DOI: 10.19663/j.issn2095-9869.20221205001 http://www.yykxjz.cn/

李文文, 徐革锋, 黄天晴, 谷伟, 刘恩慧, 王高超, 潘玉财, 周金鑫, 姜再胜, 王炳谦. 基于转录组测序筛选乌苏里白鲑肌肉生长关键候选基因研究. 渔业科学进展, 2024, 45(3): 87–100

LI W W, XU G F, HUANG T Q, GU W, LIU E H, WANG G C, PAN Y C, ZHOU J X, JIANG Z S, WANG B Q. Screening muscle growth-related genes of *Coregonus ussurinsis* Berg based on transcriptome sequencing. Progress in Fishery Sciences, 2024, 45(3): 87–100

基于转录组测序筛选乌苏里白鲑肌肉生长关键候选基因研究*

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摘要 为了挖掘调控乌苏里白鲑(*Coregonus ussurinsis* Berg)肌肉生长的关键候选基因，本研究对不同生长速度乌苏里白鲑的肌肉组织进行转录组测序，以期为乌苏里白鲑群体选育提供基础数据。首先，在同等条件下(从混合池中)随机选择乌苏里白鲑 F_2 个体进行实验分组(快长组和慢长组)。接着分别从快长组[体重为(219.20±38.66) g]和慢长组[体重为(74.30±17.86) g]中随机选取 10 尾样本，取其背部肌肉进行转录组测序。以 FDR (false discovery rate)<0.05 且$|\log_2(FC)|>1$ (FC, fold change)为条件筛选差异表达基因并对其进行 GO (gene ontology)和 KEGG (Kyoto encyclopedia of genes and genomes)富集分析，并通过 qPCR 验证转录组数据的准确性。转录组测序结果显示，共筛选出 2 211 个差异表达基因，与慢长组相比，快长组中 583 个差异基因表达上调及 1 628 个基因表达下调。GO 功能注释结果显示，差异基因主要参与细胞过程和结合过程，差异基因显著富集到 251 条 KEGG 通路(P<0.05)，其中，MAPK 信号通路(MAPK signaling pathway)、PI3K-Akt 信号通路(PI3K-Akt signaling pathway)、紧密连接(tight junction)、胰岛素信号通路(insulin signaling pathway)、糖酵解/糖异生(glycolysis/gluconeogenesis)和 PPAR 信号通路(PPAR signaling pathway)参与细胞生长。之后结合功能注释结果和 KEGG，鉴定出肌浆/内质网钙 ATP 酶基因 *atp2a1* 和 *atp2a2*、葡萄糖-6-磷酸脱氢酶基因 *g6pc*、生长因子结合蛋白 1 基因 *igfbp1* 以及肌球蛋白重链基因 *myh1*、*myh4*、*myh6*、*myh7*、*myh9* 和 *myh13* 等可能与肌肉生长密切相关的基因。本研究共筛选出 10 个可能与乌苏里白鲑肌肉生长相关的关键候选基因，为今后乌苏里白鲑分子标记辅助育种提供了基础数据。

关键词 乌苏里白鲑；转录组测序；差异表达基因；肌肉生长

中图分类号 S917 **文献标识码** A **文章编号** 2095-9869(2024)03-0087-14

乌苏里白鲑(*Coregonus ussurinsis* Berg)属鲑形目(Solmoniformes)、鲑科(Solmonidae)、白鲑亚科(Coregoninae)、白鲑属(*Coregonus*)，主要分布在俄罗斯西伯利亚、萨哈林及我国黑龙江等水域，具有明显

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收稿日期: 2022-12-05, 收修改稿日期: 2023-02-09

的洄游特性，是北方特有冷水性鱼类，也是黑龙江水系中唯一的白鲑属鱼类(张觉民, 1995; 尼科尔斯基, 1960; 罗京等, 2021; 徐革锋等, 2022)。乌苏里白鲑兼具较高的营养和经济价值，是黑龙江名贵水产品之一，但由于生活水体环境恶化、洄游通道受阻及过度捕捞等原因，乌苏里白鲑栖息地逐渐缩减，资源量显著下降，被收录至《中国濒危动物(鱼类)红皮书》中(乐佩琦等, 1998; 徐俐力, 2007; 王继隆等, 2019)。目前，对乌苏里白鲑仅开展了其群体遗传多样性、肌肉营养成分、性腺及胚胎发育、个体繁殖力以及病原体感染等相关研究(董崇智等, 1997; 马波等, 2003; 梁利群等, 2004; 李培伦等, 2015; 李虹娇等, 2017; 王继隆等, 2018; 史秀兰等, 2020; 刘恩慧等, 2022)，还缺乏对乌苏里白鲑生长方面的转录组研究，因此，有必要通过 RNA-seq 测序技术挖掘肌肉生长相关候选基因并阐明其分子调控机制。

鱼类的生长是重要的数量性状，受遗传、环境以及它们间相互作用等多种因素影响，也是养殖鱼类品质评价的重要指标之一(Fuentes *et al*, 2013; 孙雪等, 2021)。转录组测序(RNA-seq)技术能高效地对遗传调控的相关因子进行深入分析，从分子层面上对调控生长的相关基因进行筛选和鉴定。转录组测序技术已被广泛应用于鱼类生长发育(Al-Tobasei *et al*, 2017; Paneru *et al*, 2017; Lu *et al*, 2020)、免疫应激(Nguyen *et al*, 2016; Valenzuela-Miranda, 2018; Liu Z *et al*, 2020; Liang *et al*, 2021; 朱鑫海等, 2022)和遗传演化(Genet *et al*, 2014; Kang *et al*, 2017; Carruthers *et al*, 2018)等相关基因的挖掘与研究。张波等(2023)从具有生长速度差异的大黄鱼(*Larimichthys crocea*)群体筛选出3个可能与生长相关的基因；范嗣刚等(2022)从花鲈(*Lateolabrax maculatus*)中获得 10 552 个差异表达基因，筛选出 *igfbp1*、*fgf*、*mstn*、*ghr1* 等与肌肉生长发育相关的基因；Lu 等(2020)在快长和慢长的草鱼(*Ctenopharyngodon idella*)群体中发现 GH/IGF 轴、钙代谢、蛋白质和糖原合成、氧转运、细胞骨架和肌原纤维成分通路中的基因参与生长调控；Sun 等(2016)发现糖酵解、肌钙蛋白以及参与 Ca^{2+}信号传导的基因是杂交石斑鱼(*Epinephelus* spp.)中的生长优势基因。鱼类生长一直是主要的育种目标性状之一。提高乌苏里白鲑的生长速度不仅可以缩短养殖周期、增加产量，还能够促进其养殖业的发展，提高经济效益的同时满足人们的食用需求，因此，对乌苏里白鲑生长相关研究也变得更加迫切，但目前该鱼的育种工作还停留在传统选育层面，从基因层面对乌苏里白鲑进行选择育种的研究还处于空白。本研究对不同生长速度乌苏里白鲑的肌肉组织进行转录组测序，筛选与生长相关的差异表达基因和信号通路，以期为乌苏里白鲑的分子辅助育种提供基础数据。

