·论著·

基于生物信息学的心肌缺血/再灌注损伤与坏死性凋亡共表达基因分析

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摘要 目的 基于生物信息学分析心肌缺血/再灌注损伤(MI/RI)与坏死性凋亡共表达基因并验证。**方法** 基因表达图谱(GEO)数据库下载MI/RI表达谱数据(GSE67308和GSE19875),对GSE67308表达谱进行差异表达分析,筛选差异表达基因(DEGs)并进行基因集富分析和生物信号通路分析。对GSE67308表达谱数据进行免疫细胞浸润分析。利用分子标记数据库(MSigDB)和京都基因与基因组数据库(KEGG)检索坏死性凋亡相关基因,将DEGs与其交叉构建蛋白质-蛋白质相互作用(PPI)网络确定关键基因。通过单细胞测序分析平台分析关键基因在心脏各细胞类型中的表达情况,同时使用GSE19875数据集对关键基因表达进行验证。**结果** 共鉴定出1 054个DEGs,其中363个上调,691个下调。基因富集分析显示,DEGs功能主要与凋亡过程、免疫反应、细胞内信号转导调节相关; 生物信号通路分析显示,DEGs主要参与TNF、NF-κB等信号通路的调控。免疫浸润分析结果表明,MI/RI心肌组织中自然杀伤细胞、单核细胞等免疫浸润程度高。PPI网络分析显示*Il1b*、*TNF*、*Birc3*、*Ripk1*是坏死性凋亡的关键基因。单细胞测序分析表明,白细胞中关键基因表达升高。GSE19875数据集中与对照组比较,MI/RI模型组中*Il1b*、*TNF*、*Birc3*、*Ripk1*表达显著升高($P < 0.01$)。**结论** MI/RI和参与调控坏死性凋亡的TNF信号通路、NF-κB信号通路高度相关;*Il1b*、*TNF*、*Birc3*、*Ripk1*是同时参与调节MI/RI和坏死性凋亡的关键基因;IL-1b、TNf、Birc3、Ripk1可能作为坏死性凋亡关键调节因子参与MI/RI过程。

关键词 心肌缺血/再灌注损伤; 坏死性凋亡; 共表达基因; 生物信息学
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Analysis of co-expressed genes in myocardial ischemia-reperfusion injury and necrotic apoptosis utilizing bioinformatics

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Abstract Objective To identify and validate co-expressed genes associated with myocardial ischemia/reperfusion injury (MI/RI) and necrotic apoptosis by bioinformatics analysis. **Methods** Gene expression profile data for MI/RI were obtained by GSE67308 and GSE19875 datasets from the Gene Expression Omnibus (GEO) database. Differential expression analysis was conducted on the GSE67308 dataset to identify differentially expressed genes (DEGs), followed by gene set enrichment analysis and biological pathway analysis. Moreover, immune cell infiltration analysis was performed on the GSE67308 dataset. Necrotic apoptosis-related genes were retrieved from the Molecular Signatures Database and the Kyoto Encyclopedia of Genes and Genomes (KEGG). A protein-protein interaction (PPI) network was constructed by overlapping DEGs with these necrotic apoptosis-related genes to identify key genes. Furthermore, the expression patterns of these key genes across various cardiac cell types were analyzed using a single-cell sequencing analysis platform, and validation of key gene expression was performed using the GSE19875 dataset. **Results** A total of 1 054 DEGs were identified, comprising 363 upregulated and 691 downregulated genes. Gene enrichment analysis revealed that DEGs were primarily associated with processes related to apoptosis, immune responses, and intracellular signaling regulation. Moreover, biological pathway analysis demonstrated that DEGs were predominantly involved in the regulation of signaling pathways such as tumor necrosis factor (TNF) and NF-κB. Immune infiltration analysis indicated a high degree of immune infiltration, particularly with natural killer cells and monocytes, in MI/RI myocardial tissue. PPI

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network analysis identified *Il1b*, *TNF*, *Birc3*, and *Ripk1* as crucial genes in the context of necrotic apoptosis. Single-cell sequencing analysis showed the elevated expression of key genes within white blood cells. In comparison to the control group, the MI/RI model group in the GSE19875 dataset exhibited significantly increased expression of *Il1b*, *TNF*, *Birc3*, and *Ripk1* ($P < 0.01$). **Conclusion** MI/RI is strongly correlated with the TNF signaling pathway and the NF-κB signaling pathway, both of which play pivotal roles in regulating necrotic apoptosis. *Il1b*, *TNF*, *Birc3*, and *Ripk1* emerge as key genes that concurrently regulate both MI/RI and necrotic apoptosis. It is plausible that IL-1b, TNF, Birc3, and Ripk1 may serve as critical regulatory factors in the context of necrotic apoptosis during MI/RI.

