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基于转录组学探究微塑料与铜暴露对中华绒螯蟹肠道基因表达的影响*

徐建^{1,2} 冯广朋^{1,2} 严云志¹ 陈锦辉³ 吴建辉³ 杨祖长²

(1. 安徽师范大学生态与环境学院 安徽 芜湖 241002; 2. 中国水产科学研究院东海水产研究所 上海长江口渔业资源增殖和生态修复工程技术研究中心 上海 200090;
3. 上海市水生野生动植物保护研究中心 长江口水生生物监测与保护联合实验室 上海 200092)

摘要 微塑料(microplastics, MPs)是常见的污染物,而硫酸铜(CuSO_4)是水产养殖中常用的消毒剂,水体中的铜离子(Cu^{2+})可被 MPs 吸附。为探究 MPs 和 Cu^{2+} 单一及联合暴露对中华绒螯蟹(*Eriocheir sinensis*)肠道组织的影响机制,本研究选择 0.4 mg/L MPs 和 0.1 mg/L Cu^{2+} 作为实验浓度,设置 4 个处理组: 0.4 mg/L MPs 暴露组(M 组)、0.1 mg/L Cu^{2+} 暴露组(C 组)、0.4 mg/L MPs + 0.1 mg/L Cu^{2+} 联合暴露组(MC 组)和空白对照组(D 组),探究 MPs 和 Cu^{2+} 单一及联合暴露 21 d 后对中华绒螯蟹肠道的影响。采用 RNA-Seq 技术对肠道开展转录组测序,筛选差异表达基因(DEGs)进行 KEGG 富集等生物信息学分析。结果显示,与对照组相比, M 组包括 1 650 个上调 DEGs 和 1 874 个下调 DEGs; C 组包括 3 797 个上调 DEGs 和 1 073 个下调 DEGs; MC 组包括 1 492 个上调 DEGs 和 1 305 个下调 DEGs。与抗氧化、免疫和能量代谢相关的 DEGs,包括过氧化氢酶(*cat*)、三结构域蛋白(*trim*)、Toll 样受体(*tlr*)、细胞色素 P450 (*cyp450*)、碳酸酐酶(*ca*)等在 M vs D、C vs D、MC vs D 中变化显著。KEGG 富集分析显示, DEGs 主要富集于氧化磷酸化、谷胱甘肽代谢、细胞色素 P450 对异生素的代谢等通路。结果表明, MPs 与 Cu^{2+} 单一及联合暴露导致中华绒螯蟹的抗氧化、免疫和能量代谢系统失衡, MPs 和 Cu^{2+} 具有协同毒性作用,研究结果为阐明 MPs 和 Cu^{2+} 暴露对中华绒螯蟹的影响机制提供了参考。

关键词 中华绒螯蟹; 微塑料; 铜; 转录组; 能量代谢; 免疫

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微塑料(microplastics, MPs)是指粒径小于 5 mm 的塑料。近年来,由于塑料制品的广泛使用,微塑料污染俨然成为一个主要的生态和社会问题(Lebreton *et al.*, 2017)。微塑料在陆地和水生生态系统中迁移、

扩散,易对水生生态系统产生威胁(于翔等, 2021; Tian *et al.*, 2019)。由于人类活动和工业发展,环境中 80% 的微塑料来自陆地,这些陆源微塑料通过空气和渗液传播,其中 10% 进入水生生态系统,最终汇聚至海洋,

* 长江口南支东部水域中华绒螯蟹等重要物种变化趋势预测实施项目(11N42500663520242801)资助。徐建, Email: xj905173508@163.com

① 通信作者: 冯广朋, 研究员, Email: coolwindfgp@163.com

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因此,沿海地区的微塑料污染受到广泛关注(Truchet *et al*, 2023; Vo *et al*, 2021)。研究表明,在太湖、鄱阳湖、洞庭湖、洪湖等长江流域大型湖泊中的微塑料浓度显著高于河流(Zhang *et al*, 2021)。长江口是中国最大的河流入海口,受潮汐与咸淡水的交织作用,生境复杂独特(杨钧渊等, 2023)。截至2014年,长江口地表水中的微塑料丰度为 $4\ 137$ 微粒/ m^3 (Zhao *et al*, 2014)。长江口沉积物中的微塑料平均丰度为 (121 ± 9) 微粒/kg (Peng *et al*, 2017)。水环境中,微塑料的大小与藻类相似,因此,微塑料易被鱼类、甲壳类等水生生物误食(Li *et al*, 2021)。进入水生生物体内的微塑料会诱导氧化应激、免疫毒性、生殖毒性和神经毒性等损害(Wei *et al*, 2023; 杜蕴超等, 2024)。

微塑料的“载体效应”受到越来越多的关注(Magara *et al*, 2018)。研究表明,微塑料可以吸附重金属等污染物(Vo *et al*, 2021)。硫酸铜($CuSO_4$)在水产养殖中广泛使用,主要用于控制寄生虫和病原菌引起的疾病,以及控制淡水中的蓝藻水华(Tavares-Dias, 2021; Zhang *et al*, 2016)。2003年,《中国渔业生态环境状况公报》指出以 Cu^{2+} 浓度不超过 0.01 mg/L为标准,但长江口水域 Cu^{2+} 浓度高达 0.07 mg/L(杨志彪等, 2006)。2006—2017年,长江流域水体中的 Cu^{2+} 浓度最高达 0.19 mg/L(何佳等, 2019)。高浓度的铜不仅会污染水生态环境,还对水生动物具有潜在毒性(Zhang *et al*, 2022)。Lauer等(2012)研究发现,过量的铜会抑制甲壳类动物参与糖酵解等能量代谢系统,从而影响抗氧化和免疫系统。长期 Cu^{2+} 暴露会对印度对虾(*Penaeus indicus*)的谷胱甘肽系统造成抑制(Paila *et al*, 2012)。

研究表明,微塑料与其他污染物联合暴露对水生生物具有影响。微塑料和亚硝酸盐联合暴露14 d后,破坏了凡纳对虾(*Penaeus vannamei*)肠道微生物菌群平衡,导致氧化应激(Xing *et al*, 2024)。微塑料和镉共同暴露会导致黑鲫(*Carassius carassius*)肝损伤,并上调与免疫反应相关的基因转录(Wei *et al*, 2023)。目前,关于MPs和 Cu^{2+} 联合暴露对水生生物肠道组织的影响已有报道,金鱼(*Carassius auratus*)的肠道在MPs和 Cu^{2+} 联合暴露下发生炎症并抑制细胞凋亡(Zhang *et al*, 2022)。MPs和 Cu^{2+} 联合暴露会破坏斑马鱼(*Danio rerio*)肠道细胞的线粒体形态并影响肠道菌群结构(Rong *et al*, 2024)。此外,尼罗罗非鱼(*Oreochromis niloticus*)肠道微生物群的多样性和结构在MPs和 Cu^{2+} 联合暴露下也发生了变化(Zhang *et al*, 2022)。相关研究表明,微塑料会对中华绒螯蟹

(*Eriocheir sinensis*)造成损害并诱发氧化应激(Yu *et al*, 2018)。

中华绒螯蟹是长江流域渔业资源的重要物种,也是中国水产养殖业中经济价值较高的淡水蟹类(Chen *et al*, 2007)。每年生长至性成熟的中华绒螯蟹洄游至长江口进行交配和抱卵(张方圆等, 2024)。由于其底栖生活习性,中华绒螯蟹在水生环境中容易受到微塑料和重金属的影响(Yang *et al*, 2022)。中华绒螯蟹在摄食过程中,肠道直接与污染物接触,是最容易受感染的器官(Xue *et al*, 2021)。铜积累对中华绒螯蟹鳃和肝胰腺造成组织学损伤,并诱导肝胰腺的金属硫蛋白合成(Yang *et al*, 2007)。然而,关于这2种污染物联合暴露对中华绒螯蟹肠道组织的影响机制尚未见报道。

本研究采用RNA-Seq转录组测序技术对MPs与 Cu^{2+} 单一及联合暴露下中华绒螯蟹肠道的基因表达差异进行分析,揭示环境相关浓度MPs与 Cu^{2+} 对淡水生物的综合影响及其机制,以为淡水生态系统中微塑料与铜污染防治提供参考支撑,以及为水产领域的环境安全评价提供科学基础。

1 材料与方法

1.1 实验样品及处理

1.1.1 实验材料 实验用蟹选自人工养殖成蟹,平均体重为 (92.27 ± 10.34) g,于2023年11月运至实验室,置于经高锰酸钾消毒后的养殖缸中暂养2周。暂养期间,温度为 (13 ± 3) °C, pH为7.0~7.5,溶解氧 > 6 mg/L。每日18:00投喂饲料(常州海大生物饲料有限公司),日投喂量为螃蟹总重量的1%,及时清理剩余饲料。

聚苯乙烯微塑料 $[(100.0\pm 0.4)$ nm]购自天津倍思乐研究中心。 $CuSO_4\cdot 5H_2O$ ($\geq 99.0\%$),购自国药集团化学试剂有限公司,配制1 g/L的铜溶液母液置于棕色瓶中保存。

1.1.2 暴露实验 根据环境中微塑料浓度并结合前期有关微塑料对中华绒螯蟹的毒性研究,微塑料实验浓度设置为 0.4 mg/L(Liu *et al*, 2019)。根据环境中铜浓度并结合前期有关铜对中华绒螯蟹的毒性研究,铜实验浓度设为 0.1 mg/L(Ren *et al*, 2011)。

暂养结束后,选取生长良好的个体置于玻璃缸($70\text{ cm}\times 43\text{ cm}\times 50\text{ cm}$)中进行实验,以过滤后的自来水为D组(对照组),共设3个处理组:M组(微塑料暴露组)、C组(铜暴露组)和MC组(微塑料和铜共同暴露组)。每组设3个平行组,每个平行组包括20只

实验蟹(雌蟹)。实验期间持续充氧,日投喂量为中华绒螯蟹总重量的 1%,及时清理剩余饲料,每 7 d 换一次水,参考 Yang 等(2022)和 Pan 等(2023)的研究,暴露实验时间设置为 21 d。

1.1.3 样品采集 第 21 天采样,采样前 24 h 停止喂食。每组各随机取 3 只中华绒螯蟹作为生物学重复,采样前在冰上麻醉,快速分离其肠道,液氮速冻后置于-80 °C 冰箱中保存,用于后续转录测序。

1.2 总 RNA 提取、文库构建及测序

使用 TRIzol (Aidlab, 中国北京)方法提取肠道组织的总 RNA,通过 NanoDrop 2000 分光光度计(Thermo Fisher Scientific, 美国)和琼脂糖凝胶电泳检测总 RNA 的浓度、纯度和完整性。对检验合格的 RNA 样品构建文库。将构建合格的文库使用 NovaSeqX Plus 平台进行测序。

1.3 测序数据质控、组装和功能注释

采用 Fastqc (Chen *et al*, 2018)软件对原始测序数据片段(raw reads)进行质量控制,去除包含接头的

reads、序列末端低质量(质量值小于 20)的碱基、含 N 比率超过 10%的 reads。然后采用 HiSat2 软件将质控数据与参考基因组比对,进行后续分析,并进行比对结果的质量评估。

