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COVID-19 患者外周血单个核细胞 lncRNA 相关 ceRNA 调控网络的生物信息学分析

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[摘　要]　**目的：**对2019冠状病毒病(coronavirus disease 2019，COVID-19)患者外周血单个核细胞(peripheral blood mononuclear cells，PBMCs)测序数据进行生物信息学分析，分析长链非编码RNA(long non-coding RNA，lncRNA)的表达谱及竞争性内源RNA(competing endogenous RNA，ceRNA)调控网络，探讨其与COVID-19发病机制的关联。**方法：**利用R语言对从基因表达综合(Gene Expression Omnibus，GEO)数据库筛选的COVID-19相关测序数据进行基因差异表达分析并注释基因属性，鉴定出差异表达的lncRNA和信使RNA(messenger RNA，mRNA)。使用miRcode在线工具预测与差异表达的lncRNA相互作用的微RNA(microRNA，miRNA)，再利用TargetScan、miRDB和miRTarBase数据库预测miRNA下游靶基因(mRNA)，并与差异表达mRNA取交集，然后利用Cytoscape构建ceRNA调控网络。利用R语言对ceRNA调控网络中的mRNA进行基因本体论(Gene Ontology，GO)和京都基因和基因组百科全书(Kyoto Encyclopedia of Genes and Genomes，KEGG)通路富集分析。**结果：**COVID-19患者PBMCs中313个lncRNA和1 308个mRNA差异表达有统计学意义。构建了ceRNA调控网络，在该网络中差异表达的lncRNA、mRNA分别有22、76个。富集分析发现：ceRNA网络内mRNA主要参与血管发育及生成的调控、(平滑)肌细胞增殖调控、上皮细胞凋亡过程、对缺氧的应答；黏着斑、细胞-基质黏附连接、紧密连接等；及磷脂酰肌醇3激酶-蛋白激酶B(phosphatidylinositol 3 kinase-AKT，PI3K-AKT)信号通路、丝裂原活化蛋白激酶(mitogen-activated protein kinase，MAPK)信号通路、流体剪切应力与动脉粥样硬化、糖尿病并发症中的晚期糖基化终产物-晚期糖基化终产物受体(advanced glycation end products-receptor for advanced glycation end products，AGE-RAGE)信号通路等。**结论：**本研究构建了lncRNA相关的ceRNA网络，为探讨lncRNA参与COVID-19发病机制提供了一个新的视角。这些基因有可能成为潜在的治疗靶点。

[关键词]　2019冠状病毒病；长链非编码RNA；竞争性内源RNA；基因表达谱；差异表达基因；富集分析

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Bioinformatics analysis of lncRNA-related ceRNA regulatory network in peripheral blood mononuclear cells of patients with COVID-19

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Abstract **Objective:** Bioinformatics analysis of sequencing data of peripheral blood mononuclear cells (PBMCs) from patients with coronavirus disease 2019 (COVID-19) was performed to analyze expression profiles of long non-coding RNA (lncRNA) and regulatory networks of competing endogenous RNA (ceRNA) and to explore its association with the pathogenesis of COVID-19. **Methods:** Differential gene expression analysis about COVID-19-related sequencing data screened from the Gene Expression Omnibus (GEO) database was performed using R language. Gene attributes were annotated, and differentially expressed lncRNA and messenger RNA (mRNA) were identified. Online tool miRcode was used to predict microRNA (miRNA) that interacts with differentially expressed lncRNA, and TargetScan, miRDB, and miRTarBase databases were used to predict downstream target gene (mRNA) of miRNA. Afterwards, target gene and differentially expressed mRNA were intersected, followed by a ceRNA regulatory network construction using the intersected genes together with aforementioned miRNA and differentially expressed lncRNA via Cytoscape. The Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of intersected genes in the ceRNA network were performed using the R. **Results:** There were 313 lncRNAs and 1 308 mRNAs expressed differentially statistically in PBMCs of COVID-19 patients. A ceRNA regulatory network was successfully constructed, with 22 and 76 differentially expressed lncRNAs and mRNAs, respectively. Enrichment analyses found that mRNA in the ceRNA network was mainly involved in regulation of vasculature development and angiogenesis, muscle cell proliferation, regulation of smooth muscle cell proliferation, epithelial cell apoptotic process, response to hypoxia; focal adhesion, cell-substrate junction, tight junction; phosphatidylinositol 3 kinase-AKT (PI3K-AKT) signaling pathway, mitogen-activated protein kinase (MAPK) signaling pathway, fluid shear stress and atherosclerosis, advanced glycation end products-receptor for advanced glycation end products (AGE-RAGE) signaling pathway in diabetic complications, etc. **Conclusion:** Our study constructed the lncRNA-related ceRNA network, providing a neoteric perspective for exploring the pathogenic mechanism of lncRNA involved in COVID-19. These genes might become potential therapeutic targets.