Keywords myocardial ischemia/reperfusion injury; necrotic apoptosis; co-expressed gene; bioinformatics

缺血性心脏病作为最常见的心血管疾病之一，高发生率和高致死率是其主要特点。对于心肌缺血患者来说及时恢复心肌供血不仅能恢复损伤的组织功能，而且对患者预后至关重要[1]。然而，缺血心肌血液再灌注和复氧会导致心律失常、心肌细胞死亡以及内皮和微血管功能障碍等一系列心肌缺血/再灌注损伤（myocardial ischemia/reperfusion injury，MI/RI），而其他心肌组织损伤也会随MI/RI导致的氧化应激、炎症反应进一步加剧。研究[2]表明心肌梗死面积中50%组织损伤来源于MI/RI。

依据细胞的特征形态，将细胞死亡分为自噬、坏死及凋亡3种。坏死性凋亡作为新发现的程序性细胞死亡，具有坏死和凋亡双重特征，并以坏死为主要特征[3]。坏死性凋亡不依赖于半胱氨酸蛋白酶调控的蛋白质降解过程，它通过损伤细胞膜的通透性和完整性，导致损伤相关的分子模式（damage-associated molecular patterns，DAMP）和其他细胞内容物渗出，从而诱导免疫炎症反应并导致相关组织损伤进一步加剧[4]。因此，坏死性凋亡可能对MI/RI的发生发展具有调节作用。本研究基于生物信息学分析MI/RI与坏死性凋亡共表达基因并验证，旨在明确坏死性凋亡关键基因在MI/RI中潜在作用机制。

1　材料与方法

1.1　数据来源及处理

从基因表达图谱（Gene Expression Omnibus，GEO）数据库（https://www.ncbi.nlm.nih.gov/geo/）获得GSE67308数据集，数据集包含4个正常小鼠心脏组织和4个MI/RI模型小鼠心脏，使用GEO数据库提供的GEO2R分析（https://www.ncbi.nlm.nih.gov/geo/geo2r/?acc=GSE67308）对数据集进行归一化处理来筛选差异表达基因（differential expression genes，DEGs）。

1.2　基因集富集分析（gene set enrichment analysis，GSEA）

为揭示MI/RI组织和正常心脏组织之间DEGs及其与对应的生物学功能、生物信号通路的关系，对DEGs进行富集分析。分子标记数据库（molecular signatures database，MSigDB;https://www.gsea-msigdb.org/gsea/msigdb）下载分子特征数据集c5.go.bp.v7.4、c5.go.cc.v7.4、c5.go.mf.v7.4来进行功能富集分析。

1.3　京都基因与基因组数据库（Kyoto encyclopedia of genes and genomes，KEGG）信号通路富集分析

利用KEGG（https://www.kegg.jp/）中Metascape数据库（https://metascape.org/gp/index.html#/main/step1）对DEGs进行富集分析，并进行可视化处理，明确DEGs参与调控的信号通路及其内在调控关系，筛选标准为$P < 0.05$。

1.4　免疫浸润分析

利用CIBERSORT数据库（https://cibersortx.stanford.edu/）分析GSE67308数据集基因表达谱中免疫细胞丰度，以LM22数据集（收录22种免疫细胞）为对照，计算GSE67308数据集中各样本中每种免疫细胞浸润比例及细胞浸润水平。

1.5　蛋白质-蛋白质相互作用（protein-protein interaction，PPI）网络及坏死性凋亡关键基因识别

使用MSigDB（https://www.gsea-msigdb.org/gsea/msigdb）和KEGG（https://www.kegg.jp/）检索坏死性凋亡相关基因，将MI/RI DEGs与坏死性凋亡相关基因交叉，确定MI/RI DEGs与坏死性凋亡共表达基因。将共表达基因输入STRING数据库（https://cn.string-db.org/）进行可视化分析。使用Cytoscape软件中CytoHubba插件对坏死性凋亡共表达基因中关键基因进行可视化分析。

1.6　关键基因单细胞测序分析

Tabula Muris数据库（https://tabula-muris.ds.czbiohub.org/）收录了小鼠单细胞转录组数据，包含来

自20个器官和组织约100 000个细胞。设定组织类型为心脏，检索坏死性凋亡关键基因在单细胞测序结果中表达情况。

1.7　GSE19875数据集验证

GSE19875数据集（https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE19875）包含3个正常小鼠和3个MI/RI模型小鼠。GSE19875数据集作为独立验证集，来验证MI/RI中坏死性凋亡关键基因的表达情况。