1.4 基因表达水平和差异富集分析

使用 DESeq 软件对差异表达基因(DEG)进行标准化及差异表达基因检测,筛选阈值为 $P_{adj}<0.05$ 和 $|\log_2FC|>1$ (FC, fold change)。采用 Goatools 软件、Python scipy 软件包对差异基因进行 GO (gene ontology)和 KEGG (kyoto encyclopedia of genes and genomes)数据库通路富集分析(Ashburner *et al*, 2000; Kanehisa *et al*, 2004)。

1.5 实时荧光定量 PCR 验证

对筛选出的 9 个关键差异表达基因进行 qRT-PCR 验证,确保 RNA-Seq 检测结果的准确性。本研究选取 β -actin 作为内参基因,利用 Primer Premier 5.0 软件设计特异性引物,信息见表 1。每个基因进行 3 次平行实验,采用 $2^{-\Delta\Delta Ct}$ 方法计算相对表达量。

表 1 qRT-PCR 引物序列

Tab.1 Primer sequences used in qRT-PCR

基因 Gene	正向引物 Forward primer (5'~3')	反向引物 Reverse primer (5'~3')
<i>β-actin</i>	GGCATCCACGAGACCACTT	CTCCTGCTTGCTGATCCACAT
<i>cat</i>	CCTCTTCCCTTCCTTTATC	CCTGGTCGGTCTTGTAAT
<i>cox</i>	GATGAAGACTTCCAGGTTCCC	TGGACGCATTCGACGAGGC
<i>nd</i>	TACGAGGACCACCCAGAC	ATCCATAGATCCCACCAG
<i>idh</i>	TTCGCTCACTCGTCTTCC	AGTTCTTGACGCCACACA
<i>cyp450</i>	CACCACGACTCCCGCTACT	CCTCCATCAGGGCAAACC
<i>abc</i>	AGAATGGCAGTGCTAATGG	AGTTGTCAATCTTAATGTCCC
<i>v-atpase</i>	GAGGAGCACTACGACGCAAAC	TCACAATCTGGCGGGACA
<i>ctl</i>	TTACTGGCGAGCCTATGA	ACCGACTCCTTTCCCTTC
<i>gst</i>	CTCCTCTGCTTGGCTACT	TCTTCTCCTCGTCTGTCTT

2 结果

2.1 转录组测序和组装

通过 Illumina 双端测序技术对胁迫处理 21 d 后的中华绒螯蟹肠道组织进行转录组测序,产生 197 908 972 个 raw reads。过滤低质量数据后,D 组、M 组、C 组和 MC 组分别获得 51 911 548、42 924 544、57 484 980 和 43 429 502 个 clean reads,其 GC 含量分别为 42.47%、39.29%、42.59%和 42.58%,Q30 为 94.71%~95.48% (表 2)。共获得 109 644 个转录本,其中长度>1 800 的转录本数目最多,有 68 005 个(表 3)。

2.2 差异表达基因分析

如图 1 所示,在暴露 21 d 后的肠道转录组中,与对照组相比,M 组包括 1 650 个上调 DEGs 和 1 874 个下调 DEGs; C 组包括 3 797 个上调 DEGs 和 1 073 个下调 DEGs; MC 组包括 1 492 个上调 DEGs 和 1 305 个下调 DEGs。与 MC 组相比,M 组包括 1 136 个上调 DEGs 和 1 633 个下调 DEGs; C 组包括 3 505 个上调 DEGs 和 1 093 个下调 DEGs。

与抗氧化系统、免疫防御和能量代谢有关的 DEGs 在 5 个比较组之间显著差异表达。在 M vs D、C vs D、MC vs D 中,抗氧化系统中超氧化物歧化酶

表 2 转录组测序质量和序列比对
Tab.2 Quality and sequence alignment of transcriptomics sequencing

样本名 Sample	原始数据 Raw reads	原始碱基 Raw bases	质控数据 Clean reads	质控碱基 Clean bases	测序错误率 Error rate /%	Q20 /%	Q30 /%	GC 含量 GC content/%
对照组 Control group	52 479 128	7 924 348 328	51 911 548	7 702 188 470	0.0124	98.45	95.48	42.47
微塑料暴露组 Microplastic exposure group	43 376 482	6 549 848 782	42 924 544	6 426 908 088	0.0128	98.15	94.71	39.29
铜暴露组 Copper exposure group	58 129 800	8 777 599 800	57 484 980	8 468 277 808	0.0123	98.46	95.52	42.59
微塑料与铜共同暴露组 Microplastics and copper co-exposure group	43 923 562	6 632 457 862	43 429 502	6 475 778 953	0.0125	98.34	95.31	42.58

注：Q20、Q30 分别表示碱基测序质量值大于 20 和 30 的碱基占总体碱基的百分比。

Notes: Q20 and Q30 represent the percentage of bases with mass values greater than 20 and 30 in the total bases.

表 3 转录本长度分布
Tab.3 Transcript length distribution

长度 Length	0~ 200	201~ 400	401~ 600	601~ 800	801~ 1 000	1 001~ 1 200	1 201~ 1 400	1 401~ 1 600	1 601~ 1 800	>1 800	Total
数目 Number	1 501	5 278	4 374	5 324	4 967	5 353	5 195	4 915	4 732	68 005	109 644

(*sod*)均显著上调, 过氧化氢酶(*cat*)、过氧化还原蛋白(*prdx*)均显著下调; 免疫防御系统中三结构域蛋白(*trim*)、Toll 样受体(*tlr*)、补体第 1 成分(*c1*)均显著上调, 细胞色素 P450(*cyp450*)均显著下调; 能量代谢系统中碳酸酐酶(*ca*)均显著上调, 囊泡型腺苷三磷酸酶(*v-atpase*)均显著下调。

2.3 差异表达基因的 GO 富集分析

差异基因 GO 富集分析结果显示, 在 M vs D 中, DEGs 显著富集的 GO 功能主要包括 ATP 代谢过程(ATP metabolic process)、单羧酸代谢过程(monocarboxylic acid metabolic process)、嘌呤核糖核苷酸代谢过程(purine ribonucleotide metabolic process)等; 在 C vs D 中, DEGs 显著富集的 GO 功能主要包括 RNA 3'末端加工(RNA 3'-end processing)、tRNA 代谢过程(tRNA metabolic process)、rRNA 代谢过程(rRNA metabolic process)等; 在 MC vs D 中, DEGs 显著富集的 GO 功能主要包括防御响应(defense response)、免疫应答(immune response)、细胞外区域(extracellular region)等(图 2)。

2.4 差异表达基因的 KEGG 富集分析

在 M vs D 中, 肠道 1 594 个 DEGs 富集到 333 个

通路, 其中, 显著富集到 34 个通路($P_{adj}<0.05$), 主要包括化学致癌-活性氧(chemical carcinogenesis-reactive oxygen species)、氧化磷酸化(oxidative phosphorylation)、谷胱甘肽代谢(glutathione metabolism)等。在 C vs D 中, 肠道 2 445 个 DEGs 富集到 340 个通路, 其中, 显著富集到 9 个通路($P_{adj}<0.05$), 主要包括 DNA 复制(DNA replication)、抗叶酸(antifolate resistance)、ABC 转运体(ABC transporters)等。在 MC vs D 中, 肠道 1 198 个 DEGs 富集到 326 个通路, 其中, 显著富集到 14 个通路($P_{adj}<0.05$), 主要包括补体和凝血级联(complement and coagulation cascades)、细胞色素 P450 对异生素的代谢(metabolism of xenobiotics by cytochrome P450)、ABC 转运体(ABC transporters)等(图 3)。

对抗氧化系统、免疫防御和能量代谢相关的通路进行分析。谷胱甘肽代谢(glutathione metabolism)通路中, M vs D 中多数 DEGs 显著下调, 包括葡萄糖-6-磷酸脱氢酶(glucose-6-phosphate dehydrogenase, *g6pd*)、谷胱甘肽过氧化物酶(glutathione peroxidase, *gsh-px*)、谷胱甘肽 S-转移酶(glutathione S-transferase, *gst*)等; MC vs D 中 *g6pd* 表达显著下调, *gsh-px* 和 *gst* 在 C vs D 和 MC vs D 中均显著下调, 而 *gsh-px* 在 M vs MC、C vs MC 中均显著上调。

