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许氏平鲉对慢性高温胁迫响应的转录组学分析

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摘要: 为探索许氏平鲉(*Sebastes schlegelii*)高温胁迫条件下基因表达水平的变化, 利用 Illumina Hiseq 4000 测序平台分别对许氏平鲉高温处理组(温度由 18 ℃缓慢升至 27 ℃后养殖 5 d)和常温对照组(18 ℃下养殖)的鳃、肝和肠道组织进行了转录组测序。以$|\log_2 \text{fold change}| \geqslant 1$ 和 FDR (false discovery rate, 错误发现率)<0.05 作为显著差异表达基因(DEGs)筛选条件。在鳃组织中, 共筛选出 306 个 DEGs, 其中 96 个基因显著上调, 210 个基因显著下调; 在肝脏组织中, 共筛选出 806 个 DEGs, 其中 382 个基因显著上调, 424 个基因显著下调; 在肠道组织中, 共筛选出 343 个 DEGs, 其中 162 个基因显著上调, 181 个基因显著下调。3 个组织共表达的 DEGs 有 12 个。GO 功能注释分析结果显示, 鳃组织中的 DEGs 较多富集在蛋白水解作用、胞外区、结构分子活性、受体调节器活性、受体配体活性等功能中; 肝组织中 DEGs 较多富集在脂肪代谢过程、细胞氨基酸代谢过程、细胞质、氧化还原酶活性等功能中; 肠道组织中 DEGs 较多富集在氧化还原过程、胞外区、辅因子结合等功能中。运用逆转录实时荧光定量 PCR (RT-qPCR)对 10 个 DEGs 的表达量进行验证, 结果显示, RT-qPCR 结果与转录组分析结果一致, 表明转录组分析可靠。本研究为解析许氏平鲉对慢性高温胁迫应激反应的分子机制提供了转录水平上的参考数据。

关键词: 许氏平鲉; 高温胁迫; 转录组; 鳃; 肝脏; 肠道
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许氏平鲉(*Sebastes schlegelii*)广泛分布在东亚沿海, 因其营养价值较高且口味鲜美, 在中国、日本和韩国都广受欢迎[1]。日本很早就开展了许氏平鲉的养殖研究[2], 从 20 世纪 90 年代起, 中国和韩国陆续开展了许氏平鲉的网箱养殖。许氏平鲉的生长速度快, 适温范围广, 因此成为中国北方重要的网箱养殖鱼类。近几年, 我国北方各省份均开展了许氏平鲉的增殖放流, 每年放流增殖的鱼苗达数千万尾[3]。

许氏平鲉最适生长水温为 18~24 ℃[4]。在我国北方, 夏季一些养殖场的水温可达 28 ℃, 研究表明在高温季节许氏平鲉的食欲和生长速度严重下降[5]。随着全球气候变暖, 关于高温胁迫对许氏平鲉影响的研究也越来越多, 如高滨等[5]研究了高温对许氏平鲉生长和摄食的影响, 张思敏等[6]研究了高温条件下许氏平鲉肝脏新陈代谢的变化, 温海深等[7]研究了高温对许氏平鲉血液生理指标和基因表达的影响, Lyu 等[8]研究了急性高温胁迫条件下许氏平鲉肝脏中的转录组特征。在养殖环境条件下, 海水水温的增长是个缓慢的过程, 因此有必要开展许氏平鲉对慢性高温胁迫的应激机制, 为开展许氏平鲉耐高温品系的选育提供借鉴。

作为鱼的呼吸器官, 鳃与外界环境直接发生

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交流, 因此鳃成为研究鱼应对环境变化的目标器官之一, 如对梭鲈(*Sander lucioperca*)的研究表明, 高温胁迫破坏了鳃的结构并造成氧化应激反应[9]; 西伯利亚鲟(*Acipenser baerii*)在受到高温胁迫后, 鳃泡的数量增加, 上皮细胞发生变性, 退化和坏死[10]。肝脏在鱼类新陈代谢和能量储存中扮演重要角色, 因此研究肝脏对高温胁迫的应激反应也具有重要意义[11], 如高温胁迫造成褐牙鲆(*Paralichthys olivaceus*)的肝组织损伤, 发炎和氧化应激反应[12]; 高温造成虹鳟(*Oncorhynchus mykiss*)肝脏中磷脂和多不饱和脂肪酸代谢发生改变[13]。除了鳃和肝脏, 肠道在维持鱼类机体健康中也起到重要作用, 最近的研究表明, 高温胁迫造成杂交鲟(*Acipenser baerii*♀×*Acipenser schrenckii*♂)肠道发炎, 肠道中消化酶活性降低[14]。

随着高通量测序技术成本的降低, 利用高通量测序技术研究了鱼类对高温胁迫的应激机理[15-20]已有不少报道, 而关于许氏平鲉慢性高温胁迫下的转录组研究尚未见报道。本研究以慢性高温胁迫条件下的许氏平鲉为研究对象, 分析了鳃、肝脏和肠道组织的转录组变化, 筛选了差异表达的基因和关键通路, 以期为耐高温许氏平鲉的选育提供研究基础。

1 材料与方法

1.1 养殖实验及样品采集

实验用许氏平鲉养殖于山东省青岛市。随机挑选 120 尾体重(162.85±5.13) g 的健康幼鱼, (18±0.5) ℃在工厂化养殖系统中暂养两周后分为两组, 常温组和高温组, 每组 3 个重复。其中高温组按每天升温 1 ℃的节奏由 18 ℃升至 27 ℃后保持恒定, 常温组保持在(18±0.5) ℃。暂养和实验期间, 每天于 8:00 换水 1 次, 9:00 和 18:00 分别投喂 1 次, 养殖用水溶解氧≥7.5 mg/L, pH 7.8~8.2, 光照条件为自然光。