2　结果

2.1　MI/RI的DEGs

结果显示，GSE67308数据集中MI/RI 的DEGs共1 054个，其中363个上调，691个下调。见图1。

2.2　GSEA结果

结果显示，DEGs生物过程包括参与凋亡过程、细胞激活、免疫反应、细胞内信号转导调节、对生物刺激的反应；细胞组分包括细胞外基质的胶原蛋白、质膜外侧、分泌颗粒膜、分泌颗粒；分子功能包括趋化因子及其受体的结合调控、细胞因子活性、细胞因子受体结合、信号受体调节器及其结合活动。见图2。

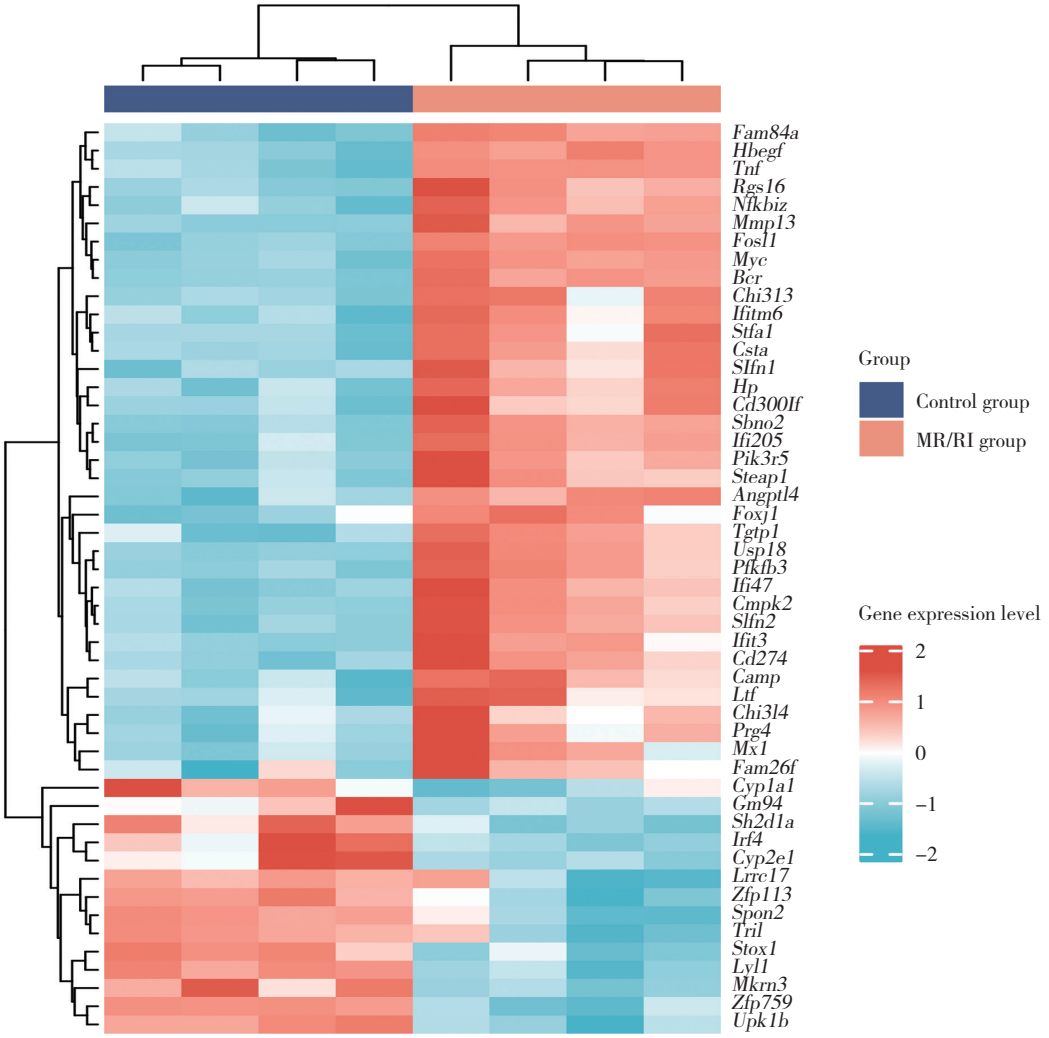
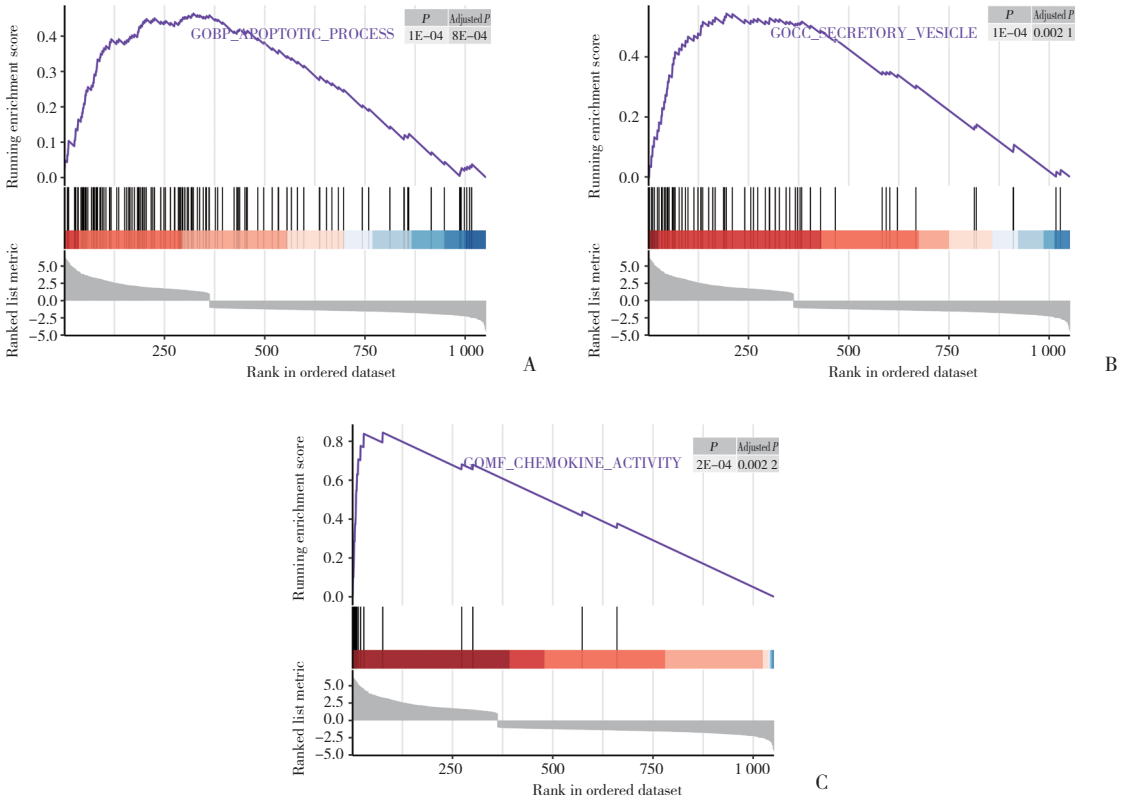