细胞色素 P450 对异生素的代谢作用(metabolism

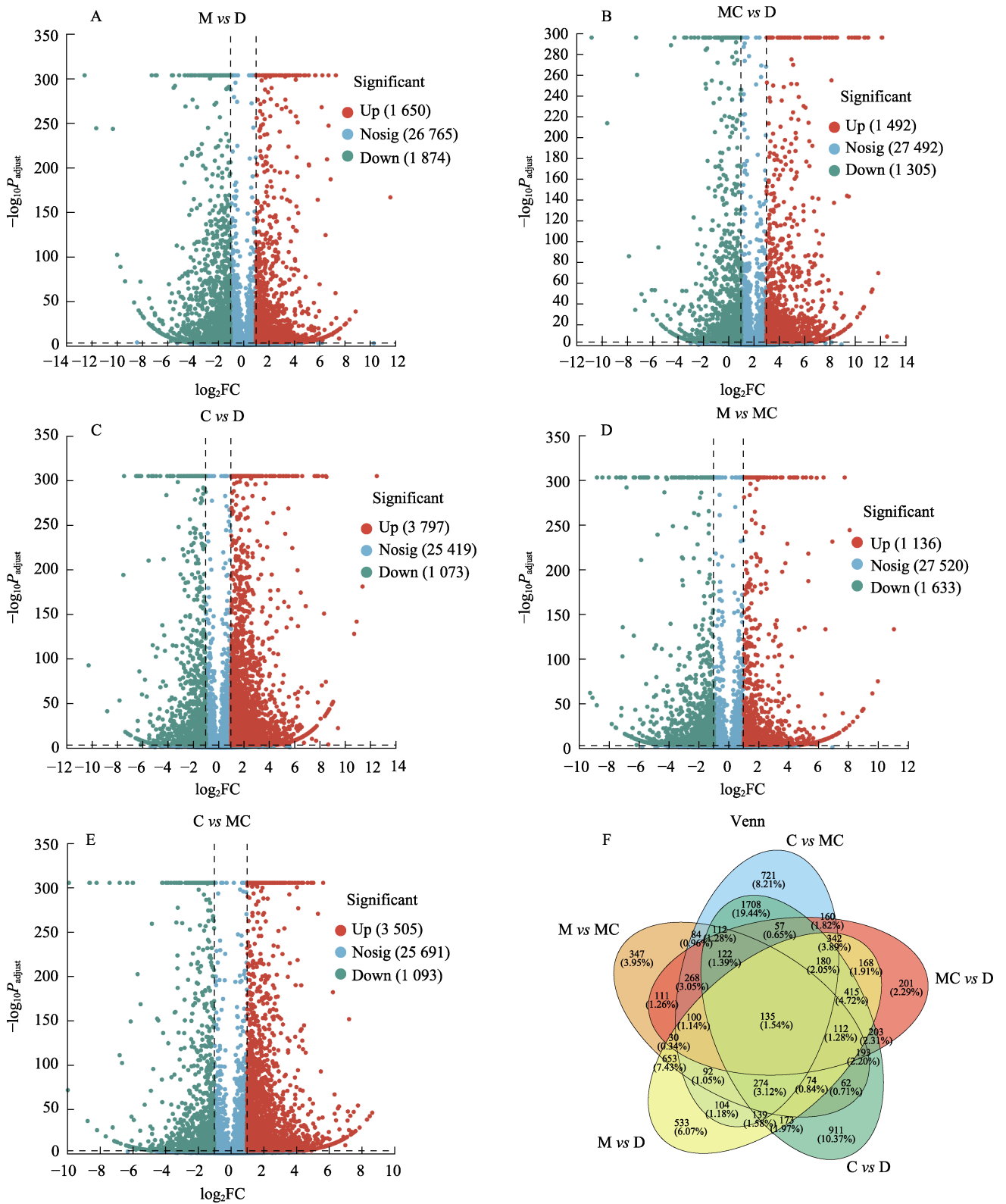
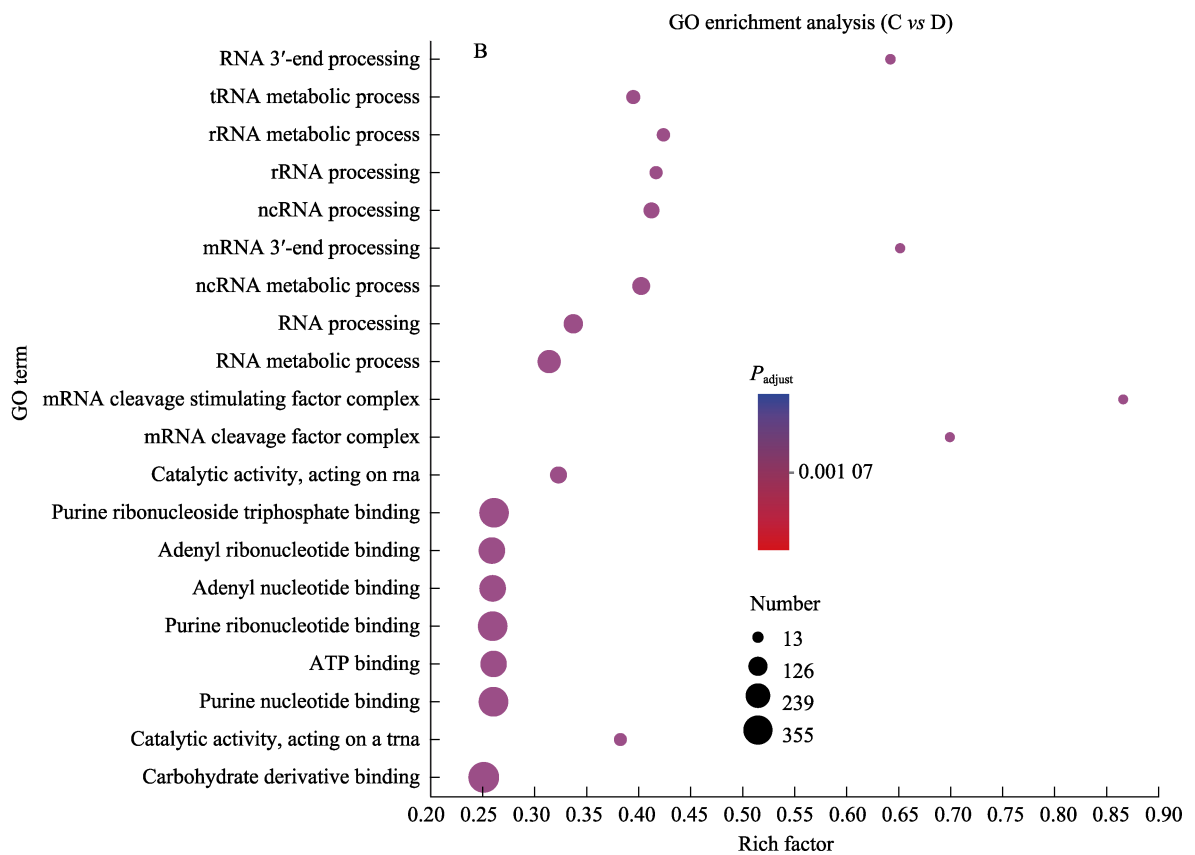
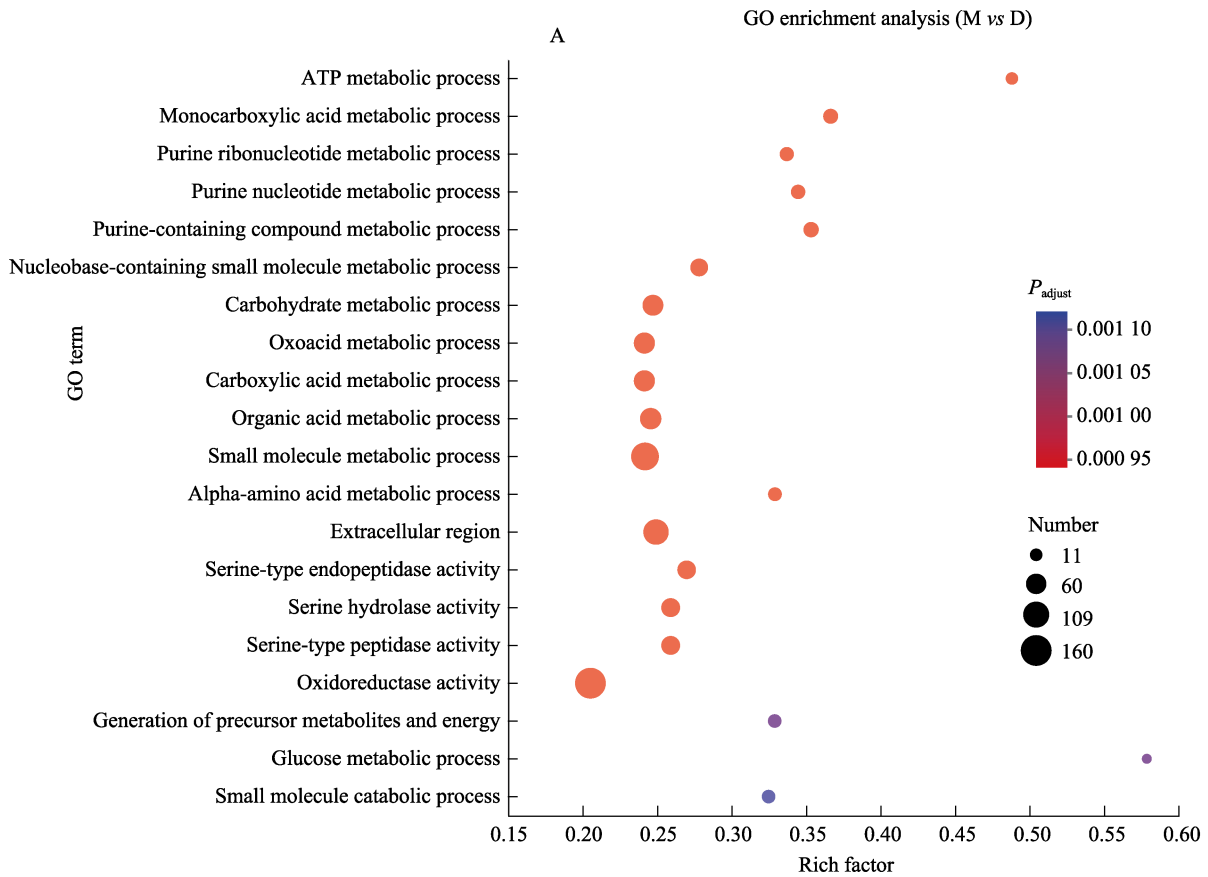


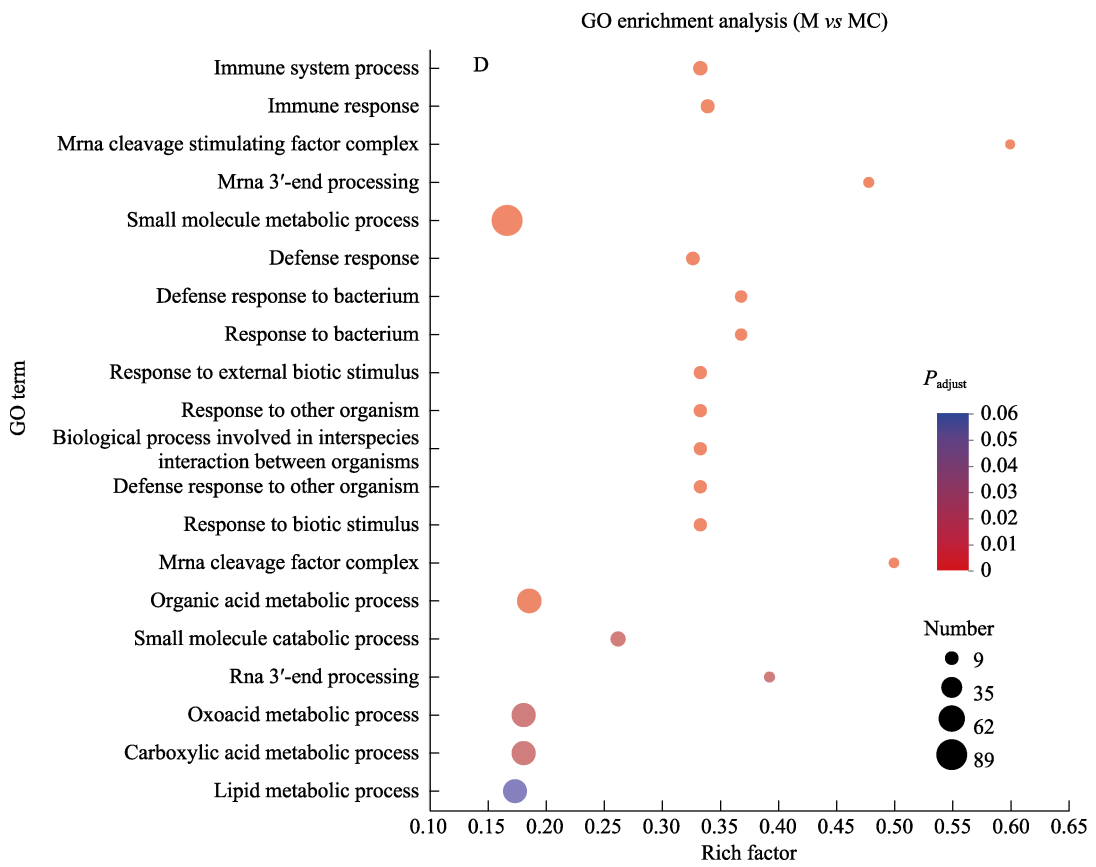
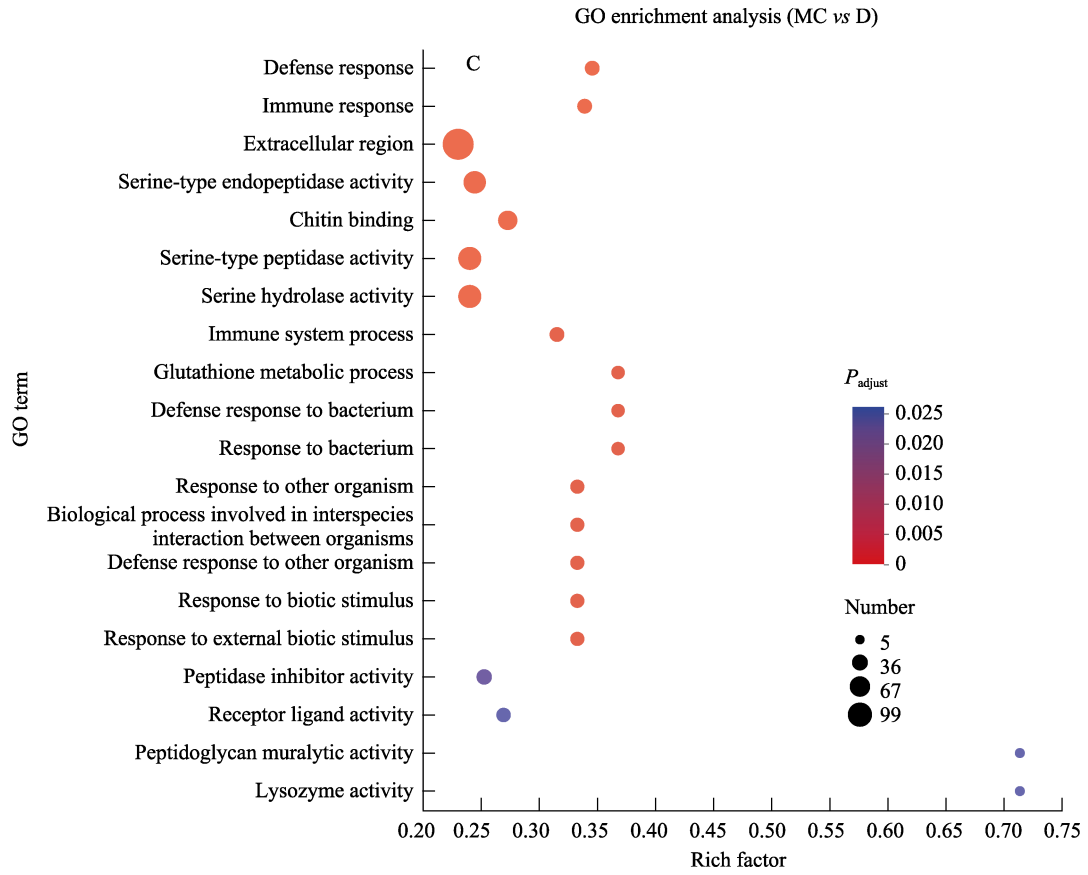
图 1 差异表达基因分析