当高温组达到 27 ℃并保持 5 d 后, 高温组和常温组每个重复随机选 3 尾鱼, 每组共 9 尾。样品经 MS-222 麻醉后, 取鳃、肝脏和肠道组织于−80 ℃保存用于 RNA 提取。每个重复的 3 尾鱼的同一组织等量混合为一个样品。

1.2 组织转录组文库构建及测序

将采集的样品委托诺禾致源生物科技有限公司进行 RNA 的提取与检测、cDNA 文库构建(NEBNext Ultra RNA Library Prep Kit for Illumina, NEB, USA), 共构建高温_鳃 1, 高温_鳃 2, 高温_鳃 3, 高温_肝 1, 高温_肝 2, 高温_肝 3, 高温_肠 1, 高温_肠 2, 高温_肠 3, 常温_鳃 1, 常温_鳃 2, 常温_鳃 3, 常温_肝 1, 常温_肝 2, 常温_肝 3, 常温_肠 1, 常温_肠 2 和常温_肠 3 共 18 个文库。构建的 cDNA 文库用 Illumina NovaSeq 6000 (Illumina, USA)平台进行测序。

1.3 差异表达基因分析及功能注释

将原始序列去除测序接头和低质量序列后得到 clean reads。使用 Bowtie 2[21]软件将 clean reads 与许氏平鲉参考基因组进行序列比对, 采用 FPKM (fragments per kilobase of exon model per million mapped reads)计算方法估计高温组和对照组的基因表达量。以$|\log_2\text{fold change}|\geqslant 1$ 和 FDR (False discovery rate, 错误发现率)<0.05 为标准, 使用 edgeR 软件筛选差异表达基因。使用 Cluster Profiler 软件对得到的所有 DEGs 进行 GO (gene ontology)功能注释和 KEGG (Kyoto encyclopedia of genes and genomes)通路富集分析。

1.4 实时荧光定量 PCR 分析

选取 10 个在 3 个组织中都差异表达的基因, 通过 RT-qPCR 技术验证转录组数据的可靠性。选择核糖体 *18S rRNA* 作为内参基因, 使用 Primer Premier 5.0 软件设计 PCR 引物, 并由生工生物工程股份有限公司合成(表 1)。每个样品设置 3 个重复, 使用 $2^{-\Delta\Delta C_t}$ 方法分析基因的相对表达量(P<0.05)。

2 结果与分析

2.1 转录组测序数据分析

利用 Illumina Hiseq 2500 高通量测序技术对许氏平鲉高温组和对照组鳃、肝脏和肠道组织开展转录组测序分析, 共获得 Raw reads 及去除杂质后的 clean reads (表 2)。共得到 815258116 个 clean reads 片段, 包含了 201.13 Gb 的序列信息, GC 含量平均值为 49.8%。碱基质量及组成分析显

表 1　实验所用的引物

Tab. 1　Primers used in the experiments

差异表达基因 DEGs	正向引物序列(5′−3′) forward (5′−3′)	反向引物序列(5′−3′) reverse (5′−3′)
serph	CCGCTCATTAACCGTTGC	CCAGGCGATTCAGGGACT
per1	CTACCCGTTTCGCCTCAC	GGTTCCCACCAAATCCTG
cry1	GCGGTTCCAGACCCTCAT	ACGCCAAACTTGTCATCG
trbr1	GATGCCAAGAAGAAGACC	TTCAGTATCCGTCCCATT
hsp90α	CAACGATGACGAGCAGTA	AAGAGCGTGATGGGATAG
hnrpc	TCTCCAAGTACGGCAAGG	TCTGTGAGGTTTCGGCTC
cxxc1	GTAGTCATCCCGTCAACCC	CTCCTGTCGGCTCAAAGA
srsf3	CTACTATGGTCCGCTCCG	CAACGTCCTTCCGTCAAG
if44l	TTCACCAAACAACCCTTCA	TTCCTGGACTGCTATACACTA
stxb	AACTGCCTCACTCTGTCTG	CATTCCACTCTACCTCCC
18S	CCTGAGAAACGGCTACCATC	CCAATTACAGGGCCTCGAAAG

表 2　常温环境与高温胁迫下许氏平鲉转录组测序数据统计

Tab. 2　Statistics of transcriptome sequencing data of black rockfish at normal and high temperature

样品名称 sample	raw reads 总数 total raw reads	clean reads 总数 total clean reads	Q20/% Q20 percentage	Q30/% Q30 percentage	GC 含量/% GC percentage
高温_鳃 1	46087140	41964372	98.36	96.75	49.73
高温_鳃 2	46597748	42961316	98.37	96.76	49.7
高温_鳃 3	43285926	40903462	98.34	96.79	49.98
高温_肝 1	39570960	39480032	98.54	97.03	51.53
高温_肝 2	45072988	40687194	98.48	96.98	51.22
高温_肝 3	43523622	40780314	98.49	96.9	50.68
高温_肠 1	40078924	40028862	98.65	97.16	51.2
高温_肠 2	48744630	47391490	99.36	97.97	49.73
高温_肠 3	42801018	42704056	98.38	96.94	50.74
常温_鳃 1	47842118	47746394	98.54	95.89	47.9
常温_鳃 2	50305382	50230260	97.98	94.46	47.63
常温_鳃 3	47373202	47257350	98.3	95.46	44.52
常温_肝 1	48594022	48486922	98.59	96	49.91
常温_肝 2	48544558	43723756	98.61	96.04	49.47
常温_肝 3	51822644	46644644	98.6	95.93	50.39
常温_肠 1	55112834	49759484	98.51	95.81	51.39
常温_肠 2	51762064	51655494	98.56	95.92	49.49
常温_肠 3	52992098	52852714	98.45	95.66	51.28

