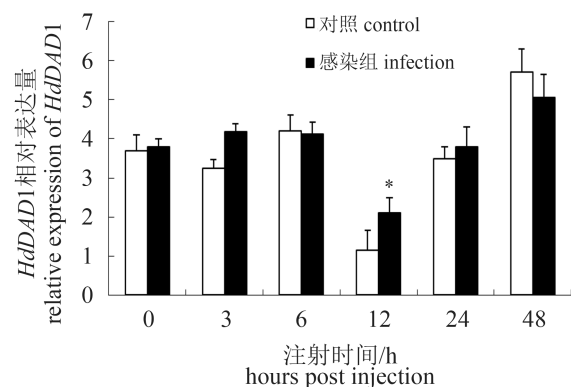


图6 *HdDAD1*基因在副溶血弧菌感染后表达变化

*. 对照组和实验组存在显著性差异($P<0.05$),下同

Fig. 6 The expression of *HdDAD1* after *V. parahaemolyticus* challenge

*. there is a significant difference between control group and experimental group ($P<0.05$), the same below

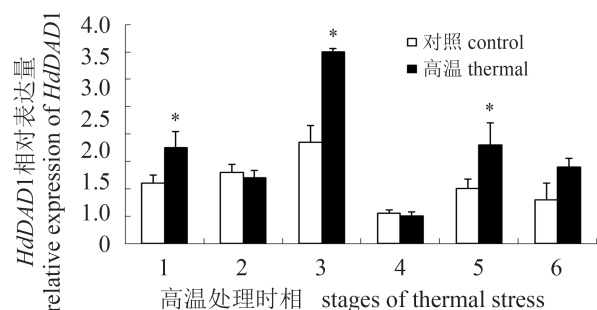


图7 *HdDAD1*基因在高温应激后表达变化

1. 28℃; 2. 31℃ (0 h); 3. 31℃ (4 h); 4. 31℃ (24 h); 5. 31℃ (96 h); 6. 31℃ (192 h)

Fig. 7 The expression of *HdDAD1* after thermal stress

的表达量没有显著性变化($P>0.05$) (图8)。

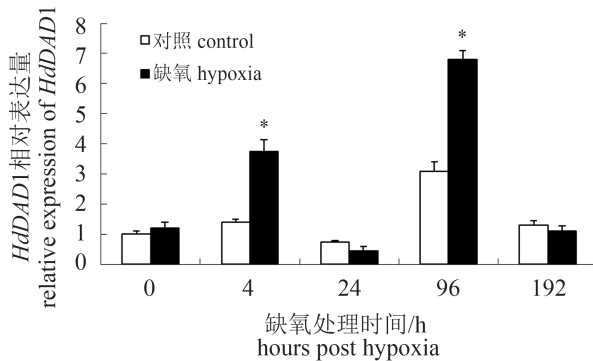


图8 缺氧处理后*HdDAD1*的表达变化

Fig. 8 The expression of *HdDAD1* after hypoxia stress

3 讨论

本研究获得杂色鲍*HdDAD1*的cDNA全长, 编码112个氨基酸, 与动物和单子叶植物的*DAD1*蛋白的氨基酸数目相同^[11]。*HdDAD1*等电点为7.72, 与动物的*DAD1* 7.38~7.83类似, 而与大部分植物8.81~9.07差别较大^[11]。*HdDAD1*与人、海胆和果蝇等物种的一致性达到70%以上, 这表明*DAD1*在蛋白一级结构上高度保守。进化树显示几种贝类的*DAD1*首先聚为一支, 表明其在贝类中具有更高的保守性。*DAD1*定位于内质网上, 具有3个保守的跨膜区, N端朝向胞质一侧, 而C端朝向内质网^[5]。抗细胞凋亡因子*DAD1*是一种内源性细胞凋亡抑制基因, 是糖基转移酶执行功能和维持其结构所必需的部分, 还能维持细胞内正常水平的糖基化。*DAD1*蛋白功能异常或表达量过低均会严重影响糖基转移酶的功能, 使细胞缺乏糖基化的蛋白质而凋亡。已证明人、拟南芥和水稻 (*Oryza sativa*) *DAD1*具有抵抗细胞凋亡的作用^[12-13]。衣藻 (*Chlamydomonas*) 细胞*DAD1*的表达量也与细胞凋亡程度具有负相关关系^[14]。这表明*DAD1*在各类生物中都能够发挥抗细胞凋亡的作用。

*HdDAD1*在所检测的杂色鲍组织中均有表达, 但是各组织的表达水平并不相同, 这表明*DAD1*是一个各组织均可以表达的基因。不同组织表达水平的差异, 可能与各组织凋亡程度不同有关^[11]。在黑森瘿蚊 (*Mayetiola destructor*)^[15]、海湾扇贝 (*Argopecten irradians*)^[16]、大腹园蛛 (*Araneus ventricosus*)^[17]、青石斑鱼 (*Epinephelus awoara*)^[18]和

脊尾白虾 (*Exopalaemon carinicauda*)^[19]等报道*DAD1*基因克隆和表达的物种中, 同样在所检测各组织中均发现*DAD1*表达。杂色鲍和黑森瘿蚊都是消化道表达量最高, 这可能是因为消化道细胞比其他组织具有更高的细胞凋亡水平, 需要*DAD1*对细胞凋亡进行较多的负调控所致^[15]。*HdDAD1*在杂色鲍鳃中表达水平也非常高, 这与脊尾白虾类似^[19]。鳃作为水生动物的呼吸器官需要过滤大量海水, 外界物质刺激水平较高, 情况类似消化道。鲍的黏液腺覆盖在鳃的外部, 同鳃一样具有较高*DAD1*的表达水平。鲍众多的免疫基因都在血细胞中表达量较高^[4, 8-9], 血细胞中*HdDAD1*表达水平较高暗示其可能参与免疫反应。