图1　MI/RI的DEGs分析热图

Fig.1　Hot map for DEGs of MI/RI

2.3　信号通路富集分析

结果显示，MI/RI的DEGs参与细胞因子产生的调节、对病原体的反应、TNF信号通路、白细胞迁移、细胞活化、对免疫反应的调节、MAPK级联反应的正调节、NF-κB信号通路、对细胞因子刺激反应等信号通路，其中通路相互作用关系较为密切的均与炎症



A, biological processes; B, cellular components; C, molecular functions.

图2　MI/RI 的DEGs 富集分析

Fig.2　Enrichment analysis of DEGs in MI/RI

反应、免疫反应相关。见图3。

2.4　免疫浸润分析

结果显示，GSE67308表达谱数据中与正常样本组织比较，MI/RI样本组织中活化后的自然杀伤细胞、CD4⁺T记忆细胞和巨噬细胞浸润比例升高，而静息状态下的自然杀伤细胞和CD8⁺T细胞比例降低。见图4。

2.5　PPI网络及关键基因

将1 054个MI/RI DEGs与159个坏死性凋亡基因取交集后，获得14个共表达基因，分别为*Il1b*、*Tnfaip3*、*Tnf*、*Birc3*、*Il33*、*Pla2g4b*、*Stat2*、*Ripk1*、*Fas*、*Jak2*、*Irf9*、*Tradd*、*Hsp90aa1*、*Ripk3*，见图5A。利用