示, 各样品碱基 Q30 为 94.46%~97.97%之间, 以上数据表明转录组测序数据量和质量都较高, 可为后续的组装分析提供可靠的原始数据。

2.2　差异表达基因分析

高温胁迫下, 在许氏平鲉 3 个组织中, 肝脏组织中差异表达基因最多, 为 806 个。在鳃组织和肠道组织中, 高温胁迫分别诱导 306 和 343 个差异表达基因。3 个组织中, 下调表达基因数目均高于上调表达基因数目。鳃、肝脏和肠道组织中的下调表达基因分别为 210、424 和 181 个。将两两比对后的差异基因进行韦恩分析(图 1), 在 3 个组织中均差异表达的基因有 12 个, 高温_鳃 *vs* 常温_鳃、高温_肝 *vs* 常温_肝和高温_肠 *vs* 常温_肠的特异性差异表达基因分别是 235、701 和 259 个。

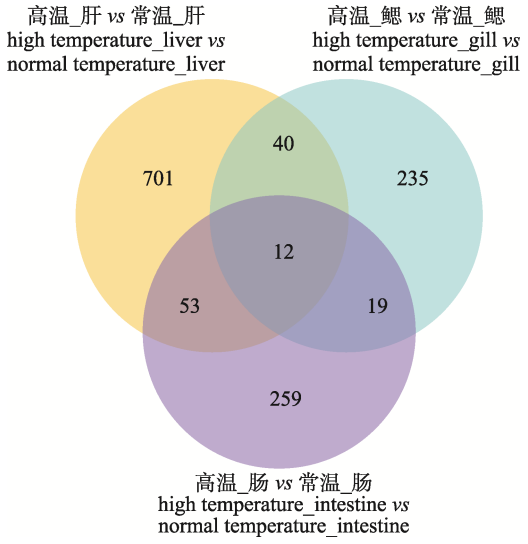


图 1 常温环境与高温胁迫下许氏平鲉转录组中差异表达基因韦恩图

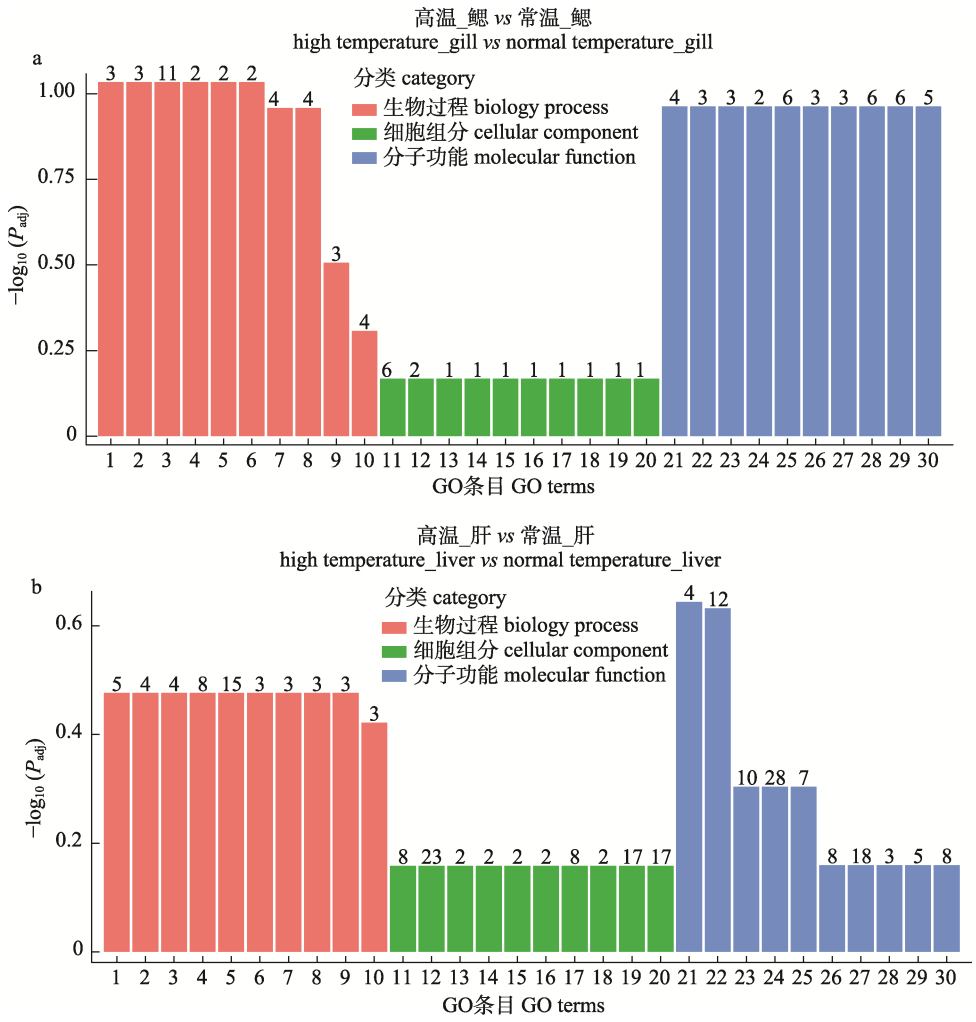
Fig. 1 Venn diagram of DEGs in the transcriptome of black rockfish at normal and high temperature

2.3 GO 功能富集分析

分别对 3 个组织中的差异表达基因进行了 GO 功能富集分析(图 2)。鳃组织中的差异表达基因较多富集在生物过程类别中的蛋白水解作用等，细胞组分类别中的胞外区等，分子功能类别中的结构分子活性、受体调节器活性、受体配体活性等。肝组织中差异表达基因较多富集在生物过程类别中的脂肪代谢过程、细胞氨基酸代谢过程等，细胞组分类别中的细胞质等，分子功能类别中的氧化还原酶活性等。肠道组织中差异表达基因较多富集在生物过程类别中的氧化还原过程等，细胞组分类别中的胞外区等，分子功能类别中的辅因子结合等。

2.4 KEGG 富集通路分析

对所有差异基因进行了 KEGG 富集通路分析，



(待续 to be continued)

(续图 2　Fig. 2 continued)