1 材料与方法

1.1 实验材料

实验用乌苏里白鲑取自中国水产科学研究院黑龙江水产研究所渤海冷水鱼试验站的第 2 代选育群体，为 2018 年 12 月同批次繁育、次年 3 月破膜成仔鱼、同池混养条件下饲育的 3 龄鱼。随机选取 10 尾大规格个体作为快长实验组，体长(24.27±1.48) cm，体重(219.20±38.66) g；随机选取 10 尾小规格个体作为慢长实验组，体长(17.61±1.80) cm，体重(74.30±17.86) g。将各组实验鱼麻醉后采集背部肌肉组织，置于液氮备用。

1.2 总 RNA 提取、cDNA 文库构建及测序

将各实验组中3尾或4尾鱼的背部肌肉等量混合后按照Trizol法提取组织中的总RNA，随后对总RNA进行质量检测：凝胶电泳检测 RNA 完整性，NanoDrop 2000 检验 RNA 的浓度和纯度，Agilent 2100 精确检验 RNA 的完整性。使用带有 Oligo dT 的磁珠通过碱基互补的方式富集 mRNA，随后将其打断成短片段经 PCR 扩增得到乌苏里白鲑转录组 cDNA 文库，共构建 6 个 cDNA 文库。将构建合格的 cDNA 文库使用 Illumina 高通量测序平台 NovaSeq 6000 进行测序。转录组文库构建和测序工作委托武汉菲沙基因信息有限公司完成。

1.3 转录组原始数据分析

经高通量测序平台获得的原始序列(raw reads)中含有接头信息、低质量及未测出的碱基，不利于转录组后续分析，故而采用 SOAPnuke 软件(v2.1.0)对原始序列进行质控，即去污染、去接头等处理后获得有效序列(clean reads)，随后计算 Q20、Q30 (质量值≥20 或 30 的碱基所占百分比)及 GC 含量。将质控后的有效序列使用 HISAT2 (Kim *et al*, 2015)、bowtie2 (Langmead, 2010)比对软件与本实验室乌苏里白鲑的参考基因组比对(NCBI 未发表)，进行基因注释。

1.4 样本相关性分析

本研究基于基因表达量 FPKM (fragments per kilobase per million bases)对样本间的相关性进行分析，以皮尔逊相关系数 r^2 作为判断标准，相关系数越接近 1，则样品间相似度越高。

1.5　差异表达基因分析及筛选

注释后的基因用 FPKM 法评估基因表达水平，以慢长组为对照组，快长组为实验组，使用 DESeq 软件对差异表达基因(DEG)进行筛选，筛选阈值为 FDR(false discovery rate)<0.05 且$|\log_2(FC)|>1$(FC, fold change)。基于差异表达基因结果，采用分布(hyper-geometric distribution)的方法对差异表达基因进行 GO (gene ontology)功能注释(Ashburner *et al*, 2000)及 KEGG (kyoto encyclopedia of genes and genomes)信号通路富集分析(Kanehisa *et al*, 2004)。

1.6　qPCR 验证

对筛选出的 10 个关键差异表达基因进行 qPCR 验证。使用反转录试剂盒(TaKaRa, 日本)反转录成 cDNA。根据基因 CDS 序列使用 Primer 5.0 软件设计 qPCR 引物，*β-actin* 为内参基因(表 1)。qPCR 使用 SYBR qPCR Mix 试剂盒(EnzyArtisan, 上海)进行操作。PCR 扩增体系为 10 μL：2 × S6 Universal SYBR qPCR mix 5 μL，正反向引物各 0.2 μL，cDNA 模板 0.5 μL，ddH_2O 4.1 μL；扩增程序：95 ℃预变性 30 s；42 个循环，循环程序为 95 ℃变性 5 s，60 ℃退火 30 s；最后，95 ℃ 15 s，60 ℃ 1 min，95℃ 15 s。使用 QuantStudio 6 Flex Real-time PCR 仪(Life Technologies, 美国)检测 qPCR 结果，采用 $2^{-\Delta\Delta Ct}$ 方法计算相对表达量。所有引物委托生工生物工程(上海)股份有限公司合成。

表 1　qPCR 引物序列

Tab.1　qPCR primer sequence

基因 ID Gene ID	基因名 Gene name	正向引物 Forward primer (5′~3′)	反向引物 Reverse primer (5′~3′)
Cus11128	*atp2a2*	TTATGAGTCTGACCTGACCTTCGT	GTCCACGTCGTCCTCCTCG
Cus27094	*atp2a1*	GTGCAATGACTCCTCTCTGGACTA	CCTTCACAAACATTTTAGCTCCG
Cus32796	*myh1*	GTCAAGGGAGTCCGCAAGTA	CTTAGACATGTGCTGGTTTGCT
Cus32832	*myh4*	TTGGAAAAACACAGGCGTTTG	TTGTAGTCCACAGTACCGGCG
Cus32788	*myh6*	CTGGTGGCGAGAAGAAGAAGGAAG	TCACTGTCTTGGCATTACCGTAAGC
Cus37534	*myh7*	GAAGTTTAAGAATAAGCAGCGGGA	CTTTGGTGACCCACTCATTTCC
Cus38339	*myh9*	GGCTACAACAACTACCGCTTCCTG	GACACCACCTTCAGCAGACCAATC
Cus32793	*myh13*	GAGAAGCCCAAGCCTGCCAAAG	CCAGCCAGCCAGTGATGTTGTAG
Cus00245	*g6pc*	GTCACCTCCATCCTAACCATCA	AGAGTGCCCCGCAGATACAG
Cus13680	*igfbp1*	CTGACCAGGTTCTATCTGCCAA	GGACACACACCAACACCTGC
LOC100136352	*β-actin*	CACAGACTACCTGATGAAGATCCTG	GTAGCACAGCTTCTCCTTGATGTC

2　结果

2.1　转录组测序和组装结果

本研究从构建的 6 个 cDNA 文库中共获得 295 605 738 条原始序列，经质量控制过滤后共获得 283 133 612 条有效序列，各样品的有效比对率在 94.43%以上，Q20 的含量在 97.80%以上，Q30 的含量在 93.90%以上，GC 碱基含量占总碱基的 49.10%以上，无明显 GC 或 AT 分离现象。将质控后的有效序列与乌苏里白鲑的参考基因组进行比对，结果显示，比对率在 94.93%以上，说明测序结果可靠，可进行后续分析(表 2)。