Fig.1 Analysis of differentially expressed genes

A~E: 差异表达基因火山图; F: 差异表达基因韦恩图

A~E: Volcano map of DEGs; F: Venn diagram of DEGs





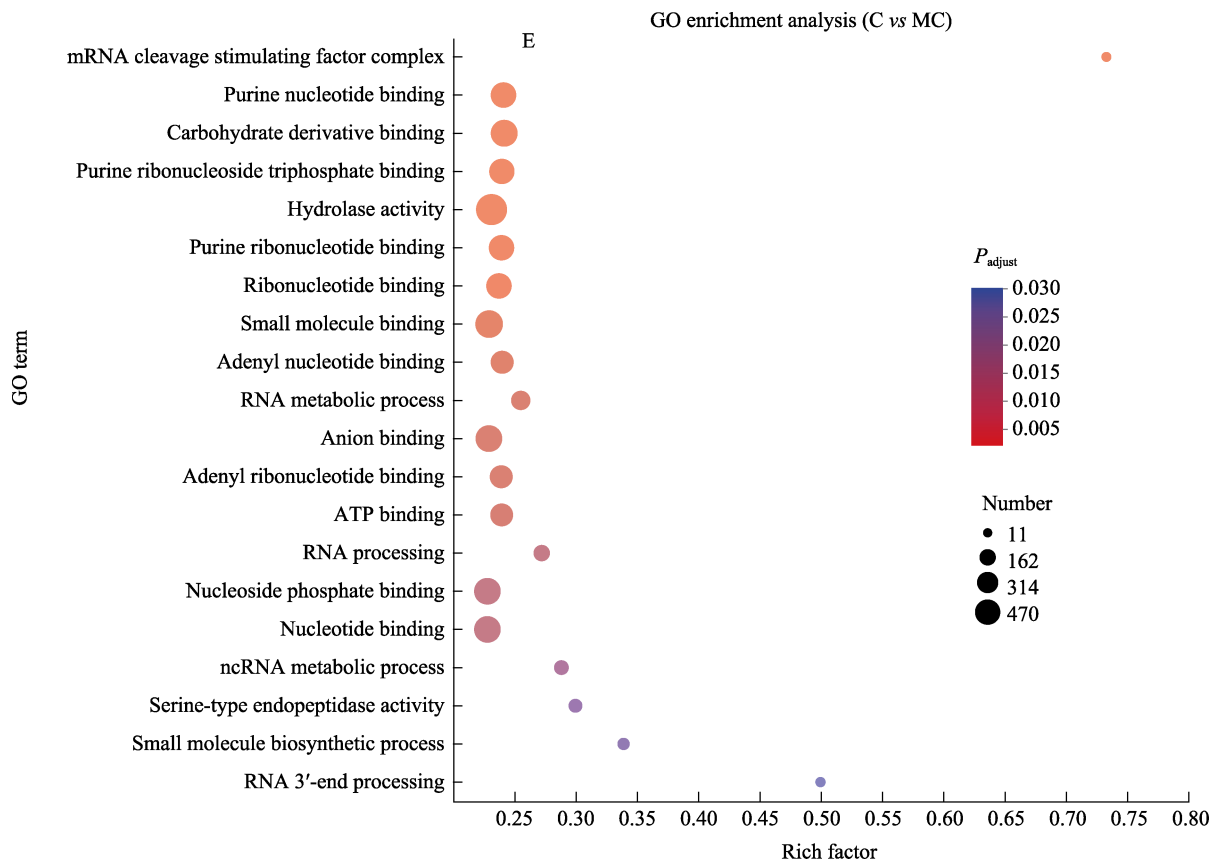


图 2 DEGs 的 GO 富集分析(富集排名前 20)

Fig.2 GO enrichment analysis of DEGs (top20)

of xenobiotics by cytochrome P450)通路中, 葡萄糖醛酸转移酶(UDP-glycosyltransferase, *ugt*)的基因在 M vs D、C vs D 中显著上调, MC vs D 中显著下调, 而在 M vs MC、C vs MC 中显著上调。*gst* 在 M vs D、C vs D、MC vs D 中均显著下调。

在补体和凝血级联通路中, 凝血因子 B (clotting factor B)基因在 M vs D、C vs D 和 MC vs D 中均显著下调, 凝血因子 G (clotting factor G)基因在 M vs D、MC vs D 中显著下调, 在 C vs D、C vs MC 中显著上调。

在氧化磷酸化通路中, M vs D 中多数 DEGs 显著下调, 包括 NADH 脱氢酶(NADH dehydrogenase, *nd*)、ATP 合酶(ATP synthase, *atpase*)、细胞色素氧化酶(cytochrome c oxidase subunit, *cox*)等; 而 C vs D 中 *cox* 表达显著上调, MC vs D 中 *cox* 表达显著下调。

2.5 qRT-PCR 验证结果

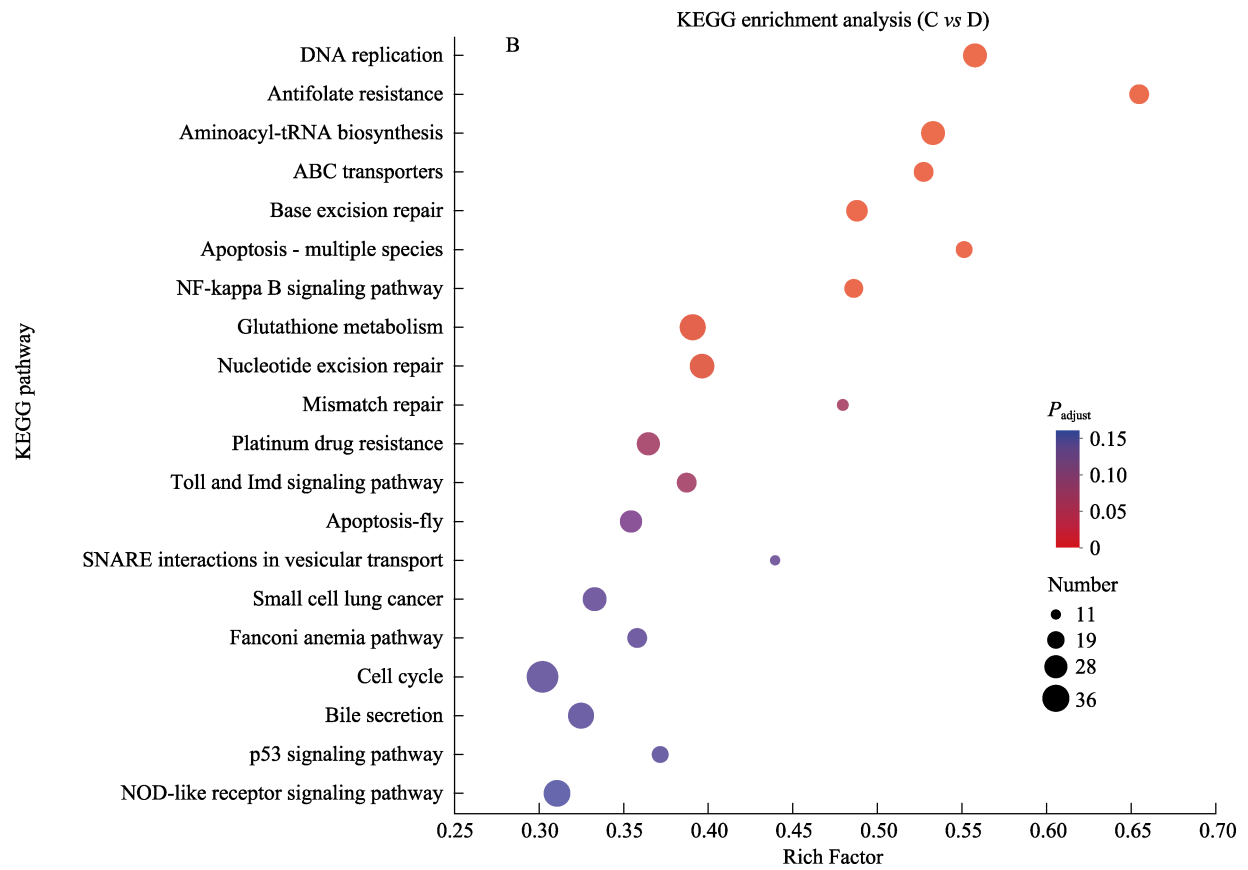
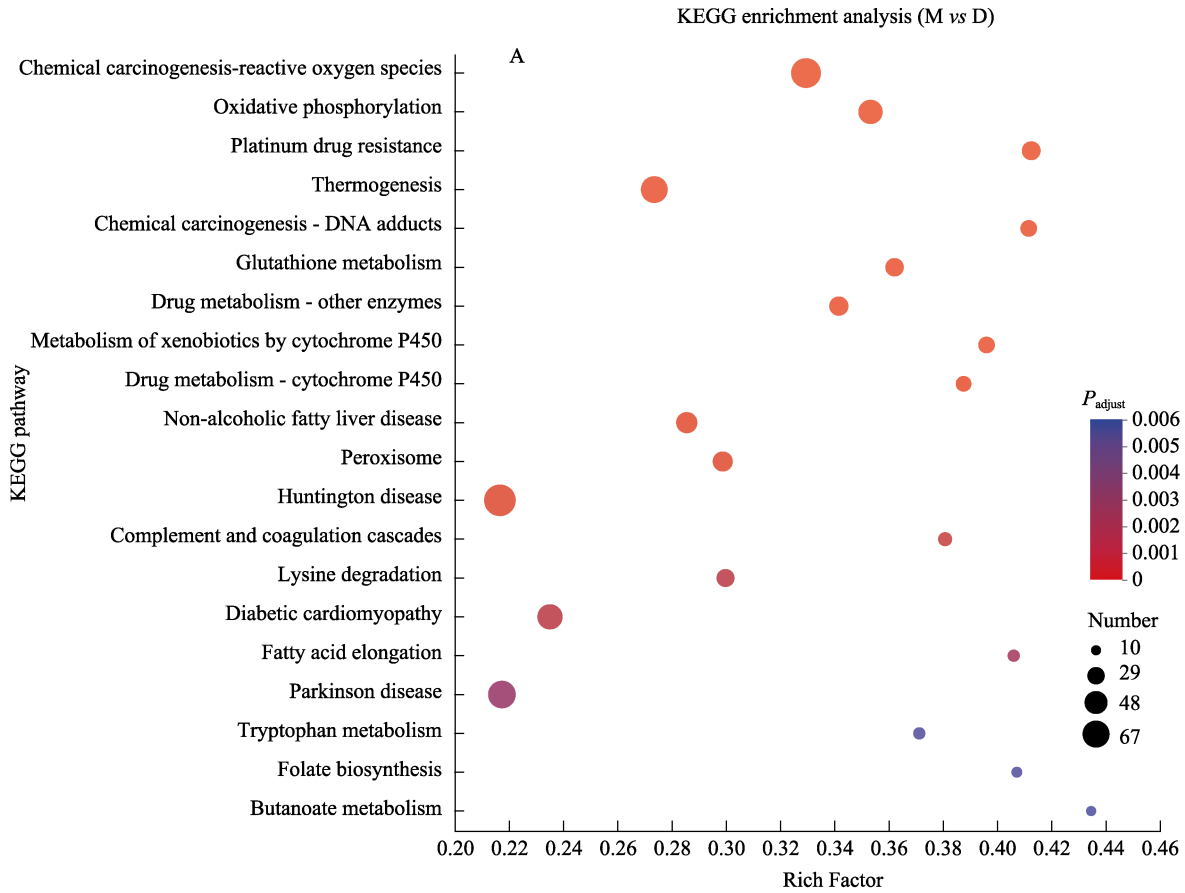
选取 9 个关键差异基因进行 qRT-PCR 验证, 结果显示, 9 个差异基因表达结果与转录组测序获得的基因表达趋势一致(图 4)。因此, qRT-PCR 验证结果证实了 RNA-seq 数据的可靠性和准确性。