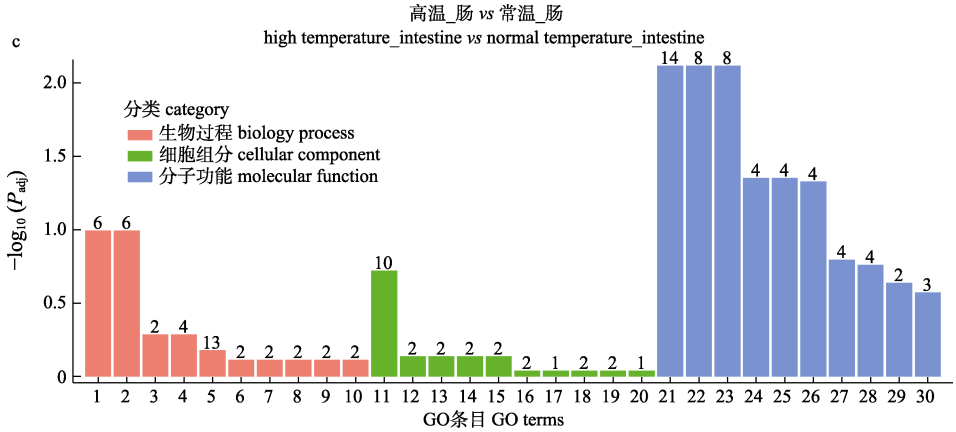


图 2　常温环境与高温胁迫下许氏平鲉转录组中差异表达基因的 GO 分析

(a) 1. 有机阴离子转运; 2. 氧化应激反应; 3. 蛋白水解作用; 4. 单羧酸转运; 5. 有机酸转运; 6. 羧酸转运; 7. 免疫应答; 8.免疫系统过程; 9. 阴离子转运; 10. 应激反应; 11. 胞外区; 12. 线粒体; 13. 质膜的组成部分; 14. 蛋白酶体核心复合物; 15. 跨膜转运复合物; 16. 转运体复合物; 17. 蛋白酶体复合物; 18. 内肽酶复合体; 19. 线粒体内膜; 20. 质膜固有成分; 21. 细胞因子活性; 22. 细胞外基质结构成分; 23. 过氧化物酶活性; 24. 氧化还原酶活性, 作用于供体的醛或氧基团; 25. 结构分子活性; 26. 氧化还原酶活性, 作为受体作用于过氧化物; 27. 抗氧化活性; 28. 受体调节活性; 29. 受体配体活性; 30. 金属内肽酶活性. (b) 1. 硫化合物代谢过程; 2. rRNA 加工; 3. rRNA 代谢过程; 4. 细胞氨基酸代谢过程; 5. 脂质代谢过程; 6. 信号转导的负调控; 7. 细胞通讯的负调控; 8. 信号的负调控; 9. 多生物过程; 10. 刺激反应的负调控; 11. 核糖体; 12. 细胞质; 13. 泡外; 14. 细胞皮层; 15. 细胞皮层部分; 16. 胞内区; 17. 核糖核蛋白复合物; 18. 铁环复合体; 19. 无边界膜复合体; 20. 胞内无边界膜复合体; 21. RNA 甲基转移酶活性; 22. 转移酶活性, 转移单碳基团; 23. 甲基转移酶活性; 24. 氧化还原酶活性; 25. 异构酶活性; 26. 铁离子结合; 27. 辅酶因子结合; 28. 转录因子结合; 29. 维生素结合; 30. 四吡咯结合. (c) 1. 免疫应答; 2. 免疫系统过程; 3. 类固醇代谢过程; 4. DNA 整合; 5. 氧化还原过程; 6. 乙醇代谢过程; 7. 有机羟基化合物代谢过程; 8. 蛋白质泛素化; 9. 小分子蛋白质偶联修饰蛋白质; 10. 转座, DNA 介导; 11. 胞外区; 12. 核小体; 13. 蛋白质-DNA 复合物; 14. DNA 包装复合体; 15. 核染色质; 16. 染色体部分; 17. 蛋白酶体核心复合物; 18. 胞外区部分; 19. 染色体; 20. 蛋白酶体复合物; 21. 辅酶因子结合; 22. 血红素结合; 23. 四吡咯结合; 24. 趋化因子活性; 25. 趋化因子受体结合; 26. G 蛋白偶联受体结合; 27. 细胞因子活性; 28. 细胞因子受体结合; 29. 水解酶活性, 作用于线性酰胺的碳氮键(而不是肽键); 30. 水解酶活性, 作用于碳氮键(但不是肽键).

柱型图上数字代表富集到该途径的差异基因数.

Fig. 2　Gene ontology analyses of DEGs in the transcriptome of black rockfish at normal and high temperature