2.2　样本相关性分析

样品相关性系数不仅可以检验生物学实验的可重复性，还可以评估差异表达基因的可靠性，是评估样本选择是否正确的重要指标。本研究通过基因表达量计算各样本之间的相关性系数，并绘制热图(图 1)。由图 1 可知，各样本间相关性系数均在 0.83 以上，表明样本间相关度和实验可靠性较高。

2.3　差异表达基因分析

依据 FPKM 算法计算基因表达量，使用 DESeq 软件(v.1.22.2)对基因表达结果进行分析，并计算基因的 FC 值。以 FDR<0.05 且$|\log_2(FC)|>1$ 作为条件筛选出乌苏里白鲑的生长候选差异基因，并绘制火山图以直观反映快长组和慢长组乌苏里白鲑肌肉组织差异表达基因的分布情况(图 2)。结果显示，在乌苏里白鲑肌肉组织中共鉴定出 2 211 个差异表达基因，其中包含 659 个新预测基因；与慢长组相比，快长组有 583 个差异基因表达上调，1 628 个差异基因表达下调。

表 2 转录组测序质控结果

Tab.2 Quality control results of transcriptome sequencing

样本组别 Sample	原始序列 Raw reads	有效序列 Clean reads	有效比对率 Effective rate/%	Q20/%	Q30/%	GC 含量 GC content/%
FM1	48 933 582	46 768 662	95.58	97.90	94.20	50.80
FM2	49 829 420	47 957 942	96.24	97.80	93.90	51.20
FM3	50 470 284	47 908 972	94.93	97.80	93.90	51.00
SM1	50 036 756	47 888 408	95.71	98.00	94.30	49.10
SM2	49 964 426	48 012 690	96.09	97.90	94.10	50.80
SM3	46 371 270	44 596 938	96.17	97.80	93.90	50.40

注：FM 为快长组肌肉组织，SM 为慢长组肌肉组织。下同。

Note: FM represents muscle of fast-growing group, and SM represents muscle of slow-growing group. The same below.

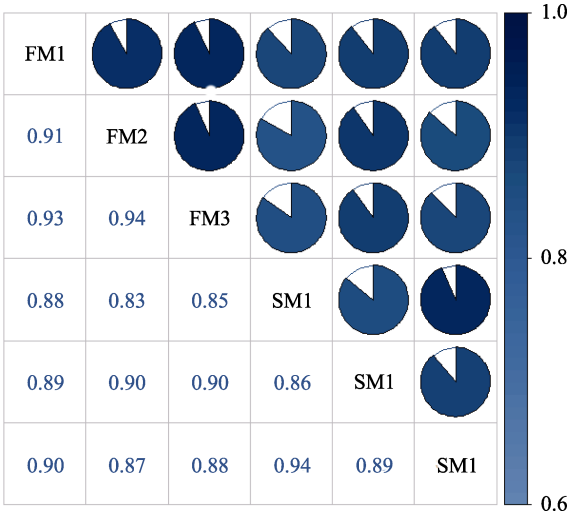

图 1 样本相关性分析

Fig.1 Sample correlation analysis

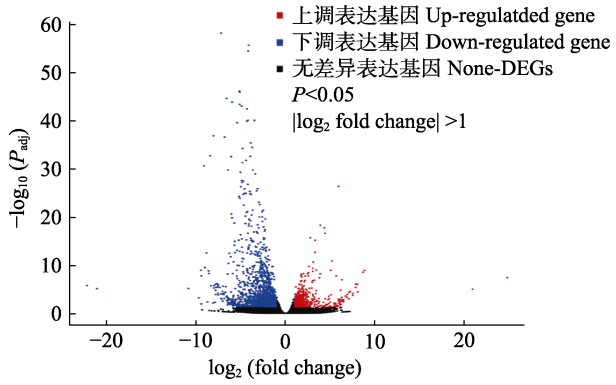

图 2 差异表达基因火山图(SM *vs* FM)

Fig.2 Volcano map of differentially expressed genes (SM *vs* FM)

2.4 差异表达基因 GO 功能富集分析

GO 功能富集分析结果显示，差异表达基因主要富集在生物学过程(biological process, BP)、分子功能(molecular function, MF)和细胞组分(cellular component, CC)的 3 620 个 GO term 中，其中，BP 最多(2 457 个)，占 67.87%；MF 次之(782 个)，占 21.6%；CC 最少(381 个)，占 10.52%。由图 3 可知，在 BP 功能中，差异表达基因主要参与细胞过程(cellular process)与代谢过程(metabolic process)，分别含有差异基因 578 和 489 个；在 MF 功能中，参与结合(binding)过程的差异表达基因最多，为 543 个，催化活性(catalytic activity)过程次之，为 364 个；在 CC 功能中，差异表达基因主要参与细胞(cell)和细胞组分(cell part)过程，分别包含 386 和 382 个差异表达基因。

2.5 差异表达基因 KEGG 通路富集分析

对差异表达基因进行 KEGG 通路富集分析的结果显示，肌肉组织中的差异表达基因被富集到 251 条已知 KEGG 通路中，其中有 73 条信号通路被显著富集(P<0.05)，快长组中上调的差异表达基因显著富集在糖酵解/糖异生(glycolysis/gluconeogenesis)、甲烷代谢(methane metabolism)、氨基酸的生物合成(biosynthesis of amino acids)、碳代谢(carbon metabolism)以及蛋白酶体(proteasome)通路等(图 4a)；下调的差异表达基因显著富集在破骨细胞分化(osteoclast differentiation)、IL-17 信号通路(IL-17 signaling pathway)、氨基酸的生物合成(biosynthesis of amino acids)、脂肪细胞因子信号通路(adipocytokine signaling pathway)以及 TNF 信号通路(TNF signaling pathway)等(图 4b)。

2.6 核心候选基因的筛选

本研究发现，乌苏里白鲑肌肉生长相关差异基因被显著富集在 MAPK 信号通路(MAPK signaling pathway)、PI3K-Akt 信号通路(PI3K-Akt signaling pathway)、紧密连接(tight junction)、胰岛素信号通路(insulin signaling pathway)、糖酵解/糖异生、PPAR 信