Keywords coronavirus disease 2019; long chain non-coding RNA; competing endogenous RNA; gene expression profile; differentially expressed genes; enrichment analysis

近年来由严重急性呼吸系统综合征冠状病毒2(severe acute respiratory syndrome coronavirus 2，SARS-CoV-2)导致的2019冠状病毒病(coronavirus disease 2019，COVID-19)席卷全球，严重危害人类健康，造成巨大的社会经济学负担。COVID-19可累及多系统[1]，发病机制复杂，且尚缺乏有效治疗措施。因而，进一步探究其致病机制及寻求治疗靶点具有重要临床意义。

非编码RNA (non-coding RNA，ncRNA)是一类不编码蛋白质但调控蛋白质表达的RNA分子，如长链ncRNA(long ncRNA，lncRNA)、微RNA(microRNA，miRNA)和环状RNA(circular RNA，circRNA)等。作为一种新型的、长度超过200个核苷酸的ncRNA，lncRNA在细胞周期的调

节、细胞命运决定、染色质重塑及加工和表观遗传学的调节中均发挥重要作用，已成为遗传研究的热点[2-3]。其可能通过与信使RNA(messenger RNA，mRNA)竞争性“海绵吸附”miRNA，抑制miRNA活性，从而微调miRNA介导的靶基因(mRNA)的表达，进而影响疾病的发生发展。

本研究分析COVID-19患者外周血单个核细胞(peripheral blood mononuclear cells，PBMCs) lncRNA表达谱及竞争性内源RNA(competing endogenous RNA，ceRNA)调控网络，探讨其与COVID-19发病机制的关联，为寻找新的治疗靶点提供理论依据。

1 数据和方法

1.1 数据下载和预处理

进入基因表达综合(Gene Expression Omnibus，GEO)数据库(https://www.ncbi.nlm.nih.gov/geo/)[4-5]搜索“COVID-19/SARS-CoV-2”相关转录组数据，获取数据集GSE152418，其来源于耶克斯国家灵长类研究中心，采用Affymetrix Human Genome U133 Plus 2.0 Array平台，以人PBMCs为研究对象，轻-重型COVID-19患者为实验组(n=17)，健康人群为对照组(n=17)，34例样本的临床信息见表1。对下载的高通量测序数据进行预处理，如主成分分析(principal component analysis，PCA)绘制散点图，评估数据可靠性，并获取基因表达矩阵信息。

1.2 基因差异表达分析

采用统计学软件R语言(R4.1.1版本)“DESeq2”包进行基因差异表达分析。差异表达基因(differentially expressed genes，DEGs)的筛选条件为|变化倍数以2为底对数化($\log_2$fold change，$\log_2$FC)|>1且校正后的P<0.05，并运用R语言绘制火山图和热图以可视化。

表1 COVID-19患者和健康对照者的临床特征

Table 1 Clinical characteristics of COVID-19 patients and healthy controls

样本名称	距症状首发的天数	性别	疾病状态	严重程度	患者所处地理位置
GSM4614985	40	男	COVID-19	康复期(轻度)	Atlanta，GA，USA
GSM4614986	2	女	COVID-19	中度	Atlanta，GA，USA
GSM4614987	23	男	COVID-19	重度	Atlanta，GA，USA
GSM4614988	15	女	COVID-19	重度	Atlanta，GA，USA
GSM4614989	9	男	COVID-19	重度	Atlanta，GA，USA
GSM4614990	16	男	COVID-19	重度	Atlanta，GA，USA
GSM4614991	9	女	COVID-19	中度	Atlanta，GA，USA
GSM4614992	9	女	COVID-19	重度	Atlanta，GA，USA
GSM4614993	15	女	COVID-19	重度	Atlanta，GA，USA
GSM4614994	8	女	COVID-19	重度	Atlanta，GA，USA
GSM4614995	9	女	COVID-19	中度	Atlanta，GA，USA
GSM4614996	13	男	COVID-19	重度	Atlanta，GA，USA
GSM4614997	18	女	COVID-19	重度	Atlanta，GA，USA
GSM4614998	21	女	COVID-19	重度	Atlanta，GA，USA
GSM4614999	16	男	COVID-19	重度	Atlanta，GA，USA
GSM4615000	15	男	COVID-19	重度	Atlanta，GA，USA
GSM4615001	12	女	COVID-19	中度	Atlanta，GA，USA
GSM4615003	NA	男	健康对照	NA	Atlanta，GA，USA
GSM4615006	NA	女	健康对照	NA	Atlanta，GA，USA
GSM4615008	NA	女	健康对照	NA	Atlanta，GA，USA