(a) 1. Organic anion transport; 2. Response to oxidative stress; 3. Proteolysis; 4. Monocarboxylic acid transport; 5. Organic acid transport; 6. Carboxylic acid transport; 7. Immune response; 8. Immune system process; 9. Anion transport; 10. Response to stress; 11. Extracellular region; 12. Mitochondrion; 13. Integral component of plasma membrane; 14. Proteasome core complex; 15. Transmembrane transporter complex; 16. Transporter complex; 17. Proteasome complex; 18. Endopeptidase complex; 19. Mitochondrial inner membrane; 20. Intrinsic component of plasma membrane; 21. Cytokine activity; 22. Extracellular matrix structural constituent; 23. Peroxidase activity; 24. Oxidoreductase activity, acting on the aldehyde or oxo group of donors; 25. Structural molecule activity; 26. Oxidoreductase activity, acting on peroxide as acceptor; 27. Antioxidant activity; 28. Receptor regulator activity; 29. Receptor ligand activity; 30. metalloendopeptidase activity. (b) 1. Sulfur compound metabolic process; 2. rRNA processing; 3. rRNA metabolic process; 4. Cellular amino acid metabolic process; 5. Lipid metabolic process; 6. Negative regulation of signal transduction; 7. Negative regulation of cell communication; 8. Negative regulation of signaling; 9. Multi-organism process; 10. Negative regulation of response to stimulus; 11. Ribosome; 12. Cytoplasm; 13. Exocyst;14. Cell cortex; 15. Cell cortex part; 16. Cytoplasmic region; 17. Ribonucleoprotein complex; 18. Tethering complex; 19. Non-membrane-bounded organelle; 20. Intracellular non-membrane-bounded organelle; 21. RNA methyltransferase activity; 22. Transferase activity, transferring one-carbon groups; 23. Methyltransferase activity; 24. Oxidoreductase activity; 25. Isomerase activity; 26. Iron ion binding; 27. Cofactor binding; 28. Transcription factor binding; 29. Vitamin binding; 30. Tetrapyrrole binding. (c) 1. Immune response; 2. Immune system process; 3. Steroid metabolic process; 4. DNA integration; 5. Oxidation-reduction process; 6. Alcohol metabolic process; 7. Organic hydroxy compound metabolic process; 8. Protein ubiquitination; 9. Protein modification by small protein conjugation; 10. Transposition, DNA-mediated; 11. Extracellular region; 12. Nucleosome; 13. Protein-DNA complex; 14. DNA packaging complex; 15. Chromatin; 16. Chromosomal part; 17. Proteasome core complex; 18. Extracellular region part; 19. Chromosome; 20. Proteasome complex; 21. Cofactor binding; 22. heme binding; 23. Tetrapyrrole binding; 24. Chemokine activity; 25. Chemokine receptor binding; 26. G protein-coupled receptor binding; 27. Cytokine activity; 28. Cytokine receptor binding; 29. Hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds, in linear amides; 30. Hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds.

The numbers on the bars indicate the number of the differentially expressed genes enriched to each GO function.

鳃组织中差异表达基因较多富集在剪接体通路等，肝组织中差异表达基因较多富集在辅因子生物合成通路等，肠道组织中差异表达基因显著富集在 HSV-1 感染通路等(图 3)。

2.5 许氏平鲉高温胁迫响应的关键基因分析

这 3 个组织中有 12 个共有差异表达基因，其中两个为未知基因，剩余 10 个基因如表 3 所示。高温_肝 *vs* 常温_肝和高温_鳃 *vs* 常温_鳃还有 40 个共有差异表达基因，高温_肝 *vs* 常温_肝和高温_肠 *vs* 常温_肠还有 53 个共有差异表达基因，高温_鳃 *vs* 常温_鳃和高温_肠 *vs* 常温_肠还有 19 个共有差异表达基因。这些基因可能是许氏平鲉高