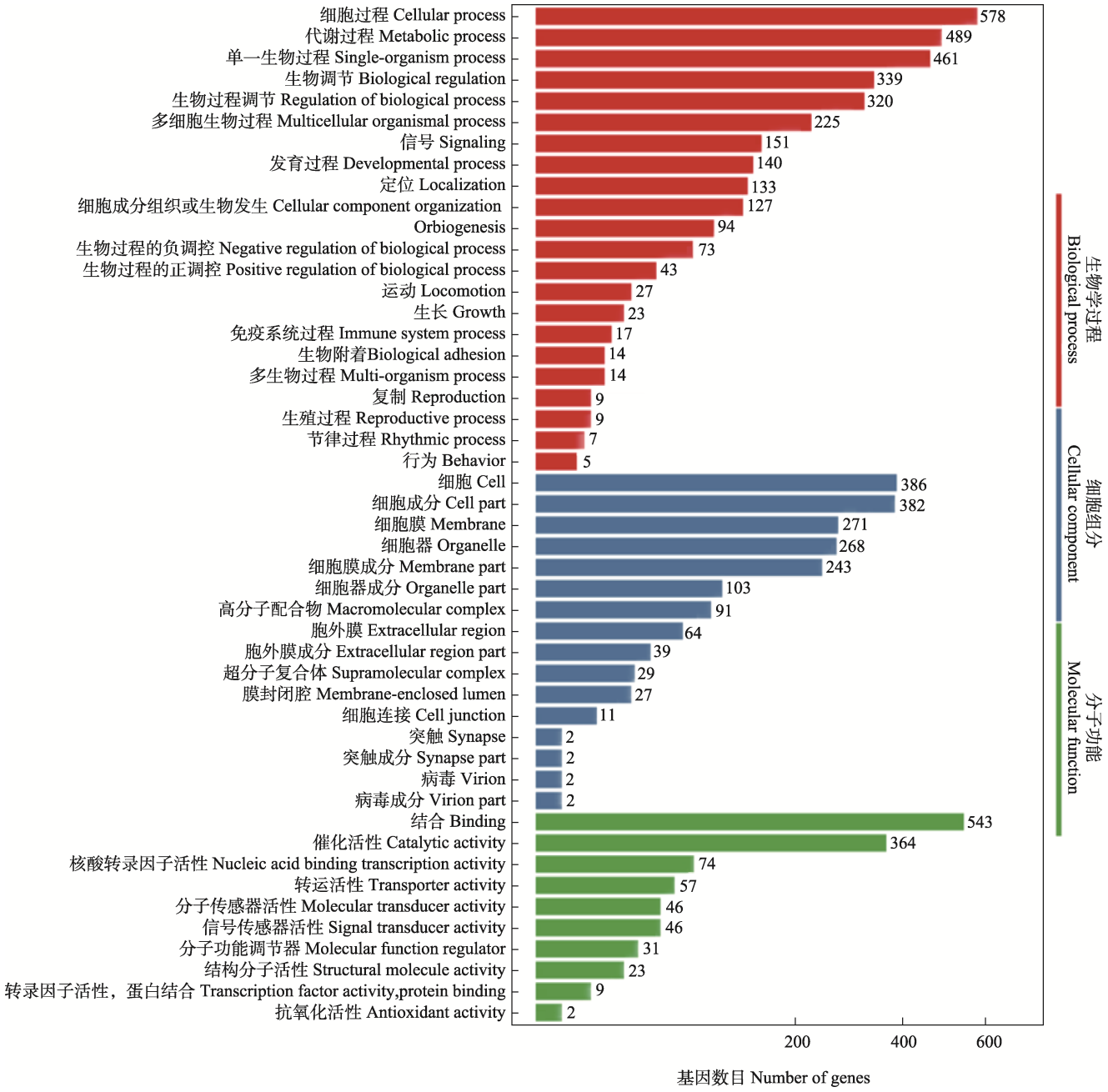

图 3　差异表达基因 GO 功能分类

Fig.3　GO functional classification of differentially expressed genes

号通路(PPAR signaling pathway)中(P<0.05)，结合 GO 功能注释和 KEGG 通路富集结果，推测差异基因显著富集的信号通路中可能含有与乌苏里白鲑肌肉生长相关的基因，结合相关文献，最终筛选出可能与乌苏里白鲑生长相关的差异基因共 31 个，如表 3 所示。

为进一步研究这些差异基因之间的相关作用关系并筛选出可能与乌苏里白鲑肌肉生长相关的关键基因，使用 STRING (Version 11.5)数据库(https://string-db.org/)构建蛋白质互作网络图(图 5)，除 *cxcl11.1* 基因外，在 31 个差异基因中共鉴定出 30 个基因编码已知蛋白质，其中 27 个蛋白质之间存在相互作用。根据蛋白网络互作数筛选出了 10 个可能与乌苏里白鲑生长相关的关键候选基因：肌浆/内质网钙 ATP 酶基因 *atp2a1* 和 *atp2a2*、葡萄糖-6-磷酸脱氢酶基因 *g6pc*、生长因子结合蛋白 1 基因 *igfbp1* 以及肌球蛋白重链基因 *myh1*、*myh4*、*myh6*、*myh7*、*myh9* 和 *myh13*。其中，快长组中 *atp2a1*、*myh1* 和 *myh13* 基因上调表达，*atp2a2*、*g6pci*、*gfbp1*、*myh4*、*myh6*、*myh7* 和 *myh9* 基因下调表达。