*DAD1*在多细胞生物的发育过程中具有重要作用^[20]。*DAD1*突变的小鼠胚胎发育延迟、畸形、中胚层发育紊乱, 表达非正常的N-糖基化蛋白^[20], 胚胎不能发育到囊胚期, 细胞凋亡水平很高^[21], 表明其在胚胎发育中具有重要作用。*HdDAD1*在发育过程中的表达变化与黑森瘿蚊类似, 二者都在幼虫阶段表达量最高, 变态后表达量急剧下降^[15]。杂色鲍和黑森瘿蚊都需要经过变态发育成成体, 变态过程中需要经历细胞分化和组织重构, 是细胞凋亡比较旺盛的阶段^[22-23]。因此, 该阶段*HdDAD1*的高表达可以控制不必要的细胞凋亡^[15]。这可能与线虫胚胎发育中*DAD1*负调控细胞凋亡具有相同作用^[24]。

*DAD1*能够响应病原的刺激。如注射脂多糖能够引起青石斑鱼*DAD1*的高表达^[18], 脊尾白虾注射WSSV和鳃弧菌 (*V. anguillarum*)后*DAD1*的表达水平显著上调^[9]。杂色鲍在注射副溶血弧菌12 h后*DAD1*表达量显著上调。而黄头病毒感染斑节对虾 (*Penaeus monodon*) 则能引起*DAD1*表达量降低^[25]。这表明*DAD1*在免疫反应中的作用具有种属特异性。其除了作为抗细胞凋亡因子外, 也可以通过促进免疫细胞的增殖来发挥作用^[26]。这也提示了*DAD1*在免疫过程中具有多种功能。

*DAD1*及其同源物在温度应激时表达量会发生显著性变化^[12, 17]。*DAD1*最初是从温度敏感仓鼠 (*Mesocricetus auratus*) 细胞系tsBN7中分离获得, tsBN7细胞在39.5 °C开始凋亡, *DAD1*可以抑制tsBN7细胞的凋亡^[12]。高温应激时, 杂色鲍血细胞数量 (total haemocyte count, THC) 显著下降^[3], 这种情况与很多甲壳动物类似^[27-29]。同样, 缺氧

应激会导致贝类^[30-34]和杂色鲍THC下降^[2, 35]。THC的减少表明血细胞发生了凋亡^[34]。杂色鲍*HdDAD1*在高温应激和缺氧应激时上调表达可以调节这些应激引起的细胞凋亡程度,防止不必要的细胞凋亡给机体带来更大的损伤^[15]。

应激导致*DAD1*表达水平的变化是一种普遍的现象。除了上述病原应激、高温应激和缺氧应激外,黑森瘿蚊幼虫在饲喂抗性小麦(*Triticum aestivum*)时*DAD1*表达水平要高于饲喂易感性小麦^[15]。海湾扇贝在受到机械损伤后*DAD1*的表达量也会上升^[16]。在拟南芥中过表达*DAD1*可以耐受高浓度H₂O₂的处理^[36]。而将雪莲(*Saussurea involucrata*)*DAD1*的基因转到烟草(*Nicotiana tabacum*)中,能提高烟草的抗旱性和抗盐性^[37]。Mittapalli等^[15]认为机体在内稳态保持正常时具有适当的细胞凋亡程度,当应激破坏了内稳态时细胞凋亡程度随之升高;所以作为调控细胞凋亡的*DAD1*也随之上调表达。这可能是*DAD1*在应激过程中上调表达的原因之一。

本研究克隆了杂色鲍*HdDAD1*的全长cDNA,分析了该基因在不同组织、不同发育阶段、副溶血弧菌感染、高温应激和缺氧应激下的表达特征。结果表明该基因在发育和应激过程中具有重要作用,这为杂色鲍发育和应激过程中细胞凋亡程度的研究提供了参考资料。作为高度保守的内源性抑制细胞凋亡基因,*DAD1*的生物学功能与抵抗胁迫应激等密切相关^[11]。*HdDAD1*基因的克隆及表达分析可以为鲍优良种质资源的开发和利用提供理论基础资料。

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Molecular cloning and expression of defender against cell death1 from *Haliotis diversicolor* in development, vibrio infection, thermal stress and hypoxia stress

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Abstract: The defender against cell death 1 (DAD1) is a regulatory protein to inhibit the apoptosis process. The full-length cDNA of *Haliotis diversicolor* DAD1 (*HdDAD1*) was cloned by RACE according to DAD1 partial sequence of transcriptome. *HdDAD1* was 553 bp, with a 339 bp open reading frame encoding a protein of 112 aa (*HdDAD1*). *HdDAD1* contains a conserved DAD domain, with 3 transmembrane spans. Quantitative real-time PCR results indicated that *HdDAD1* could be detected in all examined tissues, with the highest expression level in digestive tract, gill, hemocyte, mucus gland and kidney. There was also expression of *HdDAD1* in different developmental stages. *HdDAD1* expression level of larva stages was the highest, that of gastrula stage was middle, that of other stages was the lowest. *HdDAD1* could respond bacterial challenge/thermal/hypoxia stress. There was significant up-regulation of *HdDAD1* at 12 h after *Vibrio parahaemolyticus* challenge. *HdDAD1* was significantly up-regulated, when water temperature rose from 25 to 28 °C. The expression of *HdDAD1* also significantly increased, when water temperature was 31 °C for 4 and 96 h. At 4 and 96 h post hypoxia stress, *HdDAD1* significantly increased, too.

Key words: *Haliotis diversicolor*; defender against cell death 1 (*DAD1*); gene expression; development; stress

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