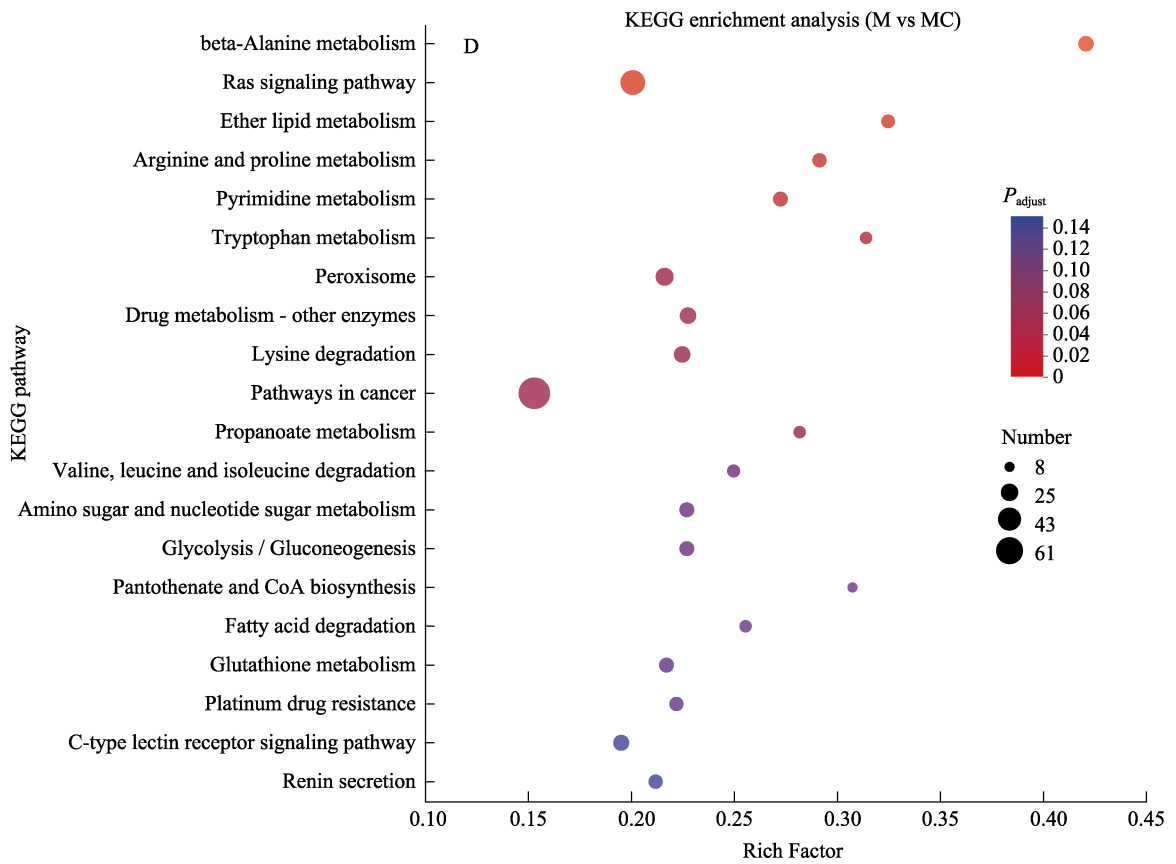
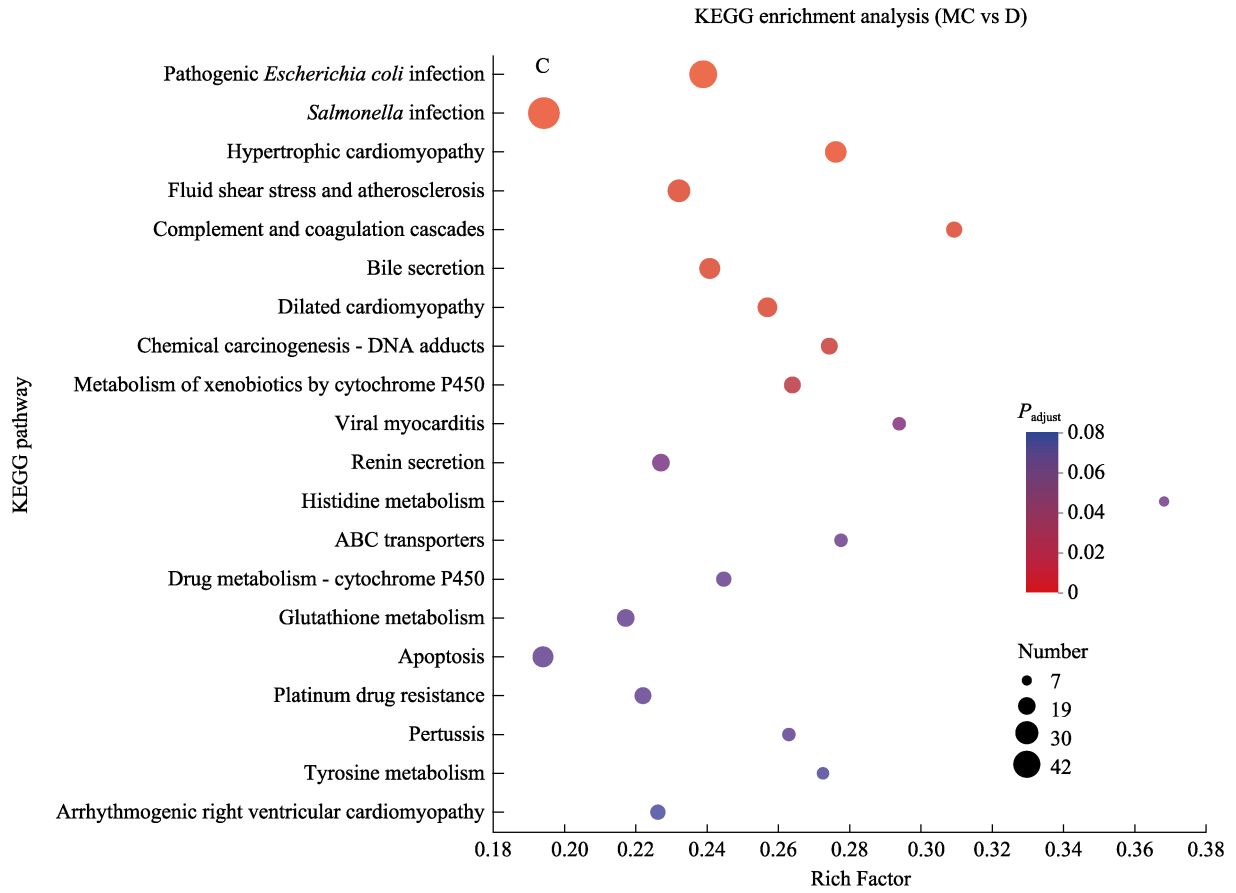
3 讨论

3.1 抗氧化性能

氧化应激是微塑料在生物体中诱导应激和毒性的主要机制(Magni *et al*, 2018)。在微塑料胁迫下, 中华绒螯蟹体内会富集微塑料, 增加活性氧(ROS)水平, 进而激活细胞抗氧化防御系统以抵御氧化应激(Sarasamma *et al*, 2020)。Cu²⁺暴露会诱发水生动物的氧化应激, 例如, 暴露于 Cu²⁺会增强凡纳对虾(Qian *et al*, 2020)和蓝蟹(*Callinectes sapidus*)(Brouwer *et al*, 1998)肝胰腺中 SOD 和 GSH-Px 等抗氧化酶的活性。

KEGG 富集分析显示, 有 25、27 和 15 个与谷胱甘肽代谢通路相关的 DEGs 分别在 M vs D、C vs D 和 MC vs D 中富集。谷胱甘肽代谢是抗氧化应激的重要抗氧化系统之一, 在调节氧化应激方面起着重要作用(Wu *et al*, 2004)。谷胱甘肽过氧化物酶基因(*gsh-px*)编码 GSH-Px 酶, 可催化谷胱甘肽(GSH)和过氧化氢(H₂O₂)生成谷胱甘肽二硫醚(GS-SG)和水(H₂O), 并防止氧化应激对细胞的不利影响(Liang *et al*, 2022)。*g6pd* 的主要功能是产生 NADPH, NADPH 是对抗氧