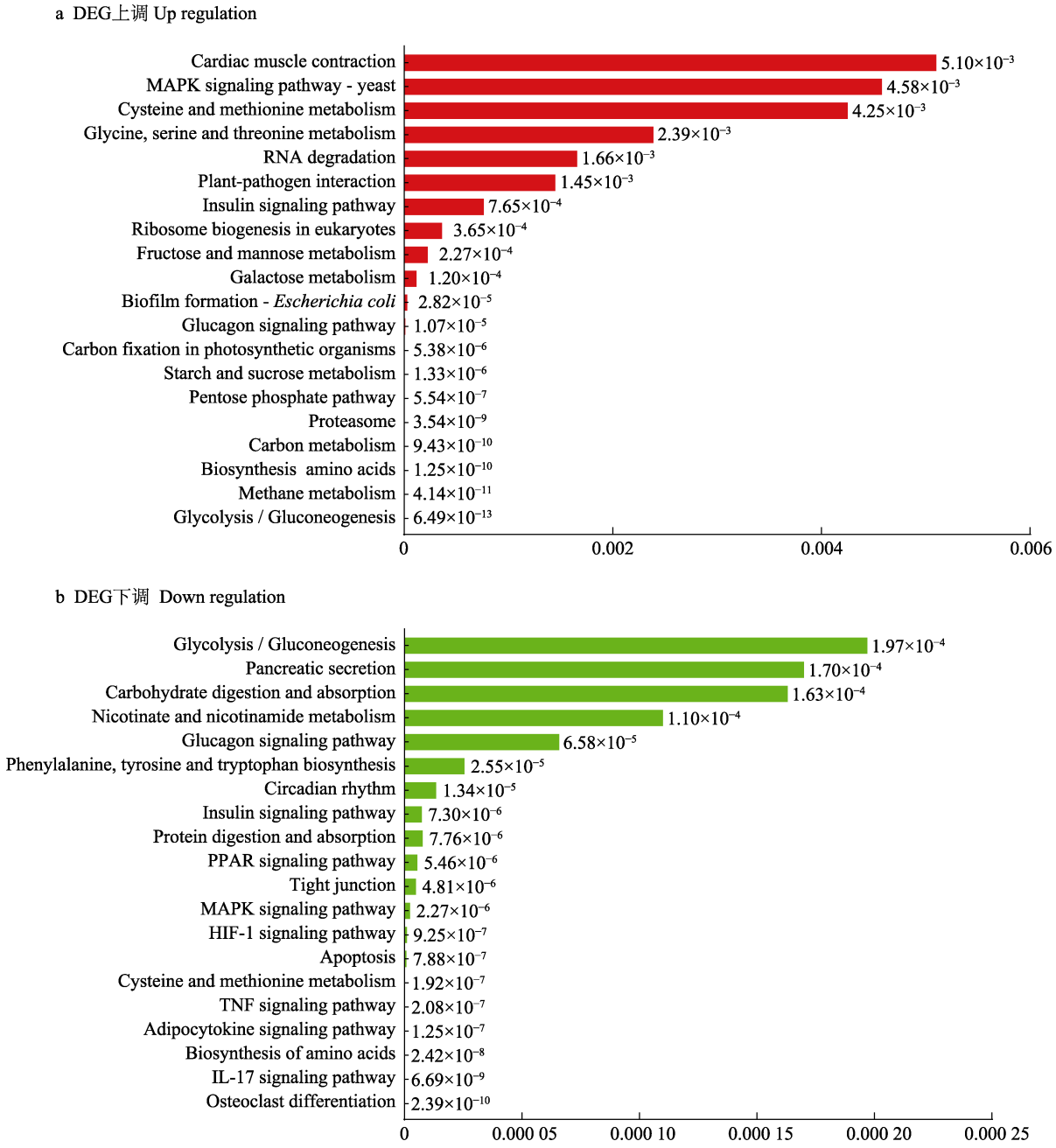

图 4 差异表达基因 KEGG 富集分析

Fig.4 KEGG enrichment analysis of differentially expressed genes

2.7 qPCR 验证

为验证乌苏里白鲑肌肉转录组测序结果的可靠性，对筛选出的 10 个与生长相关的关键候选基因，以 *β-actin* 为内参进行 qPCR 验证，结果显示(图 6)，10 个差异基因表达结果与转录组测序获得的基因表达结果趋势一致，证明本研究通过转录组测序获得的结果准确，所筛选出的核心候选基因可靠，可用于后续功能验证和分子标记的开发。

3 讨论

肌肉生长是评价养殖产品质量和经济效益的重要标准之一，也是优良品种选育过程中不可忽视的指标。乌苏里白鲑是黑龙江省的名贵冷水鱼，因其肉质鲜美广受消费者喜爱。本研究前期发现，同批次繁殖、相同环境条件下养殖的乌苏里白鲑个体规格存在较大差异，为揭示其生长速度显著差异的潜在遗传机制，本研究采用高通量测序技术对快长组和慢长组的

乌苏里白鲑进行了转录组测序, 筛选出差异表达基因并进行 GO 和 KEGG 富集分析,挖掘出了可能与乌苏里白鲑肌肉生长相关的 10 个候选基因。

atp2a1 和 *atp2a2* 是 ATP2As 基因家族的成员, 编码负责调节钙跨胞内膜转运的肌浆/内质网 Ca^{2+}-ATP 酶(SERCA), 能够将胞浆中的 Ca^{2+}回收至肌浆网腔和内质网腔中, 从而肌肉由收缩状态恢复为松弛状态, 在控制细胞生长和分化过程中发挥着重要作用(Chemaly *et al*, 2018)。*atp2a1* 基因在成肌细胞分化过程中表达上调, 编码的 SERCA1 蛋白参与快肌纤维收缩过程,其基因突变显著影响虹鳟(*Oncorhynchus mykiss*)的生长速度(Salem *et al*, 2012)。*atp2a2* 基因主要在人体心脏、脑和Ⅰ型骨骼肌(慢肌纤维)发育过程中表达,其编码的 SERCA2 包含 SERCA2a、SERCA2b 和 SERCA2c 三种亚型(Hino *et al*, 2007; Hovnanian, 2007; Periasamy *et al*, 2007), *atp2a2* 基因能够减轻小鼠细胞 Ca^{2+}失调造成的营养不良(Goonasekera *et al*, 2011), SERCA 含量下降时会延缓心肌舒张, 导致心肌收缩功能下降, 最终造成心脏衰竭(李红艳等, 2020)。本研究发现, 在快长组肌肉组织中 *atp2a1* 基