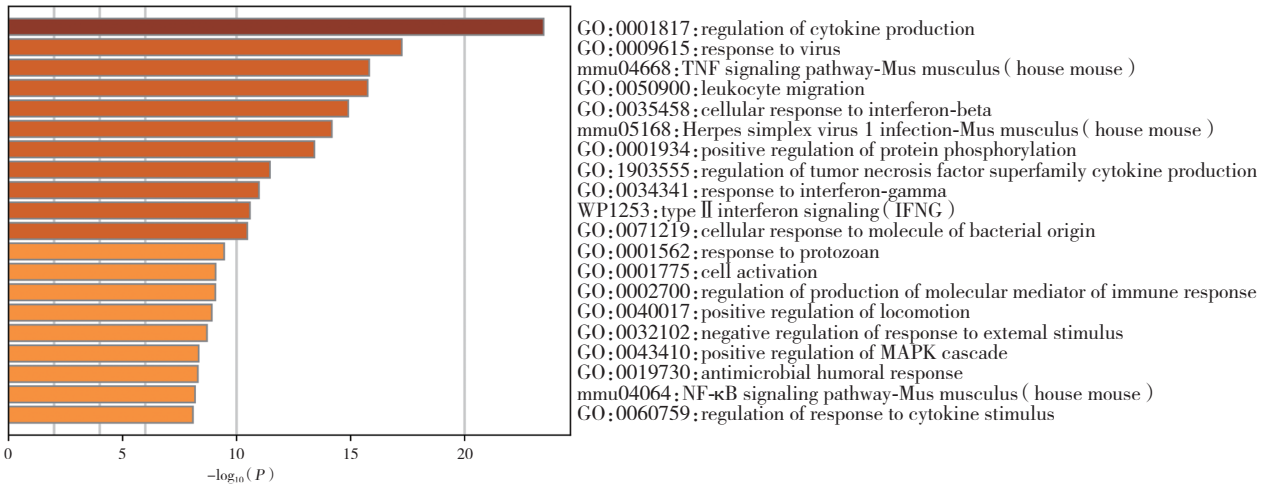


图3　MI/RI的DEGs通路富集分析

Fig.3　Pathway enrichment analysis of DEGs in MI/RI

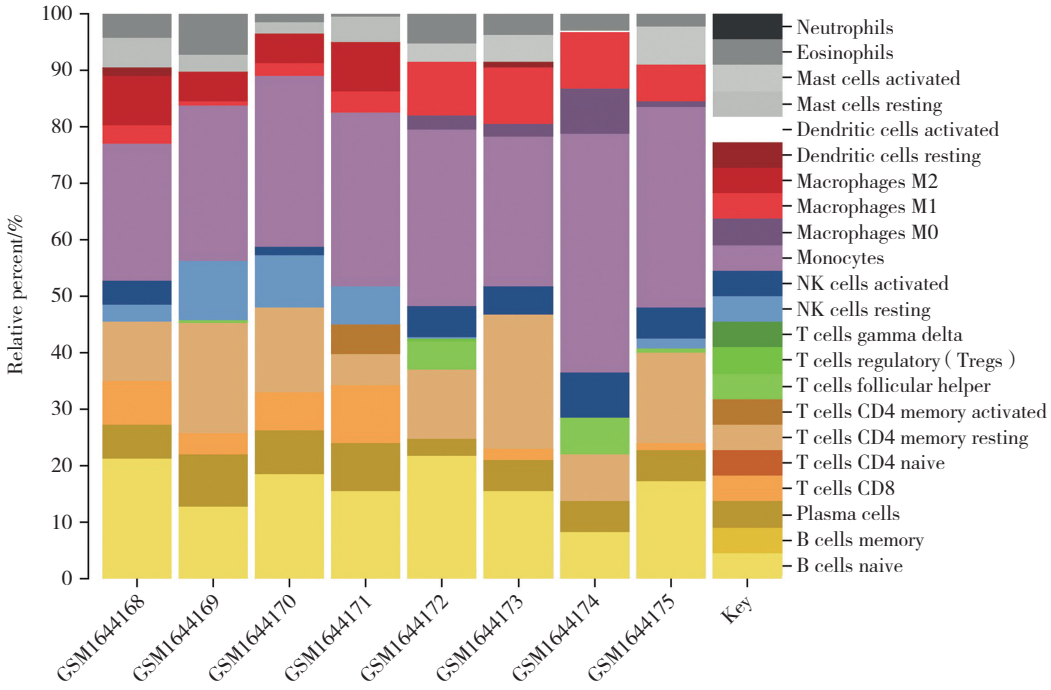


图4　免疫浸润分析

Fig.4　Immuno-infiltration analysis

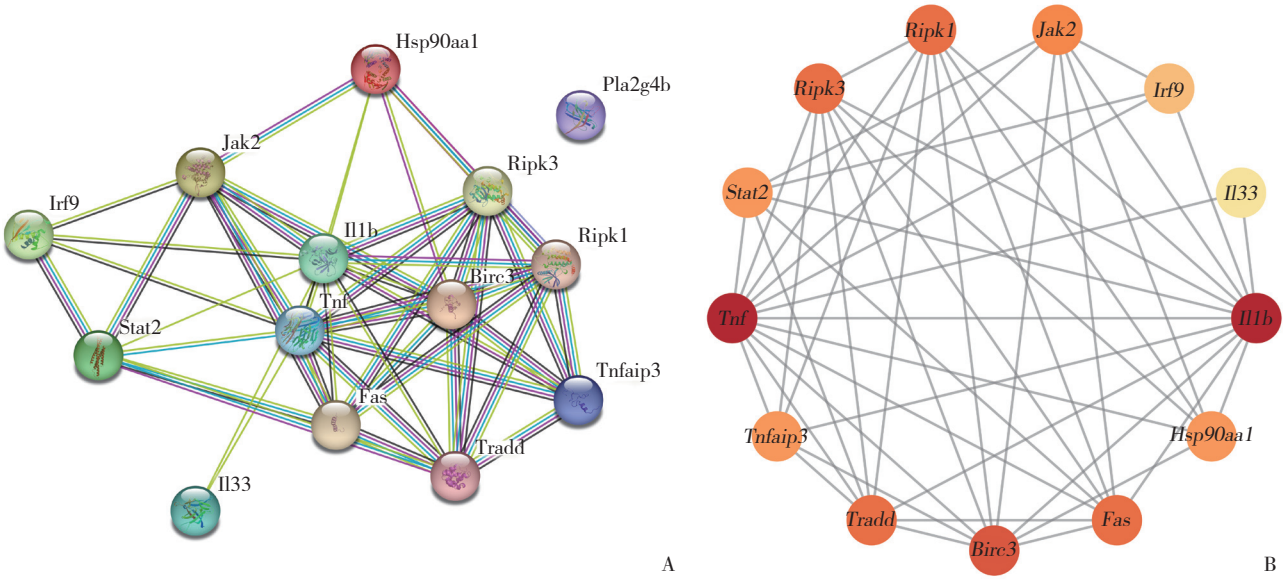
STRING构建PPI网络，获得14个节点和48条边；对其作用关系进行分析，确定*Il1b*、*Tnf*、*Birc3*、*Ripk1*为关键基因，见图5B。