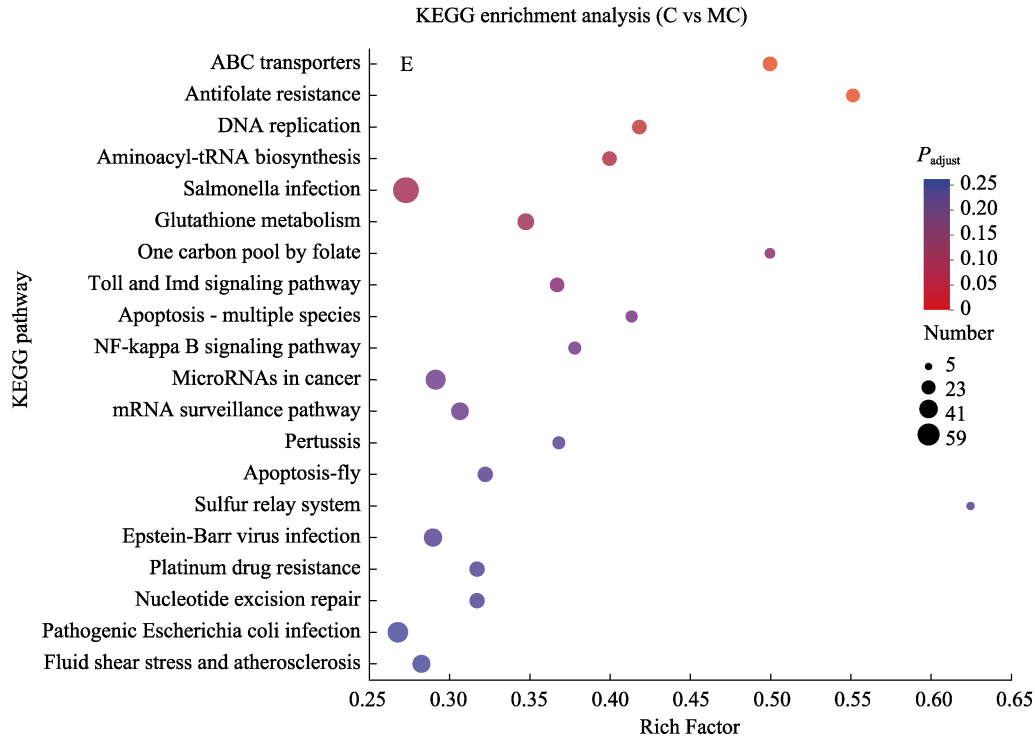


图 3 DEGs 的 KEGG 富集分析(富集排名前 20)
Fig.3 KEGG enrichment analysis of DEGs (top20)

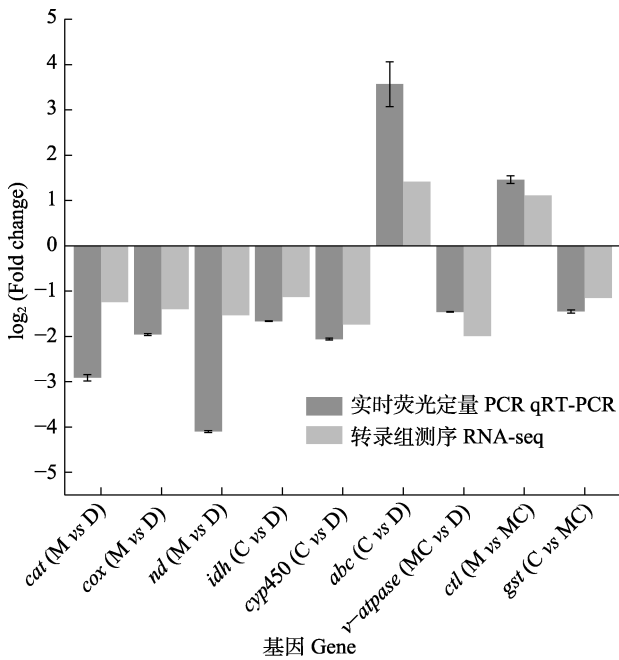


图 4 差异表达基因的 qRT-PCR 及转录组的比较分析
Fig.4 Comparison of DEGs by qRT-PCR and transcriptome analysis

化剂的关键电子供体, *gst* 可保护细胞免受氧化应激和细胞死亡等影响(Saetan *et al*, 2020)。 *g6pd* 在 M vs D、MC vs D 中显著下调, *gsh-px* 和 *gst* 在 M vs D、C vs D 和 MC vs D 中均显著下调, *gsh-px* 在 M vs MC、

C vs MC 中均显著上调。这可能表明无论是 MPs 还是 Cu^{2+} 单一或联合暴露均对谷胱甘肽代谢通路产生了抑制, 而 MPs 与 Cu^{2+} 联合暴露对 *gsh-px* 的抑制作用更强。

CAT 通常在需氧生物的组织细胞中去除多余的 H_2O_2 , 从而减轻氧化损伤(Olsvik *et al*, 2005; Wang *et al*, 2018)。在本研究中, M 组、C 组和 MC 组的 *cat* 表现出显著降低的水平, 可能导致过量的 ROS 产生。ROS 诱导脂质过氧化, 破坏细胞成分和线粒体功能, 降低 ATP 水平, 从而激活线粒体和内质网途径介导的细胞凋亡(Zhang *et al*, 2023)。MPs 与 Cu^{2+} 的单一和联合暴露均导致了中华绒螯蟹抗氧化防御系统的失衡。这与河蚬(*Corbicula fluminea*)应对微囊藻毒素和微塑料胁迫时的情况相似(Zhang *et al*, 2023)。

3.2 免疫防御

肠道是各种有毒物质进入水生动物体内的重要途径(Maharajan *et al*, 2016)。MPs 和 Cu^{2+} 共同暴露会诱发肠道杯状细胞病变, 进而诱导免疫反应(Zhang *et al*, 2022)。CYP450 是主要参与药物、内固醇、环境污染物等外源性物质和内生性物质代谢的酶系, 其中约有三分之一的 CYP450 参与外源性物质代谢过程, 可用于评估环境污染(Guengerich *et al*, 2013; Zhang *et al*, 2012)。KEGG 富集分析结果表明, 药物

代谢-细胞色素 P450 通路以及细胞色素 P450 对异生素的代谢通路在 M vs D、MC vs D 中均显著富集。CYP450 通路中编码 UGT 的基因在 M vs D、C vs D 中显著上调, MC vs D 中显著下调, 而在 M vs MC、C vs MC 中显著上调。推测可能是 MPs 与 Cu^{2+} 联合暴露比单一暴露对 *ugt* 的抑制作用更强, *ugt* 基因表达下调。编码 GST 的基因在 M vs D、C vs D、MC vs D 中均显著下调, 说明 *gst* 基因表达在 3 个实验组中均受到抑制。CYP450 属于生物转化的 I 相解毒酶, 而 GST 和 UGT 属于 II 相解毒酶(陈璐等, 2024)。异生素的吸收、代谢和细胞排泄过程可分为 4 个阶段, GST 和 UGT 通过与药物代谢酶(DMEs)介导的生物转化第一阶段的代谢产物结合, 防止共轭反应, 从而发挥解毒作用(Estevés *et al.*, 2021)。鱼类通过葡萄糖醛酸化途径代谢外源性物质, 从而保护自身(Wang *et al.*, 2014)。在小鼠的暴露实验中, 胁迫后的 GST 表达显著上调(Zhang *et al.*, 2012)。本研究中, *cyp450* 在 M vs D、C vs D 和 MC vs D 中均显著下调。*cyp450* 在水生生物的免疫毒性反应中发挥重要作用, 许多环境污染物可诱导 *cyp450* 的异常调节(Burkina *et al.*, 2015)。暴露于微塑料和菲(phenanthrene)单一及联合胁迫 24 d 后, 斑马鱼鳃中 *cyp450* 基因的表达显著上调(Xu *et al.*, 2021)。

凝血系统是一种主要的血源性蛋白水解级联反应机制。凝血级联激活后, 由丝氨酸蛋白酶介导的一系列序列裂解事件随之展开。凝血酶从其凝血酶原中被激活, 催化纤维蛋白的聚合, 将可溶性纤维蛋白原转化为不溶性纤维蛋白, 从而形成血凝块(Felisbino *et al.*, 2021)。KEGG 富集分析结果表明, 补体和凝血级联通路在 M vs D、MC vs D 中显著富集, 凝血因子 B 基因在 M vs D、C vs D 和 MC vs D 中均显著下调, 凝血因子 G 基因在 M vs D、MC vs D 中显著下调, 在 C vs D、C vs MC 中显著上调。推测 MPs 单一暴露、MPs 与 Cu^{2+} 联合暴露对凝血因子 G 的抑制作用比 Cu^{2+} 单一暴露更强。凝血因子 B 和 G 在甲壳类动物的凝血系统中发挥关键作用, 当病原体入侵时, 甲壳类动物通过凝血系统的级联反应产生血凝块, 从而提高免疫防御能力(Shen *et al.*, 2023)。

综上, 肠道作为免疫系统的第一道防线, 免疫防御相关通路及基因(*cyp450*、*ugt*、*gst*、凝血因子 B 和 G 等)表达发生变化, 可能与肠道屏障修复、炎症反应调节等相关机制有关(Dalmo *et al.*, 1997; Günther *et al.*, 2018)。MPs 和 Cu^{2+} 单一或联合暴露均抑制了中华绒螯蟹的免疫防御系统。

3.3 能量代谢

水生动物摄食的微塑料会在肠道中停留, 不仅会阻碍食物的正常吸收, 还会导致能量和脂质代谢失调, 并通过调节能量消耗的细胞通路来维持细胞能量稳态(Cedervall *et al.*, 2012; Dong *et al.*, 2016; Jost *et al.*, 2015; Sarasamma *et al.*, 2020)。肠道是水生动物重要的消化器官, MPs 和 Cu^{2+} 共同暴露会改变肠道菌群的结构, 导致代谢紊乱和疾病发生(Rong *et al.*, 2024; Zhang *et al.*, 2022)。氧化磷酸化是生物体中能量代谢的重要过程, 在线粒体内膜上进行, 通过产生线粒体膜电位, 合成 ATP 并驱动线粒体蛋白和代谢物的转运, 将氧化(放能)和磷酸化(储能)偶联在一起(刘思琪, 2020)。KEGG 富集分析结果显示, 在 M vs D 中, 41 个与氧化磷酸化通路相关的 DEGs 全部显著下调, 而在 C vs D 和 MC vs D 中, 均只有 7 个与氧化磷酸化通路相关的 DEGs 富集。*cox* 在 M vs D 和 MC vs D 中表达下调, 在 C vs D 中表达上调。*atpase* 仅在 M vs D 中表达显著下调。*cox* 编码线粒体呼吸链中的终端酶——细胞色素氧化酶, 其将电子从细胞色素转移到分子氧, 形成线粒体内膜上的电子梯度, 决定线粒体含量和活性(Galati *et al.*, 2009)。*cox* 表达降低可能对线粒体呼吸链活动和细胞稳态产生重大影响(Zhang *et al.*, 2023)。*atpase* 是编码 ATP 合酶的基因, 该酶通过磷酸化过程合成 ATP, 供给机体能量。破坏氧化磷酸化途径可能会降低线粒体膜电位并增加 ROS 的产生, 线粒体功能异常会减少 ATP 的产生(Fan *et al.*, 2019)。

GO 分析表明, 与能量代谢相关的 ATP 代谢过程、碳水化合物代谢过程、草酸代谢过程等在 M vs D 中显著富集。而在 C vs D 和 MC vs D 中, 与能量代谢相关的显著富集通路较少。研究结果表明, MPs 暴露对氧化磷酸化通路具有抑制作用, 并显著影响能量代谢系统, 而 Cu^{2+} 暴露及 MPs 与 Cu^{2+} 联合暴露对能量代谢系统的影响较小。