表 3　候选差异表达基因注释

Tab.3　Annotation of candidate differentially expressed genes

基因 ID Gene ID	基因名 Gene name	*P* 值 *P*-value
Cus32796	肌球蛋白重链 1 Myosin heavy chain 1, *myh1*	2.75×10^{-11}
Cus32832	肌球蛋白重链 4 Myosin heavy chain 4, *myh4*	4.98×10^{-10}
Cus32788	肌球蛋白重链 6 Myosin heavy chain 6, *myh6*	1.99×10^{-4}
Cus37534	肌球蛋白重链 7 Myosin heavy chain 7, *myh7*	7.74×10^{-7}
Cus38339	肌球蛋白重链 9 Myosin heavy chain 9, *myh9*	1.29×10^{-3}
Cus32793	肌球蛋白重链 13 Myosin heavy chain 13, *myh13*	1.34×10^{-3}
Cus32818	肌球蛋白重链 15 Myosin heavy chain 15, *myh15*	1.86×10^{-5}
Cus25495	C-X-C 基序趋化子 11 Chemokine (C-X-C motif) ligand 11, duplicate 1, *cxcl11.1*	9.24×10^{-4}
Cus33036	转录激活因子 4 Activating transcription factor 4, *atf4*	1.35×10^{-10}
Cus09210	转录激活因子 7 Activating transcription factor 7, *atf7*	1.28×10^{-9}
Cus40180	核转录因子 RELA proto-oncogene, NF-kB subunit, *rela*	8.06×10^{-5}
Cus05452	成纤维细胞生长因子 1 Fibroblast growth factor 1, *fgf1*	9.08×10^{-29}
Cus13680	生长因子结合蛋白 1 Insulin like growth factor binding protein 1, *igfbp1*	2.56×10^{-11}
Cus34244	一磷酸腺苷激活蛋白激酶-γ3 Protein kinase AMP-activated non-catalytic subunit gamma 3, *prkag3*	3.95×10^{-4}
Cus33489	蛋白激酶 3 AKT serine/threonine kinase 3, *akt3*	1.25×10^{-4}
Cus11870	丙酮酸激酶 Pyruvate kinase M1/2, *pkm*	8.46×10^{-5}
Cus23574	Na^+/K^+-ATP 酶 α3 亚基 ATPase Na^+/K^+ transporting subunit alpha 3, *atp1a3*	1.83×10^{-9}
Cus27094	肌浆/内质网钙 ATP 酶 1 Sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase transporting 1, *atp2a1*	3.47×10^{-6}
Cus11128	肌浆/内质网钙 ATP 酶 2 Sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase transporting 2, *atp2a2*	1.75×10^{-3}
Cus29966	转铁蛋白受体 Transferrin receptor, *tfrc*	8.24×10^{-5}
Cus33302	磷酸肌醇-3-激酶 5 受体 Phosphoinositide-3-kinase regulatory subunit 5, *pik3r5*	4.05×10^{-4}
Cus27589	脂肪酸合成酶 Fatty acid synthase, *fasn*	5.30×10^{-5}
Cus23453	糖原合成酶 2 Glycogen synthase 2, *gys2*	2.58×10^{-4}
Cus27723	磷酸甘油酸变位酶-1 Phosphoglycerate mutase 1, *pgam1*	4.48×10^{-4}
Cus02461	磷酸烯醇式丙酮酸羧基酶 Phosphoenolpyruvate carboxykinase 1, *pck1*	6.85×10^{-9}
Cus00245	葡萄糖-6-磷酸脱氢酶 Glucose-6-phosphatase, catalytic, *g6pc*	1.40×10^{-8}
Cus15210	谷氨酸氨连接酶 Glutamate-ammonia ligase, *glul*	2.20×10^{-6}
Cus20143	精氨酸酶 2 Arginase 2, *arg2*	8.19×10^{-25}
Cus25173	F 框蛋白 34 F-box protein 34, *fbxo34*	9.78×10^{-12}
Cus05922	原癌基因 Jun proto-oncogene, AP-1 transcription factor subunit, *jun*	6.24×10^{-33}
Cus39186	cAMP 应答元件结合蛋白 5 cAMP responsive element binding protein 5, *creb5*	4.15×10^{-12}

因表达量显著上调，*atp2a2* 表达显著下调，可能是通过调节肌浆/内质网中 Ca^{2+}通道影响心肌和骨骼肌纤维的收缩与舒张，从而提高乌苏里白鲑神经系统的兴奋性，进而提高乌苏里白鲑的心肌功能与运动能力，通过加速血液循环与骨骼肌收缩对乌苏里白鲑的生长产生了影响。