2.6　关键基因的单细胞测序分析

单细胞测序分析显示，在心脏各细胞分类中，*Il1b*在白细胞高表达；*Tnf*在白细胞和内皮细胞高表达；*Birc3*在内皮细胞、成纤维细胞和白细胞中高表达；*Ripk1*在内皮细胞、成纤维细胞、白细胞、肌纤维母细胞中高表达。见图6。

2.7　GSE19875数据集中关键基因的验证

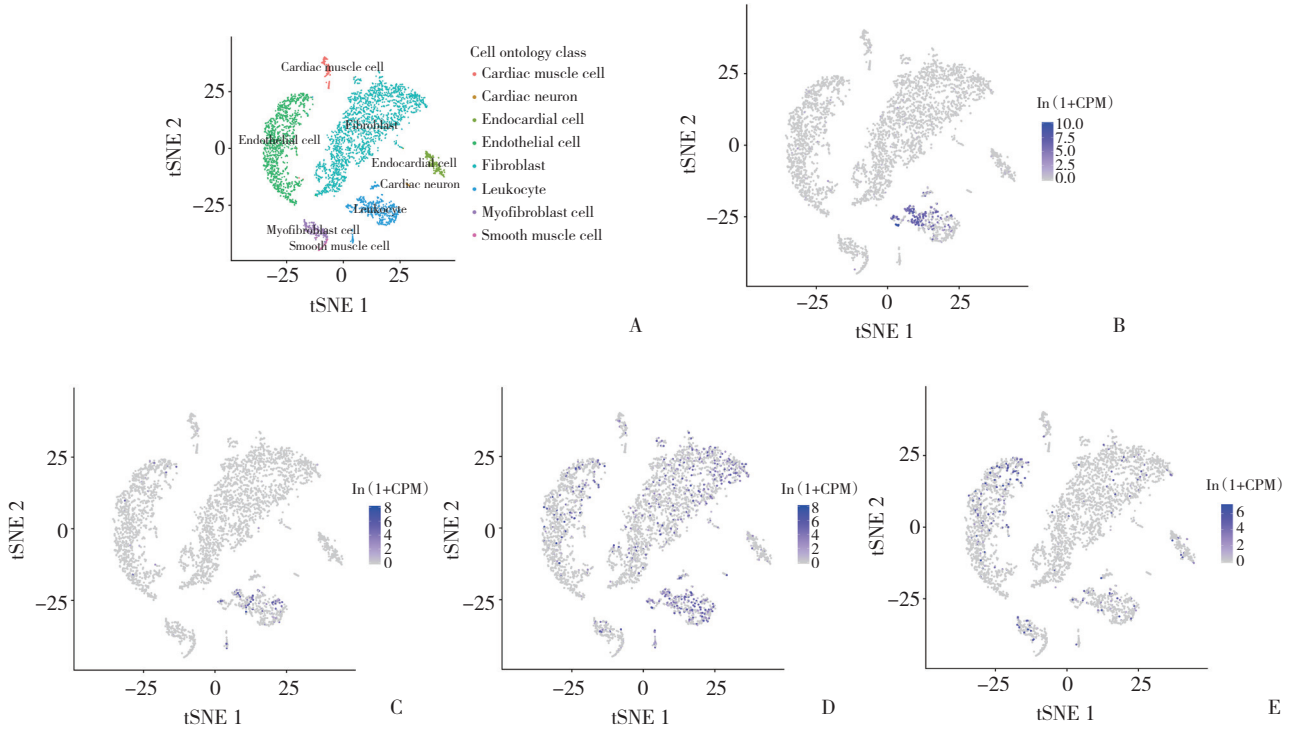
结果显示，与对照组比较，MI/RI模型组中*Il1b*、*Tnf*、*Birc3*、*Ripk1*表达显著升高（均$P<0.01$），见图7。