4 结论

MPs 与 Cu^{2+} 单一及联合暴露对中华绒螯蟹肠道组织的影响研究表明, 在 M、C 和 MC 组中, 肠道组织的谷胱甘肽代谢通路、免疫防御相关通路及基因(*cyp450*、*ugt*、凝血因子 B 和 G 等)均受到抑制。在 M vs D 中, 氧化磷酸化通路中富集的 DEGs 达 41 个, MPs 暴露对该通路的抑制作用强于 Cu^{2+} 暴露及 MPs 与 Cu^{2+} 共同暴露。研究结果揭示了 MPs 和 Cu^{2+} 对中华绒螯蟹抗氧化、免疫防御和能量代谢的影响机制, 为中华绒螯蟹养殖和环境毒性研究提供了理论参考。

参 考 文 献

- ASHBURNER M, BALL C A, BLAKE J A, *et al.* Gene ontology: Tool for the unification of biology. *Nature Genetics*, 2000, 25(1): 25–29
- BROUWER M, BROUWER T H. Biochemical defense mechanisms against copper-induced oxidative damage in the blue crab, *Callinectes sapidus*. *Archives of Biochemistry and Biophysics*, 1998, 351(2): 257–264
- BURKINA V, ZLABEK V, ZAMARATSKAIA G, *et al.* Effects of pharmaceuticals present in aquatic environment on Phase I metabolism in fish. *Environmental Toxicology and Pharmacology*, 2015, 40(2): 430–444
- CEDERVALL T, HANSSON L A, LARD M, *et al.* Food chain transport of nanoparticles affects behaviour and fat metabolism in fish. *PLoS One*, 2012, 7(2): e32254
- CHEN D, ZHANG M. Non-volatile taste active compounds in the meat of Chinese mitten crab (*Eriocheir sinensis*). *Food Chemistry*, 2007, 104(3): 1200–1205
- CHEN L, YANG Z, XU J H, *et al.* Effect of diflubenzuron exposure on gene expression in *Cyprinus carpio* liver based on transcriptomics. *Progress in Fishery Sciences*, 2024, 45(3): 129–139 [陈璐, 杨臻, 徐锦华, 等. 基于转录组学探究除虫脲暴露对鲤肝脏基因表达的影响. *渔业科学进展*, 2024, 45(3): 129–139]
- CHEN S, ZHOU Y, CHEN Y, *et al.* fastp: An ultra-fast all-in-one fastq preprocessor. *Bioinformatics*, 2018, 34(17): i884–i890
- DALMO R A, INGEBRIGTSEN K, BØGWALD J. Non-specific defence mechanisms in fish, with particular reference to the reticuloendothelial system (RES). *Journal of Fish Diseases*, 1997, 20(4): 241–273
- DONG Y, ZHANG S, WOODS A. Ecological relevance of energy metabolism: Transcriptional responses in energy sensing and expenditure to thermal and osmotic stresses in an intertidal limpet. *Functional Ecology*, 2016, 30(9): 1539–1548
- DU Y C, REN J Y, TENG J, *et al.* Combined effects of elevated temperature and polystyrene microplastics on hemocyte function, immune-related gene expression, and energy metabolism of *Crassostrea gigas*. *Progress in Fishery Sciences*, 2024, 45(1): 161–171 [杜蕴超, 任晶莹, 滕佳, 等. 升温与聚苯乙烯微塑料复合暴露对长牡蛎血细胞功能、免疫基因表达和能量代谢的影响. *渔业科学进展*, 2024, 45(1): 161–171]
- ESTEVEZ F, RUEFF J, KRANENDONK M. The central role of cytochrome P450 in xenobiotic metabolism—a brief review on a fascinating enzyme family. *Journal of Xenobiotics*, 2021, 11(3): 94–114
- FELISBINO M B, ZIEMANN M, KHURANA I, *et al.* Valproic acid influences the expression of genes implicated with hyperglycaemia-induced complement and coagulation pathways. *Scientific Reports*, 2021; 11: 2163
- FAN Y, YANG Q, YANG Y, *et al.* Sirt6 suppresses high glucose-induced mitochondrial dysfunction and apoptosis in podocytes through AMPK activation. *International Journal of Biological Sciences*, 2019, 15(3): 701–713
- GALATI D, SRINIVASAN S, RAZA H, *et al.* Role of nuclear-encoded subunit Vb in the assembly and stability of cytochrome *c* oxidase complex: Implications in mitochondrial dysfunction and ROS production. *Biochemical Journal*, 2009, 420(3): 439–449
- GUENGERICH F P. Cytochrome P450 activation of toxins and hepatotoxicity. *Drug-induced liver disease*. Academic Press, 2013: 15–33
- GÜNTHER J, SEYFERT H M. The first line of defence: Insights into mechanisms and relevance of phagocytosis in epithelial cells. *Seminars in Immunopathology*, 2018, 40(6): 555–565
- HE J, SHI D, WANG B B, *et al.* Ecological risk assessment and water quality standard evaluation of 10 typical metals in eight basins in China. *China Environmental Science*, 2019, 39(7): 2970–2982 [何佳, 时迪, 王贝贝, 等. 10 种典型重金属在八大流域的生态风险及水质标准评价. *中国环境科学*, 2019, 39(7): 2970–2982]
- JOST A, KESHWANI S S, ABOU-HANNA J J. Activation of AMP-activated protein kinase in response to temperature elevation shows seasonal variation in the zebra mussel, *Dreissena polymorpha*. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, 2015, 182: 75–83
- KANEHISA M, GOTO S, KAWASHIMA S, *et al.* The KEGG resource for deciphering the genome. *Nucleic Acids Research*, 2004, 32(suppl_1): D277–D280
- LAUER M M, DE OLIVEIRA C B, YANO N L I, *et al.* Copper effects on key metabolic enzymes and mitochondrial membrane potential in gills of the estuarine crab *Neohelice granulata* at different salinities. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, 2012, 156(3/4): 140–147
- LEBRETON L C M, VAN D Z J, DAMSTEEG J W, *et al.* River plastic emissions to the world's oceans. *Nature Communications*, 2017, 8(1): 15611
- LI B, LIANG W, LIU Q, *et al.* Fish ingest microplastics unintentionally. *Environmental Science and Technology*, 2021, 55(15): 10471–10479
- LIANG Y S, WU R X, NIU S F, *et al.* Liver transcriptome analysis reveals changes in energy metabolism, oxidative stress, and apoptosis in pearl gentian grouper exposed to acute hypoxia. *Aquaculture*, 2022, 561: 738635
- LIU S Q. Deficiency in oxidative phosphorylation activates adaptive responses to maintain mitochondrial membrane potential. Doctoral Dissertation of Peking Union Medical College, Chinese Academy of Medical Sciences, 2020 [刘思琪. 氧化磷酸化缺陷激活适应性反应维持线粒体膜电位. 中国医学科学院北京协和医学院博士研究生学位论文, 2020]
- LIU Z, YU P, CAI M, *et al.* Effects of microplastics on the innate immunity and intestinal microflora of juvenile

- Eriocheir sinensis*. Science of the Total Environment, 2019, 685: 836–846
- MAGARA G, ELIA A C, SYBERG K, *et al.* Single contaminant and combined exposures of polyethylene microplastics and fluoranthene: Accumulation and oxidative stress response in the blue mussel, *Mytilus edulis*. Journal of Toxicology and Environmental Health, Part A, 2018, 81(16): 761–773
- MAGNI S, GAGNE F, ANDRE C, *et al.* Evaluation of uptake and chronic toxicity of virgin polystyrene microbeads in freshwater zebra mussel *Dreissena polymorpha* (Mollusca: Bivalvia). Science of the Total Environment, 2018, 631: 778–788
- MAHARAJAN A, KITTO M R, PARURUCKUMANI P S, *et al.* 2016. Histopathology biomarker responses in Asian sea bass, *Lates calcarifer* (Bloch) exposed to copper. Journal of Basic and Applied Zoology, 2016, 77: 21–30
- OLSVIK P A, KRISTENSEN T, WAAGBO R, *et al.* mRNA expression of antioxidant enzymes (SOD, CAT and GSH-Px) and lipid peroxidative stress in liver of Atlantic salmon (*Salmo salar*) exposed to hyperoxic water during smoltification. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 2005, 141(3): 314–323
- PAILA R V, YALLAPRAGADA P R, THATIPAKA S D R. Response of glutathione system and carotenoids to sublethal copper in the postlarvae of *Penaeus indicus*. Ecotoxicology and Environmental Safety, 2012, 75: 127–133
- PAN Y, QIAN J, MA X, *et al.* Response of moulting genes and gut microbiome to nano-plastics and copper in juvenile horseshoe crab *Tachypleus tridentatus*. Marine Environmental Research, 2023, 191: 106128
- PENG G, ZHU B, YANG D, *et al.* Microplastics in sediments of the Changjiang estuary, China. Environmental Pollution, 2017, 225: 283–290
- QIAN D, XU C, CHEN C, *et al.* Toxic effect of chronic waterborne copper exposure on growth, immunity, anti-oxidative capacity and gut microbiota of Pacific white shrimp *Litopenaeus vannamei*. Fish and Shellfish Immunology, 2020, 100: 445–455
- REN F, JIANG H, SUN J, *et al.* Cloning, characterization, expression, and copper sensitivity of the metallothionein-1 gene in the Chinese mitten crab, *Eriocheir sinensis*. Molecular Biology Reports, 2011, 38: 2383–2393
- RONG J, YUAN C, YIN X, *et al.* Co-exposure of polystyrene nanoplastics and copper induces development toxicity and intestinal mitochondrial dysfunction *in vivo* and *in vitro*. Science of the Total Environment, 2024, 930: 172681
- SAETAN W, TIAN C, YU J, *et al.* Comparative transcriptome analysis of gill tissue in response to hypoxia in silver sillago (*Sillago sihama*). Animals, 2020, 10(4): 628
- SARASAMMA S, AUDIRA G, SIREGAR P, *et al.* Nanoplastics cause neurobehavioral impairments, reproductive and oxidative damages, and biomarker responses in zebrafish: throwing up alarms of wide spread health risk of exposure. International Journal of Molecular Sciences, 2020, 21(4): 1410
- SHEN C, FENG G, ZHAO F, *et al.* Integration of transcriptomics and proteomics analysis reveals the molecular mechanism of *Eriocheir sinensis* gills exposed to heat stress. Antioxidants, 2023, 12(12): 2020
- TAVARES-DIAS M. Toxic, physiological, histomorphological, growth performance and antiparasitic effects of copper sulphate in fish aquaculture. Aquaculture, 2021, 535: 736350
- TIAN L, CHEN Q, JIANG W, *et al.* A carbon-14 radiotracer-based study on the phototransformation of polystyrene nanoplastics in water versus in air. Environmental Science: Nano, 2019, 6(9): 2907–2917
- TRUCHET D M, BUZZI N S, MOULATLET G M, *et al.* Macroecotoxicological approaches to emerging patterns of microplastic bioaccumulation in crabs from estuarine and marine environments. Science of the Total Environment, 2023, 870: 161912
- VO H C, PHAM M H. Ecotoxicological effects of microplastics on aquatic organisms: A review. Environmental Science and Pollution Research, 2021, 28: 44716–44725
- WANG Y, HUANG H, WU Q. Characterization of the zebrafish *Ugt* repertoire reveals a new class of drug-metabolizing *UDP* glucuronosyltransferases. Molecular Pharmacology, 2014, 86(1): 62–75
- WANG Y, ZHAO H, GUO M, *et al.* Arsenite renal apoptotic effects in chickens co-aggravated by oxidative stress and inflammatory response. Metallomics, 2018, 10(12): 1805–1813
- WEI W, YANG Q, XIANG D, *et al.* Combined impacts of microplastics and cadmium on the liver function, immune response, and intestinal microbiota of crucian carp (*Carassius carassius*). Ecotoxicology and Environmental Safety, 2023, 261: 115104
- WU G, LUPTON J R, TURNER N D, *et al.* Glutathione metabolism and its implications for health. Journal of Nutrition, 2004, 134(3): 489–492
- XING Y F, ZHU X Y, HUANG J H, *et al.* Toxic effects of microplastics and nitrite exposure on intestinal histology, digestion, immunity, and microbial community of shrimp *Litopenaeus vannamei*. Marine Pollution Bulletin, 2024, 200: 116077
- XU K, ZHANG Y, HUANG Y, *et al.* Toxicological effects of microplastics and phenanthrene to zebrafish (*Danio rerio*). Science of the Total Environment, 2021, 757: 143730
- XUE Y, FENG L, XU Z, *et al.* The time-dependent variations of zebrafish intestine and gill after polyethylene microplastics exposure. Ecotoxicology, 2021, 30: 1997–2010
- YANG J Y, CHEN J H, ZHONG J S, *et al.* Species composition and diversity of fish larvae and juveniles in the water area of Chongming Dongtan, Yangtze River estuary. Journal of Shanghai Ocean University, 2023, 32(4): 829–840 [杨钧渊, 陈锦辉, 钟俊生, 等. 长江口崇明东滩水域仔稚鱼种类组