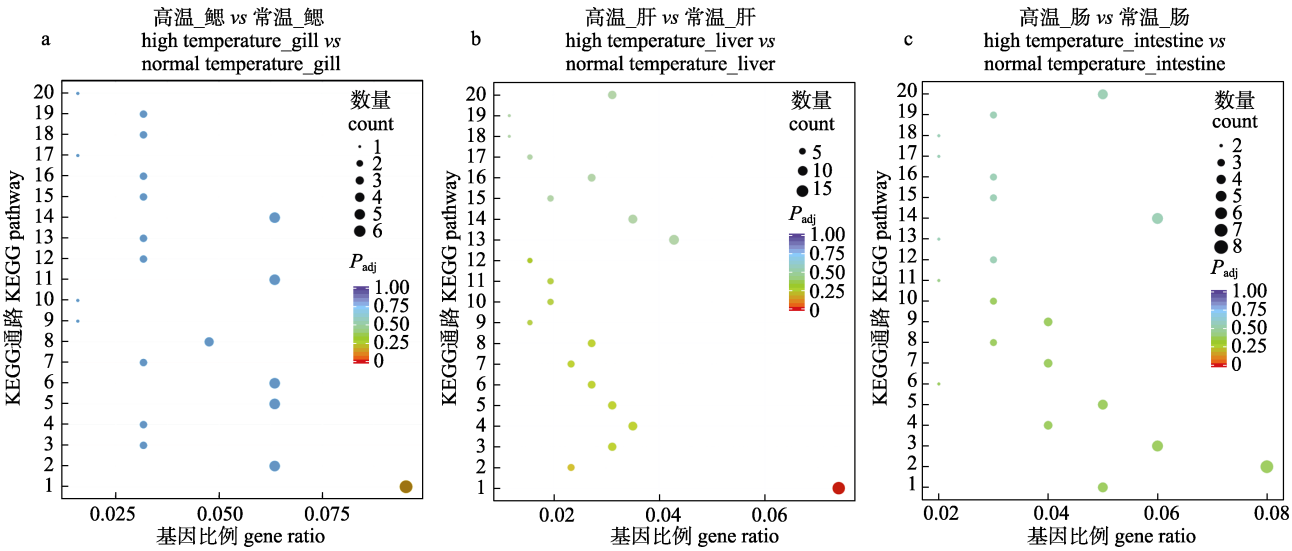


图 3 常温环境与高温胁迫下许氏平鲉转录组差异表达基因的 KEGG 富集分析

(a) 1. 亚麻酸代谢; 2. PPAR 信号通路; 3. 糖异生/糖酵解; 4. 苯丙氨酸代谢; 5. 药物代谢-其他酶; 6. 半胱氨酸和蛋氨酸代谢; 7. 单纯疱疹病毒感染; 8. 细胞质 DNA 感应通路; 9. 丙氨酸、天冬氨酸和谷氨酸代谢; 10. 细胞粘附分子; 11. 硫代谢; 12. 苯丙氨酸、酪氨酸和色氨酸的生物合成; 13. 心肌收缩; 14. 类固醇激素生物合成; 15. 坏死性凋亡; 16. 细胞因子受体相互作用; 17. 药物代谢-细胞色素 P450; 18. 细胞色素 P450 对外源性药物的代谢作用; 19. 碳代谢; 20. 剪接体. (b) 1. 核苷酸代谢; 2. 氮代谢; 3. 苯丙氨酸代谢; 4. 精氨酸生物合成; 5. PPAR 信号通路; 6. 药物代谢-细胞色素 P450; 7. 甘油磷脂代谢; 8. 碳代谢; 9. 亚油酸代谢; 10. 戊糖和葡萄糖醛酸之间的相互转化; 11. 乙醛酸盐和二羧酸盐代谢; 12. 叶酸一碳库; 13. 半胱氨酸和蛋氨酸代谢; 14. 细胞色素 P450 对外源性药物的代谢作用; 15. 花生四烯酸代谢; 16. 真核生物中的核糖体生物发生; 17. 氨基酸的生物合成; 18. 嘧啶代谢; 19. 酪氨酸代谢; 20. 辅因子的生物合成. (c) 1. 嘌呤代谢; 2. 花生四烯酸代谢; 3. 亚油酸代谢; 4. α-亚麻酸代谢; 5. 鞘脂类代谢; 6. 嘧啶代谢; 7. 吞噬体; 8. 酪氨酸代谢; 9. 谷胱甘肽代谢; 10. 组氨酸代谢; 11. 药物代谢-细胞色素 P450; 12. PPAR 信号通路; 13. 甘氨酸、丝氨酸和苏氨酸代谢; 14. 甘油脂代谢; 15. 苯丙氨酸代谢; 16. 核苷酸代谢; 17. 醚脂质代谢; 18. 剪接体; 19. 单纯疱疹病毒感染; 20. 药物代谢-其他酶.

Fig. 3 KEGG enrichment analysis of DEGs in the transcriptome of black rockfish at normal and high temperature

(a) 1. Alpha-Linolenic acid metabolism; 2. PPAR signaling pathway; 3. Glycolysis / Gluconeogenesis; 4. Phenylalanine metabolism; 5. Drug metabolism-other enzymes; 6. Cysteine and methionine metabolism; 7. Herpes simplex virus 1 infection; 8. Cytosolic DNA-sensing pathway; 9. Alanine, aspartate and glutamate metabolism; 10. Cell adhesion molecules; 11. Sulfur metabolism; 12. Phenylalanine, tyrosine and tryptophan biosynthesis; 13. Cardiac muscle contraction; 14. Steroid hormone biosynthesis; 15. Necroptosis; 16. Cytokine-cytokine receptor interaction; 17. Drug metabolism-cytochrome P450; 18. Metabolism of xenobiotics by cytochrome P450; 19. Carbon metabolism; 20. Spliceosome. (b) 1. Nucleotide metabolism; 2. Nitrogen metabolism; 3. Phenylalanine metabolism; 4. Arginine biosynthesis; 5. PPAR signaling pathway; 6. Drug metabolism-cytochrome P450; 7. Glycerophospholipid metabolism; 8. Carbon metabolism; 9. Linoleic acid metabolism; 10. Pentose and glucuronate interconversions; 11. Glyoxylate and dicarboxylate metabolism; 12. One carbon pool by folate; 13. Cysteine and methionine metabolism; 14. Metabolism of xenobiotics by cytochrome P450; 15. Arachidonic acid metabolism; 16. Ribosome biogenesis in eukaryotes; 17. Biosynthesis of amino acids; 18. Pyrimidine metabolism; 19. Tyrosine metabolism; 20. Biosynthesis of cofactors. (c) 1. Purine metabolism; 2. Arachidonic acid metabolism; 3. Linoleic acid metabolism; 4. alpha-Linolenic acid metabolism; 5. Sphingolipid metabolism; 6. Pyrimidine metabolism; 7. Phagosome; 8. Tyrosine metabolism; 9. Glutathione metabolism; 10. Histidine metabolism; 11. Drug metabolism-cytochrome P450; 12. PPAR signaling pathway; 13. Glycine, serine and threonine metabolism; 14. Glycerolipid metabolism; 15. Phenylalanine metabolism; 16. Nucleotide metabolism; 17. Ether lipid metabolism; 18. Spliceosome; 19. Herpes simplex virus 1 infection; 20. Drug metabolism-other enzymes.

表 3　许氏平鲉高温胁迫响应的关键基因

Tab. 3　Key genes that respond to high temperature stress in black rockfish

基因名称 gene name	高温_鳃 *vs* 常温_鳃 high temperature_gill *vs* normal temperature_gill	高温_肝 *vs* 常温_肝 high temperature_liver *vs* normal temperature_liver	高温_肠 *vs* 常温_肠 high temperature_intestine *vs* normal temperature_intestine	注释 annotation
serph	2.828	3.250	3.732	丝氨酸蛋白酶抑制剂 H1
per1	−1.465	−2.026	−2.540	昼夜节律蛋白 1
cry1	1.499	−1.771	−1.829	隐花色素蛋白 1
trbr1	−5.838	−4.761	−4.944	T 细胞受体 β 链
hsp90α	2.522	1.893	2.467	热休克蛋白 90α
hnrpc	2.191	1.468	1.576	异质核糖核蛋白
cxxc1	1.517	1.476	1.977	cxxc 型锌指蛋白 1
srsf3	1.066	1.254	1.606	富丝氨酸/精氨酸剪接因子 3
if44l	1.353	1.430	3.809	干扰素诱导蛋白 44
stxb	−3.648	1.332	−4.094	石首鱼毒素 β 亚基

温胁迫响应的关键基因。

2.6　实时荧光定量 PCR 结果

从转录组分析结果中随机挑选 10 条差异表达基因, 采用逆转录实时荧光定量 PCR (RT-qPCR)相对定量法检测高温胁迫组与对照组 3 个组织中基因转录的差异, 并与转录组的分析结果进行比较。结果如图 4 所示, *serph*、*hsp90α*、*hnrpc*、*cxxc1*、*srsf3*、*if44l*等基因在 3 个组织中都上调表达, *per1*、*trbr1* 在 3 个组织中都下调表达, *cry1* 在鳃中上调表达, 在肝脏和肠道中下调表达, *stxb* 在鳃和肠道中下调表达, 在肝脏中上调表达, RT-qPCR 表达结果与转录组表达分析结果一致。