A，PPI network；B，visualization of key genes.

图5　PPI 网络和关键基因

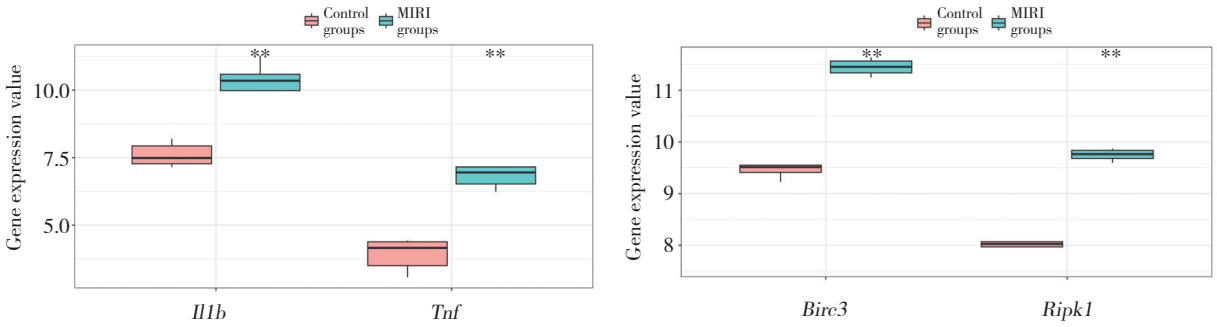
Fig.5　PPI network and key genes



A, ontology classification of mouse cardiac cells; B, *Il1b*; C, *Tnf*; D, *Birc3*; E, *Ripk1*.

图6　关键基因的单细胞测序分析

Fig.6　Single-cell sequencing validation of key genes



$^{**}P < 0.01$ vs. control group.

图7　GSE19875数据集中*Il1b*、*Tnf*、*Birc3*、*Ripk1*基因表达

Fig.7　Expression of *Il1b*, *Tnf*, *Birc3*, and *Ripk1* in the GSE19875 dataset

3　讨论

坏死性凋亡在动脉粥样硬化、MI/RI和心肌梗死等心血管疾病的病理生理过程中发挥重要作用。坏死性凋亡与细胞凋亡不同，往往是由于外部刺激而导致的细胞被动死亡[5]。研究[6-7]表明，坏死性凋亡可以由Toll样受体（Toll-like receptors，TLR）配体以及某些病原体诱导，其中以TNF诱导，由RIPK1和RIPK3介导的坏死性凋亡通路最具代表性。本研究结果显示，MI/RI的 DEGs参与凋亡、细胞因子活性以及对病原体反应等病理生理过程。

本研究结果显示，*Il1b*、*Tnf*、*Birc3*、*Ripk1*是坏死性凋亡的关键调控基因，参与调控MI/RI的发生发展。TNF信号通路和NF-κB信号通路能够调节炎性细胞因子，在组织损伤、免疫反应中发挥促炎作用，消除感染因子并协调伤口愈合。坏死性凋亡分子机制均涉及TNF，研究[8-9]表明TNF及其受体（TNF receptor，TNFR）可以诱导RIPK1、TNFR 相关死亡结