- 成及多样性. 上海海洋大学学报, 2023, 32(4): 829–840]
- YANG Z B, ZHAO Y L, LI N, *et al.* Effect of waterborne copper on the microstructures of gill and hepatopancreas in *Eriocheir sinensis* and its induction of metallothionein synthesis. *Archives of Environmental Contamination and Toxicology*, 2007, 52: 222–228
- YANG Z B, ZHAO Y L, ZHOU Z L, *et al.* Effects of water-borne copper on activities of metabolic enzymes in *Eriocheir sinensis*. *Oceanologia et Limnologia Sinica*, 2006, 37(2): 118–124 [杨志彪, 赵云龙, 周忠良, 等. 水体铜对中华绒螯蟹(*Eriocheir sinensis*)代谢酶活力的影响. 海洋与湖沼, 2006, 37(2): 118–124]
- YANG Z, ZHU L, LIU J, *et al.* Polystyrene microplastics increase Pb bioaccumulation and health damage in the Chinese mitten crab *Eriocheir sinensis*. *Science of the Total Environment*, 2022, 829: 154586
- YU P, LIU Z, WU D, *et al.* Accumulation of polystyrene microplastics in juvenile *Eriocheir sinensis* and oxidative stress effects in the liver. *Aquatic Toxicology*, 2018, 200: 28–36
- YU X, XU L L, LIU Q, *et al.* Abundance, shape, and chemical composition of microplastics within ten common marine species in Xiangshan Bay. *Progress in Fishery Sciences*, 2021, 42(4): 9–18 [于翔, 许莉莉, 刘强, 等. 象山湾常见海洋生物体内微塑料的丰度、形态和成分组成研究. 渔业科学进展, 2021, 42(4): 9–18]
- ZHANG B H, DING Z G, LI H Q, *et al.* Algicidal activity of *Streptomyces eurocidicus* JXJ-0089 metabolites and their effects on *Microcystis* physiology. *Applied and Environmental Microbiology*, 2016, 82(17): 5132–5143
- ZHANG C, YE L, WANG C, *et al.* Toxic effect of combined exposure of microplastics and copper on goldfish (*Carassius auratus*): Insight from oxidative stress, inflammation, apoptosis and autophagy in hepatopancreas and intestine. *Bulletin of Environmental Contamination and Toxicology*, 2022, 109(6): 1029–1036
- ZHANG F Y, WANG R X, YANG G, *et al.* Application of environmental DNA technology in monitoring the broodstock resources of *Eriocheir sinensis* in the Yangtze River estuary. *Acta Hydrobiologica Sinica*, 2024, 48(6): 950–957 [张方圆, 王汝贤, 杨刚, 等. 环境 DNA 技术在长江口中华绒螯蟹亲蟹资源监测中的应用. 水生生物学报, 2024, 48(6): 950–957]
- ZHANG F, LI D, YANG Y, *et al.* Combined effects of polystyrene microplastics and copper on antioxidant capacity, immune response and intestinal microbiota of Nile tilapia (*Oreochromis niloticus*). *Science of the Total Environment*, 2022, 808: 152099
- ZHANG J, JIE W, CHENG G, *et al.* Transcriptome analysis of response mechanism to microcystin-LR and microplastics stress in Asian clam (*Corbicula fluminea*). *Fish and Shellfish Immunology*, 2023, 139: 108875
- ZHANG Y, ZHANG X, WU B, *et al.* Evaluating the transcriptomic and metabolic profile of mice exposed to source drinking water. *Environmental Science and Technology*, 2012, 46(1): 78–83
- ZHANG Z, DENG C, DONG L, *et al.* Microplastic pollution in the Yangtze River Basin: Heterogeneity of abundances and characteristics in different environments. *Environmental Pollution*, 2021, 287: 117580
- ZHAO S, ZHU L, WANG T, *et al.* Suspended microplastics in the surface water of the Yangtze estuary system, China: First observations on occurrence, distribution. *Marine Pollution Bulletin*, 2014, 86(1/2): 562–568

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Microplastic and Copper Exposure Effects on Intestinal Gene Expression in *Eriocheir sinensis*: A Transcriptomics-Based Investigation

XU Jian^{1,2}, FENG Guangpeng^{1,2①}, YAN Yunzhi¹, CHEN Jinhui³, WU Jianhui³, YANG Zuchang²

(1. School of Ecology and Environment, Anhui Normal University, Wuhu 241002, China;

2. Shanghai Yangtze Estuary Fishery Resources Proliferation and Ecological Restoration Engineering Technology Research Center, East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shanghai 200090, China;

3. Joint Laboratory for Monitoring and Conservation of Aquatic Living Resources in the Yangtze Estuary, Shanghai Aquatic Wildlife Conservation and Research Center, Shanghai 200092, China)

Abstract Over 80% of marine litter is composed of plastic waste, which reaches the oceans via atmospheric transport, surface runoff, and human activities such as shipping and fishing. These plastics

① Corresponding author: FENG Guangpeng, Email: coolwindfgp@163.com

degrade into microplastics (MPs)—prevalent environmental pollutants <5 mm, which possess a “carrier effect”, enabling them to adsorb contaminants such as heavy metals. CuSO_4 —frequently used in aquaculture to manage diseases and cyanobacterial blooms—can introduce excess Cu^{2+} into aquatic environments, adversely affecting water quality and aquatic life. The Chinese mitten crab (*Eriocheir sinensis*) is vital to freshwater aquaculture in China. Mature *E. sinensis* migrate to the Yangtze River estuary annually for reproduction; however, estuarine ecosystems are becoming increasingly disturbed, making mitten crabs vulnerable to MPs and heavy metal contamination. The intestinal tract, which directly interacts with ingested pollutants, is particularly susceptible. Previous studies have examined MP or Cu^{2+} effects on *E. sinensis* independently, however, their combined effects remain underexplored. Here, MPs (0.4 mg/L) and Cu^{2+} (0.1 mg/L) were selected as the experimental concentrations with four treatment groups: Group M (0.4 mg/L MP-exposed); Group C (0.1 mg/L Cu^{2+} -exposed); Group MC (0.4 mg/L MP + 0.1 mg/L Cu^{2+} combined-exposed); and Group D (blank control group) to investigate the effects of MPs and Cu^{2+} on the intestinal tract of *E. sinensis* after 21 days of single and combined exposure. Transcriptome sequencing of the intestinal tissues was conducted using Illumina's high-throughput platform, generating 197,908,972 raw reads. Post-quality filtering yielded clean reads across the four groups, with 94.71%–95.48% Q30 scores. In total, 109,644 transcripts were identified, with 68,005 exceeding 1800 bp in length. Differential expression analysis revealed 1,650 and 1,874, 3,797 and 1,073, and 1,492 and 1,305 upregulated and downregulated differentially expressed genes (DEGs) in Groups M, C, MC, respectively. Notably, DEGs associated with antioxidant defense, immune response, and energy metabolism differed significantly among the comparison groups. Catalase (*cat*) and peroxiredoxin (*prdx*) were downregulated, whereas trim, toll-like receptor (*tlr*) and complement component 1 (*c1*) were upregulated in the immune system. Cytochrome P450 (*cyp450*) was significantly downregulated across the treatment groups. Energy metabolism DEGs also varied, with carbonic anhydrase (*ca*) up-regulated and vesicular adenosine triphosphatase (*v-atpase*) down-regulated. KEGG pathway enrichment analysis indicated that in Groups M vs D, 1,594 DEGs in the intestine were mapped to 333 pathways. Among these, 34 pathways were significantly enriched, including oxidative phosphorylation and glutathione metabolism. In Groups C vs D, 2,445 DEGs in the intestine were mapped to 340 pathways. Among these, nine pathways were significantly enriched, primarily involving DNA replication and ABC transporters. In Groups MC vs D, 1,198 DEGs in the intestine were mapped to 326 pathways. Among these, 14 pathways were significantly enriched, including the complement and coagulation cascades and metabolism of xenobiotics by cytochrome P450. The DEGs were predominantly enriched in pathways related to oxidative phosphorylation, glutathione metabolism, xenobiotic metabolism by cytochrome P450, and ABC transporters. These findings indicate that both individual and combined exposure to MPs and Cu^{2+} disrupts the antioxidant, immune, and energy metabolic systems of *E. sinensis*. The glutathione metabolic pathway was particularly inhibited in Groups M, C, and MC. MPs and Cu^{2+} may affect the expression of *cyp450* and related genes (*ugt*), potentially compromising the immune function of *E. sinensis*. Notably, *ugt* was significantly upregulated in Groups M vs D and downregulated in Groups MC vs D. The number of DEGs linked to the oxidative phosphorylation pathway varied across comparisons, with 41 DEGs in Groups M vs D and seven in Groups C vs D and MC vs D being enriched in this pathway. MPs may affect the oxidative phosphorylation pathway by inhibiting the expression of *cox* and *atpase* genes. In contrast, exposure to Cu^{2+} alone and co-exposure to MPs had comparatively smaller impacts on this pathway. The mechanisms underlying the stress response of *E. sinensis* to MPs and Cu^{2+} exposure were further elucidated in this study. The results of this study revealed the mechanisms of MPs and Cu^{2+} 's effects on the antioxidant, immune defence, and energy metabolism of *Eriocheir sinensis*, which provides an important theoretical basis for the study of *E. sinensis* culture and environmental toxicity.

Key words *Eriocheir sinensis*; Microplastics; Copper; Transcriptome; Energy metabolism; Immunity