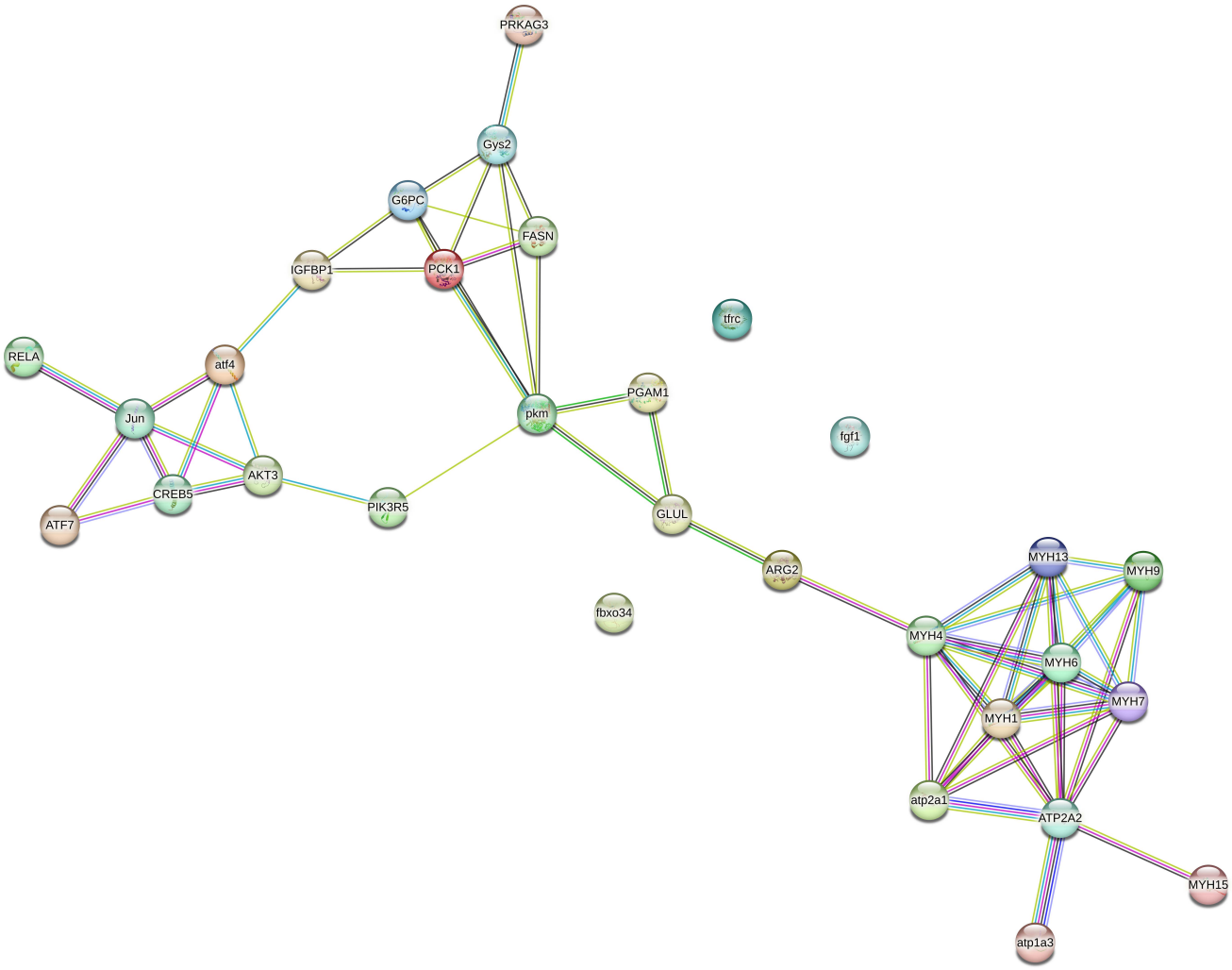

图 5 差异基因蛋白互作网络分析

Fig.5 Protein-protein network interaction analysis of differential gene

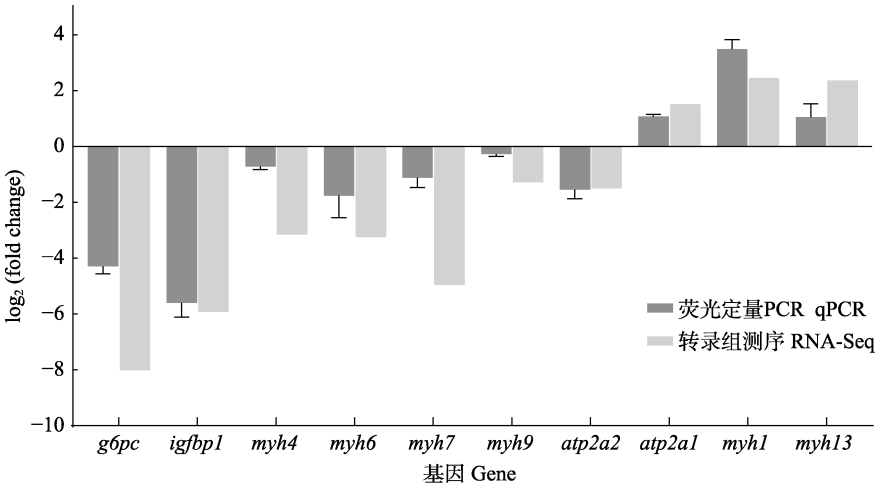

图 6 差异表达基因 qPCR 验证结果

Fig.6 qPCR results of differentially expressed genes

MYHs 基因家族是调控肌肉生长发育的关键基因，其编码的肌球蛋白重链是肌球蛋白(Myosin) Ⅱ类分子中的关键亚基，也是骨骼肌中含量最丰富的蛋白质，在肌肉生长过程中发挥重要作用(陈之航等, 2017)；*myh* 在不同类型的骨骼肌纤维中起作用，是肌肉纤维功能特性的主要决定因素，如 *myh7* 主要在慢肌纤维中表达，*myh1* 和 *myh2* 主要在快肌纤维中表达(Gauvry *et al*, 1996; Weiss *et al*, 1999)。本研究发现，在参与乌苏里白鲑生长调节的 6 个 *myhs* 亚型中，快长组中显著上调表达基因为 *myh1* 和 *myh13*。*myh1* 及其编码的肌球蛋白-1 (Myosin-1)与脂肪生成有关，其表达上调可增加慢肌纤维的数量，增强肌肉的抗氧化能力，提高运动耐力(Talbot *et al*, 2016; Ahn *et al*, 2018; Czapiewski *et al*, 2022)；*myh13* 基因编码特异性眼外肌肌球蛋白重链，参与齿鲸(toothed whales)肌原纤维活性的调节(Senevirathna *et al*, 2021)。除此之外，本研究中 *myh4*、*myh6*、*myh7* 和 *myh9* 基因在快长组中表达显著下调；*myh4* 基因是成肌分化的标志基因，编码骨骼肌生长所必须的ⅡB 型肌球蛋白重链纤维(Lv *et al*, 2020)，*myh6* 基因所编码的肌球蛋白较其他肌球蛋白具有更高的 ATP 酶活性，在突触传递过程中发挥重要作用(Buga *et al*, 2008; Miao *et al*, 2015)，*myh7* 基因突变会引起骨骼肌或心肌方面疾病(何一旻等, 2017)，同时，推测该基因在肌肉生长发育中具有调控作用(Feinstein-Linial *et al*, 2016; Zhang *et al*, 2016)；在鱼类中，*myh4* 和 *myh6* 基因是参与黑鲷(*Acanthopagrus schlegelii*)生长调控的关键基因(Lin *et al*, 2021)，*myh6* 和 *myh7* 基因在快速生长的兰州鲶(*Silurus lanzhouensis*)肌肉组织中表达下调(Xiao *et al*, 2022)；*myh9* 基因编码非肌肉肌球蛋白ⅡA 重链，且能促进肌纤维分化，其表达量与草鱼(*Ctenopharyngodon idellus*)肌肉质地有关(Pecci *et al*, 2018; Xu *et al*, 2020)。不同亚型的 *myh* 基因特异性表达不同肌纤维类型，与快肌纤维相比，慢肌纤维具有更高的有氧运动耐受力和胰岛素敏感性，通过上调快肌表达基因增加快肌纤维的数量及横截面积，提高运动能力，而慢肌纤维能提高肌肉运动的持续时间(Yang *et al*, 2020)。本研究发现，不同亚型 *myh* 基因通过调节其表达影响乌苏里白鲑的肌肉生长，可将 MYH 基因家族的多个亚型作为乌苏里白鲑生长关键候选基因。

g6pc 编码葡萄糖-6-磷酸酶(glucose-6-phosphatase, G-6-pase)催化亚基，该酶是内质网的标志酶，能够催化 6-磷酸葡萄糖水解产生葡萄糖和磷酸，是糖酵解和糖异生途径的关键酶，在维持血糖稳态中起着重要作用(钱云霞等, 2011; Liu S *et al*, 2020)。注射生长抑制素的金钱鱼(*Scatophagus argus*)体内 *g6pc* 基因表达量显著上调，进而激活糖酵解/糖异生过程，消耗大量 ATP，导致其生长所需能量不足，从而抑制金钱鱼的生长(Tian *et al*, 2022)。同样在对草鱼的研究中发现，*g6pc* 基因表达下调能够促进糖原分解从而提高饲料中碳水化合物的利用率(Yue *et al*, 2021)。*g6pc* 基因主要参与能量与物质代谢过程，在机体代谢和生长的协调过程中发挥重要作用。本研究发现，*g6pc* 基因在快长组的乌苏里白鲑中显著下调，*g6pc* 基因表达能激活糖酵解途径、加速糖原利用、消耗更多能量、减少有机物的积累，该基因高表达时可能会阻碍乌苏里白鲑的生长，可将其作为生长相关候选基因，进一步对其进行功能验证及分子标记的挖掘。