构域(TRADD)、细胞凋亡蛋白抑制剂(cIAP1、cIAP2)、TRAF2等蛋白共同组成复合物Ⅰ,其中RIPK1作为坏死性凋亡的关键调节因子被cIAP1/2 多泛素化处理后,进一步诱导激活NF-κB通路,最终通过RIPK1、RIPK3和MLKL的相互作用诱导坏死性凋亡。此外,TNF和TLR3/TLR4同样也可以通过激活RIPK1、RIPK3和MLKL参与调控坏死性凋亡。研究[10]发现TNF诱导的坏死性凋亡需要c-Jun N末端激酶(c-Jun N-terminal kinase,JNK)活性及其支架功能,JNK1和JNK2在调节TNF和TLR介导的坏死性凋亡中表现出激酶依赖性,阻断JNK激酶活性可显著降低TNF和TLR诱导的坏死性凋亡。因此,通路富集分析结果显示MI/RI DEGs可能在TNF信号通路和NF-κB信号通路介导下参与调控坏死性凋亡,影响MI/RI的病理发展过程。自然杀伤细胞、$CD4^+$T细胞和巨噬细胞能够改善局部免疫细胞微环境,参与调控MI/RI进程,其中自然杀伤细胞可能通过细胞因子IFN-γ/TNF-α/IL-12与巨噬细胞相互作用,进而增强活性来扩大心脏梗死区域的炎症反应,而$CD4^+$T细胞水平在心肌梗死后迅速升高,参与心肌梗死后的组织清除、疤痕形成和免疫应答[11-12],与免疫浸润分析结果一致。

RIPK1/RIPK3/MLKL轴作为坏死性凋亡的经典调控途径,通过调控坏死性凋亡参与MI/RI的发生发展。MI/RI小鼠模型心肌组织中RIPK1、RIPK3和MLKL的表达上调,在使用抑制剂后RIPK1和RIPK3磷酸化水平以及MLKL表达水平降低,坏死性凋亡显著抑制,同时心肌梗死面积减少[13]。与野生型小鼠比较,*RIPK3*基因敲除小鼠心脏在心肌梗死后炎症反应减弱、活性氧生成降低,因此RIPK3介导的坏死性凋亡可能会加剧心肌梗死对心肌细胞的损伤[14]。同样在MI/RI小鼠模型中,敲除RIPK3后能够显著减少心肌梗死面积[15]。进一步研究[16]发现,坏死性凋亡通过改变线粒体结构和功能来影响MI/RI,细胞实验表明氧气供应的短暂缺失后立刻供氧会刺激RIPK3表达显著上调,导致RIPK3依赖性线粒体损伤和细胞坏死,影响调节线粒体分裂的关键蛋白(DRP1)表达及活性改变,从而促进心肌细胞坏死性凋亡。因此,MI/RI过程中心肌细胞氧化应激损伤可能与RIPK3和DRP1相互作用所介导的坏死性凋亡密切相关。

IL-1b和TNF作为典型的炎性细胞因子,广泛参与由坏死性凋亡引发的炎症和免疫反应。细胞受损破裂后随着核DNA、线粒体和蛋白质等DAMP渗出,激活并形成各种炎症小体复合物,从而导致IL-1b、TNF等细胞因子和炎症介质释放[17-18]。此外,研究[19]发现坏死性凋亡主要调节因子MLKL活化也可以触发并形成炎症小体,诱导IL-1b产生和表达。通路分子Birc3 在TNF信号通路的激活过程中起关键作用,Birc3通过减少TNFR1 促凋亡信号复合物的形成来抑制TNF刺激的细胞凋亡,而且还通过NF-κB 诱导激酶的泛素化调控NF-κB 信号通路的激活,参与坏死性凋亡的调节[20]。RIPK1是细胞死亡和炎症的关键调节因子,通过与MLKL相互作用来调节坏死性凋亡,其作用机制是当NF-κB活化被抑制时会激活caspase-8诱导RIPK1裂解并导致细胞凋亡,caspase-8表达被抑制后能够通过去泛素化活化RIPK1,最终促进坏死性凋亡,导致细胞膜破裂和细胞死亡。在心肌梗死大鼠模型心脏中,使用RIPK1特异性抑制剂后MI/RI导致的梗死程度降低[21-22]。

综上所述,MI/RI和参与调控坏死性凋亡的TNF信号通路、NF-κB信号通路高度相关;*Il1b*、*Tnf*、*Birc3*、*Ripk1*是MI/RI和坏死性凋亡的共表达基因;IL-1b、TNF、Birc3、Ripk1可能作为坏死性凋亡关键调节因子参与MI/RI过程。本研究仅为生物信息学研究,未进行相关实验验证,因此今后需要纳入更多测序样本并结合动物实验来进一步论证。

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