3　讨论

温度是影响鱼类生长的重要环境因素之一, 此外还对鱼类的发育、代谢、繁殖等生命过程有着直接影响。李军等[22]在 4~27.3 ℃的水温条件下均观察到许氏平鲉的摄食, 但当水温高于 25 ℃时, 摄食强度很低, 冯东岳[23]报道当夏季水温高于 22 ℃时许氏平鲉摄食强度明显减弱, 生长缓慢。上述研究中许氏平鲉生长的适温范围不同, 可能是因为实验条件不同所致。本研究中高温组水温为 27 ℃, 超过了许氏平鲉生长的适温范围, 许氏平鲉的形态、生理和生化都会发生变化。对比常温组, 高温组的 3 个组织中下调基因数均高于上调基因数, 这和卡氏雅罗鱼(*Squalius carolitertii*)的研究结果一致[24], 其原因可能是在高温条件下, 许氏平鲉减少分配用于生长的能量, 以用于维持机体平衡[25]。

通常来说, 基因表达具有组织特异性, 同时, 鱼类不同器官对高温胁迫的应激反应也不同[26]。本研究中许氏平鲉肝脏中差异表达基因数目明显高于鳃中和肠道中差异表达基因数目, 这可能与器官在机体内负责的生理功能不同有关。研究表明, 肝脏是鱼类调节新陈代谢包括氨基酸和碳水化合物代谢的重要器官, 控制着许多受热应激影响的生理过程[27-28]。以往的研究也表明, 在极端环境下肝脏中基因表达的变化相对其他器官更大[29]。本研究中, 通过对肝脏中差异表达基因进行 GO 功能富集分析显示, 差异表达基因较多富集的 GO Term 包括脂肪代谢过程、细胞氨基酸代谢过程等, 这说明和其他鱼类如长颌姬鳉虎鱼(*Gillichthys mirabilis*)[30]、大黄鱼(*Larimichthys crocea*)[31]、虹鳟[32]、俄罗斯鲟(*Acipenser gueldenstaedtii*)[33]等一样, 对代谢过程的调节是许氏平鲉对高温胁迫应激反应的重要组成部分。许氏平鲉在应对长时间高温胁迫的过程中, 机体内的能量消耗和新陈代谢不断增强, 差异基因可能更多地通过代谢通路相互作用的方式共同应答高温胁迫。

热休克蛋白(HSP)又称应激蛋白, 是一类高度保守的蛋白, 几乎所有生物在受到温度胁迫时都会大量表达热休克蛋白来抵抗伤害[34]。HSP 家族不仅在变性蛋白的重折叠中发挥作用, 也负责

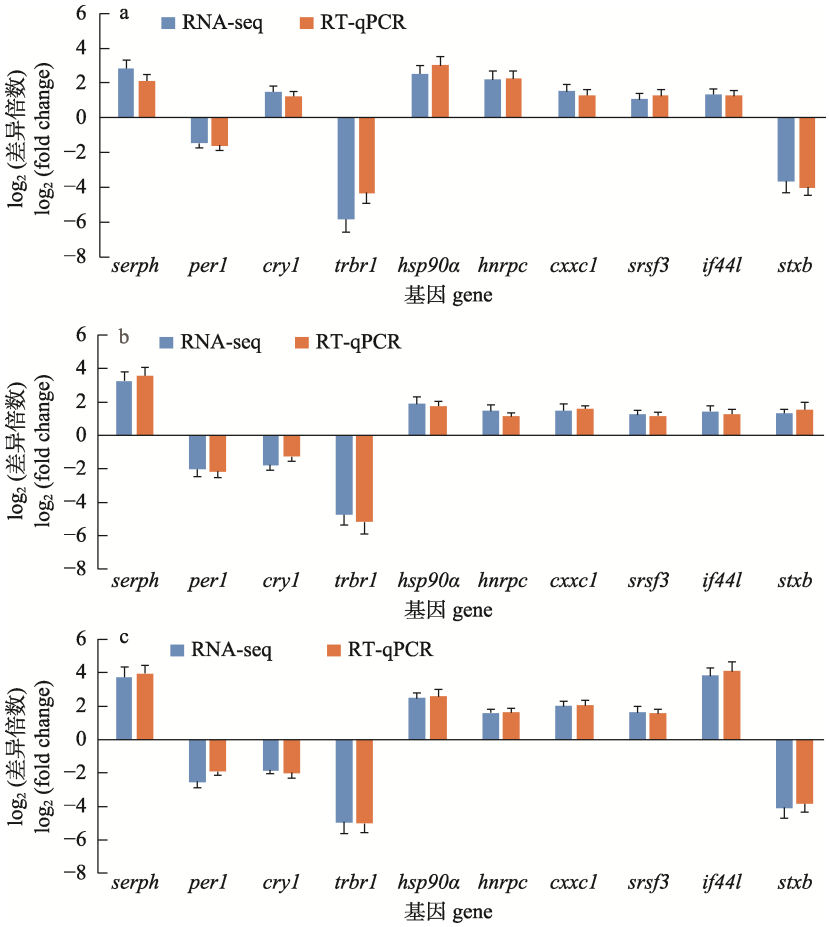


图 4 10 个差异表达基因的 RT-qPCR 验证

a. 鳃; b. 肝脏; c. 肠道.

Fig. 4 Validation of the DEGs by RT-qPCR

a. Gill; b. liver; c. intestine.