IGF 结合蛋白(IGFBPs)和 IGF、IGF 受体(IGFR)以及 IGFBP 水解酶四部分共同构成胰岛素样生长因子(insulin-like growth factors, IGFs)，IGFs 参与细胞分裂与分化、生物体代谢、生长与繁殖等过程，是 GH/IGF 生长轴(生长激素/胰岛素样生长因子轴)的关键因子，也是动物生长发育过程中的重要调控因子(黄建峰等, 2011; Hakuno *et al*, 2015)。IGFBPs 是一种分泌性蛋白，在人和哺乳动物中已鉴定出 6 种亚型(IGFBP1~IGFBP6)，它与 IGF 形成复合体后可延长 IGF 的半衰期，并激活胰岛素受体，抑制低血糖的发生(Duan *et al*, 2005; 赵艳等, 2015)。*igfbp1* 是 IGFBPs 家族中第一个被发现和鉴定的成员，其结构与功能已在多个物种中进行克隆和研究，其表达水平与鲑鱼的体重、体长、生长速率等指标呈负相关(Shimizu *et al*, 2006)；在对虹鳟(Kocmarek *et al*, 2014)和斑马鱼(*Danio rerio*) (Opazo *et al*, 2017)的研究中发现，*igfbp1* 基因均在小规格个体中表达上调。本研究发现，与慢长组相比，快长组 *igfbp1* 基因表达下调，这与斑马鱼、虹鳟等研究结果一致，可将其作为乌苏里白鲑生长相关候选基因进行后续验证。

4　结论

本研究通过对不同生长速度的乌苏里白鲑肌肉组织进行转录组测序，共发现了 2 211 个差异表达基因，其中包含 659 个新预测基因；583 个差异基因在快长组中表达显著上调，1 628 个差异基因表达显著下调。此外，GO 和 KEGG 富集分析发现，与生长相关的基因被显著富集在 MAPK 信号通路、PI3K-Akt 信号通路、紧密连接、胰岛素信号通路、糖酵解/糖异生、PPAR 信号通路中。进一步对筛选出候选差异

表达基因进行 PPI 蛋白互作网络分析，确定了 10 个与生长相关的核心候选基因：*atp2a2*、*atp2a1*、*g6pc*、*igfbp1*、*myh1*、*myh4*、*myh6*、*myh7*、*myh9* 和 *myh13*。本研究为乌苏里白鲑生长性状相关的分子调控机制及分子标记辅助育种的研究提供了基础资料。

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(编辑　冯小花)

Screening Muscle Growth-Related Genes of *Coregonus ussurinsis* Berg Based on Transcriptome Sequencing

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Abstract　*Coregonus ussurinsis* Berg is a rare cold-water fish found in Heilongjiang Province, which has high nutritional and economical value. The growth traits of fish are critical breeding target traits, and improving the growth efficiency of cultured fishes has always been a major issue for researchers. As an endangered fish, very limited research has been conducted on *C. ussurinsis*, and studies on its growth and development are still lacking. Therefore, investigating gene expression in *C. ussurinsis* muscles would significantly contribute to our understanding of their muscle development. RNA-Seq was used to find and study the specific genes and pathways of muscle development under different conditions. Recently, transcriptome sequencing has been applied to diverse animal populations, aiding in the selection of candidate genes related to important traits by comparing the global gene expression profiles between different animal populations with specific characteristics. This study aims to understand the genetic basis of muscle development in *C. ussurinsis* at the transcriptome level and to provide new insights into growth and development. To explore the molecular regulation mechanism of growth traits of *C. ussurinsis*, F_2 individuals of *C. ussurinsis* were randomly selected from the mixed pool for test grouping (fast-growing group and slow-growing group). The dorsal muscle tissue was clipped from 10 fast-growing individuals (219.20±38.66 g, weight) and 10 slow-growing individuals (74.30±17.86 g, weight) for transcriptome sequencing to construct six cDNA libraries. High-throughput sequencing from Illumina NovaSeq 6000 and bioinformatics was used to determine the abundances and characteristics of transcripts. The differentially expressed genes were screened with FDR (false discovery rate)<0.05 and $|\log_2\text{FoldChange}|>1$; the functions of these differentially expressed genes (DEGs) were annotated and analyzed by the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) database to identify the genes and genetic pathways related to the development of muscle in *C. ussurinsis*. Moreover, to verify the sequencing results, real-time fluorescence quantitative PCR (qRT-PCR) was used to detect the expression levels of DEGs. The results showed that the correlation coefficients of all the samples used for transcriptome sequencing were above 0.83, indicating high correlation between the samples and experimental reliability. Transcriptome sequencing results showed that a total of 295 605 738 raw reads were assembled from the six cDNA libraries, and 283 133 612 clean reads were obtained after quality control. Q20 and Q30 sequences accounted for above 97.80% and 93.90%, respectively, and the

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content of GC bases accounted for more than 49.1% of the total bases. Through comparison with the genome using EdgeR software, which was used to analyze the differences in gene expression, 2 211 DEGs were preliminarily obtained from muscle, including 659 novel genes. Compared with the slow-growing group, 583 differential genes were up-regulated, and 1 628 differential genes were down-regulated in the fast-growing group. Function enrichment analysis found that the DEGs participated in 3 620 GO terms. Among them, 2 457 biological processes were primarily involved in cellular and metabolic processes; there were 782 molecular functions, primarily involved in binding function and catalytic activity processes, and 381 cellular components, primarily involved in cell and cell component processes. The enrichment analysis of the KEGG pathway found that a total of 251 signal pathways were obtained, among which 73 were significantly enriched (P<0.05). Among them, the up-regulated DEGs were mainly involved in glycolysis/gluconeogenesis, methane metabolism, and biosynthesis of amino acids, while the down-regulated DEGs were mainly involved in osteoclast differentiation, IL-17 signaling pathway, and biosynthesis of amino acids. The genes related to muscle growth were significantly (P<0.05) enriched in the MAPK signaling pathway, PI3K-Akt signaling pathway, tight junction, insulin signaling pathway, glycolysis/gluconeogenesis, and PPAR signaling pathway. These pathways might be closely related to muscle growth. Combined with the GO functional annotation, the KEGG pathway enrichment, and the annotation results, 31 potential growth-related candidate genes were preliminarily screened. Protein-protein interaction networks were used to further analyze the relationship between these differential genes. It was found that *atp2a2*, *atp2a1*, *g6pc*, *igfbp1*, *myh1*, *myh4*, *myh6*, *myh7*, *myh9*, and *myh13* might be closely related to muscle growth regulation, and these 10 genes can be used as crucial candidate genes for the growth regulation of *C. ussurinsis*. The qRT-PCR validation of 10 randomly selected differential genes showed consistent gene expression trends with the transcriptome sequencing results, which indicated that the results obtained by transcriptome sequencing in this study were accurate. A total of 10 growth-related essential candidate genes were screened in this study; these genes affect the growth of *C. ussurinsis* by regulating their expression levels in muscle tissue. These results provide vital information for the further understanding of the molecular basis and marker-assisted breeding of the growth regulation of *C. ussurinsis*.

Key words *Coregonus ussurinsis* Berg; RNA-seq; Differentially expressed genes; Muscle growth