降解没有折叠的损伤蛋白质[35], 强化细胞的修复功能并提高细胞对应激的耐受水平。*hsp* 通过促进应激蛋白的合成来应答环境的突然变化从而维持机体平衡[36]。虹鳟[15], 大黄鱼[31], 河豚(*Takifugu obscurus*)[37]和斑尾刺鳅虎鱼(*Acanthogobius ommaturus*)[36]等鱼类在受到温度胁迫后, HSP 家族基因表达水平都有所提高。HSP90 是热休克蛋白家族重要的分子之一, 有研究报道其除了具有分子伴侣功能外, 还参与到机体免疫调节中[38]。在本研究中, *hsp90α* 基因在 3 个组织中都显著上调表达。许氏平鲉受到急性高温胁迫后, HSP70、HSP90 和 HSP40 和其他 HSP 蛋白家族的表达显著升高[8]。HSP90 对于蛋白质的折叠和聚合很重要, 参与到调整细胞内折叠、易位和聚集的动力学分配, 特别是在高温或者低温胁迫引起的损害中。本研究中, 只观察到 *hsp90α* 上调表达, 可能是因为本研究中温度缓慢升高, 而且高温持续一段时间取样, 因为 *hsps* 的 mRNA 转换比较快, 在升温过程中已经完成蛋白质的合成。

本研究中, 丝氨酸蛋白酶抑制剂基因 *serph* 显著升高, 这和俄罗斯鲟[33]以及太平洋鲑(*Oncorhynchus* spp.)[39]中的研究结果一致。丝氨酸蛋白酶抑制剂具有通过调节丝氨酸蛋白酶和半胱氨酸蛋白酶活性来调节蛋白酶级联反应的作用, 从而参与了包括炎症反应、血液凝结、消化作用、补体激活、激素转运和纤维蛋白溶解等基本生物过程, 在机体免疫调节及防御中发挥着重要作用[40]。本研究中, 昼夜节律蛋白基因 *per1* 在 3 个器官中也差异表达, 这与卡氏雅罗鱼的研究一致[24]。一些自然环境的变化是由昼夜交替造成的,

这些环境变化具有周期性, 生物也进化出周期性的基因表达方案以应对环境的周期性变化[41]。昼夜节律蛋白基因表达的变化可能对鱼类的行为包括进食、繁殖以及一些生理活动, 如新陈代谢等具有显著影响[42]。

本研究利用 Illumina Hiseq 高通量测序技术对许氏平鲉鳃、肝脏和肠道组织在温度缓慢升高后的 cDNA 文库进行测序, 建立了许氏平鲉慢性热应激转录组数据库, 识别了一些与慢性高温应激相关的关键基因与代谢通路, 为今后许氏平鲉热应激胁迫分子调控机制研究提供基础数据, 也为许氏平鲉耐高温品系的选育工作提供理论依据。

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Transcriptomic responses to chronic heat stress in black rockfish (*Sebastes schlegelii*)

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Abstract: Black rockfish (*Sebastes schlegelii*) inhabit the coastal regions of the northwestern Pacific Ocean, including the East China Sea, Yellow Sea, and the coastal areas of Korea and Japan. It is highly favored by consumers because of its delicate flavor and high nutritional content. Black rockfish is among the most economically cultivated marine species in northern China. The juveniles were raised in offshore net cages. The optimum temperature for black rockfish growth ranges from 18 ℃ to 24 ℃. In summer, the seawater temperature sometimes exceeds 28 ℃ in black rockfish farms. As global warming intensifies, long-term exposure to high temperatures in summer will affect the growth and survival of black rockfish cultured in net cages, indicating that the effects of chronic heat stress on black rockfish merit further research. However, the molecular mechanisms underlying the responses of black rockfish to chronic heat stress remain largely unknown. Understanding these mechanisms will improve fish welfare and farm production. Marker genes to monitor heat stress are required to identify heat-resistant fish. In this study, we conducted RNA-seq analysis to characterize the genes and pathways involved in chronic thermal stress responses in the gills, liver, and intestines of black rockfish. Healthy black rockfishes were cultured at a normal temperature (18 ℃) and a high temperature (27 ℃). For the chronic heat stress treatment, water temperature was increased from 18 ℃ to 27 ℃ at a constant rate of 1 ℃ per day, and maintained for 10 days. The gill, liver, and intestinal tissues were used as experimental materials in both the heat stress and normal groups. Total RNA was extracted, and 18 mRNA libraries were constructed and sequenced using the Illumina HiSeq-4000 technology platform. Differentially expressed genes were analyzed using edgeR. Bioinformatic analysis was performed on the GO and KEGG functions of the differentially expressed genes, and the key differentially expressed genes were further validated using RT-qPCR. In total, 306 annotated differentially expressed genes (DEGs) were identified in the gill, of which 96 and 210 were up- and downregulated, respectively. In total, 806 annotated differentially expressed genes (DEGs) were identified in the liver, of which 382 were upregulated and 424 were downregulated. A total of 343 annotated differentially expressed genes (DEGs) were identified in the intestine, among which 162 and 181 were up- and downregulated, respectively. A Venn diagram showed that 12 DEGs were shared among three tissues. And 40, 53, and 49 DEGs were shared between liver and gill, liver and intestine, gill and intestine, respectively. Furthermore, GO functional enrichment analysis revealed that the DEGs were mainly enriched in proteolysis, extracellular region, structural molecule activity, receptor regulator activity, and receptor-ligand activity in the gill; in lipid metabolic process, cellular amino acid metabolic process, cytoplasm, and oxidoreductase activity in the liver; and in oxidation-reduction process, extracellular region, and cofactor binding in the intestine. Among the up-regulated genes under heat stress included the heat shock proteins 90α, period circadian protein homolog 2, serpin H1, and the down-regulated genes included period circadian protein homolog 1. Ten DEGs were subjected to reverse transcription quantitative PCR (RT-qPCR) for relative quantification to assess the differences in gene expression between the normal and high temperature groups. The expression trends observed in the RT-qPCR analysis were consistent with those identified in the RNA-seq data, which confirmed the reliability of the transcriptomic sequencing results. These results provide abundant data for further studies on the molecular mechanisms of the chronic heat stress response in *Sebastes schlegelii*.

Key words: *Sebastes schlegelii*; heat stress; transcriptome; gill; liver; intestine

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