续表1

样本名称	距症状首发的天数	性别	疾病状态	严重程度	患者所处地理位置
GSM4615011	NA	男	健康对照	NA	Atlanta，GA，USA
GSM4615014	NA	女	健康对照	NA	Atlanta，GA，USA
GSM4615016	NA	男	健康对照	NA	Atlanta，GA，USA
GSM4615019	NA	女	健康对照	NA	Atlanta，GA，USA
GSM4615022	NA	男	健康对照	NA	Atlanta，GA，USA
GSM4615025	NA	女	健康对照	NA	Atlanta，GA，USA
GSM4615027	NA	男	健康对照	NA	Atlanta，GA，USA
GSM4615030	NA	女	健康对照	NA	Atlanta，GA，USA
GSM4615032	NA	男	健康对照	NA	Atlanta，GA，USA
GSM4615033	NA	女	健康对照	NA	Atlanta，GA，USA
GSM4615034	NA	女	健康对照	NA	Atlanta，GA，USA
GSM4615035	NA	男	健康对照	NA	Atlanta，GA，USA
GSM4615036	NA	女	健康对照	NA	Atlanta，GA，USA
GSM4615037	NA	男	健康对照	NA	Atlanta，GA，USA

NA，无法获取的。

NA, not available.

1.3 DEGs 属性注释

利用R语言“org.Hs.eg.db”包(3.10.0版本)和Ensembl 101版本注释文件对DEGs进行属性注释，区分出差异表达的lncRNA和编码蛋白的mRNA。

1.4 预测 miRNA 和靶基因并构建 ceRNA 网络

使用miRcode在线工具(http://www.mircode.org/)预测与差异表达的lncRNA相互作用的miRNA，再利用TargetScan(https://www.targetscan.org/)、miRDB(https://mirdb.org/)和miRTarBase(https://mirtarbase.cuhk.edu.cn)数据库对miRNA下游靶基因(mRNA)进行预测，并与测序数据中差异表达的mRNA取交集，后利用Cytoscape3.7.2软件[6]构建ceRNA可视化调控网络。

1.5 GO 和 KEGG 富集分析

利用R语言“clusterProfiler”包(3.14.3版本)对ceRNA网络中靶基因实施基因本体论(Gene Ontology，GO)和京都基因与基因组百科全书(Kyoto Encyclopedia of Genes and Genomes，KEGG)通路富集分析[7-8]。在生物学过程(biological process，BP)、细胞组分(cellular component，CC)和分子功能(molecular function，MF)(http://geneontology.org/)方面对基因进行功能注释，并探讨DEGs参与的信号通路。P<0.05为有统计学意义。

2 结果

2.1 基因差异表达分析

对原始数据进行PCA分析，如图1A所示，提示样本数据的可靠性。与健康人群相比，轻-重型COVID-19患者PBMCs中313个lncRNA和1 308个mRNA差异表达有统计学意义。其中，lncRNA上调的有256个，下调的有57个；mRNA上调的有1 133个，下调的有175个。基因差异表达分析如火山图和热图(图1B、1C)所示。按$|\log_2FC|$降序排序，上下调各前10的lncRNA和mRNA分别如表2、3所示。

2.2 ceRNA 网络构建

预测miRNA和mRNA后，构建ceRNA网络(图2)。在该图中，红色“V”形、绿色三角形、粉色圆形分别代表lncRNA、miRNA、mRNA。在该网络中，差异表达的lncRNA有22个，差异表达的mRNA有76个，与lncRNA、mRNA靶向作用的miRNA有29个。

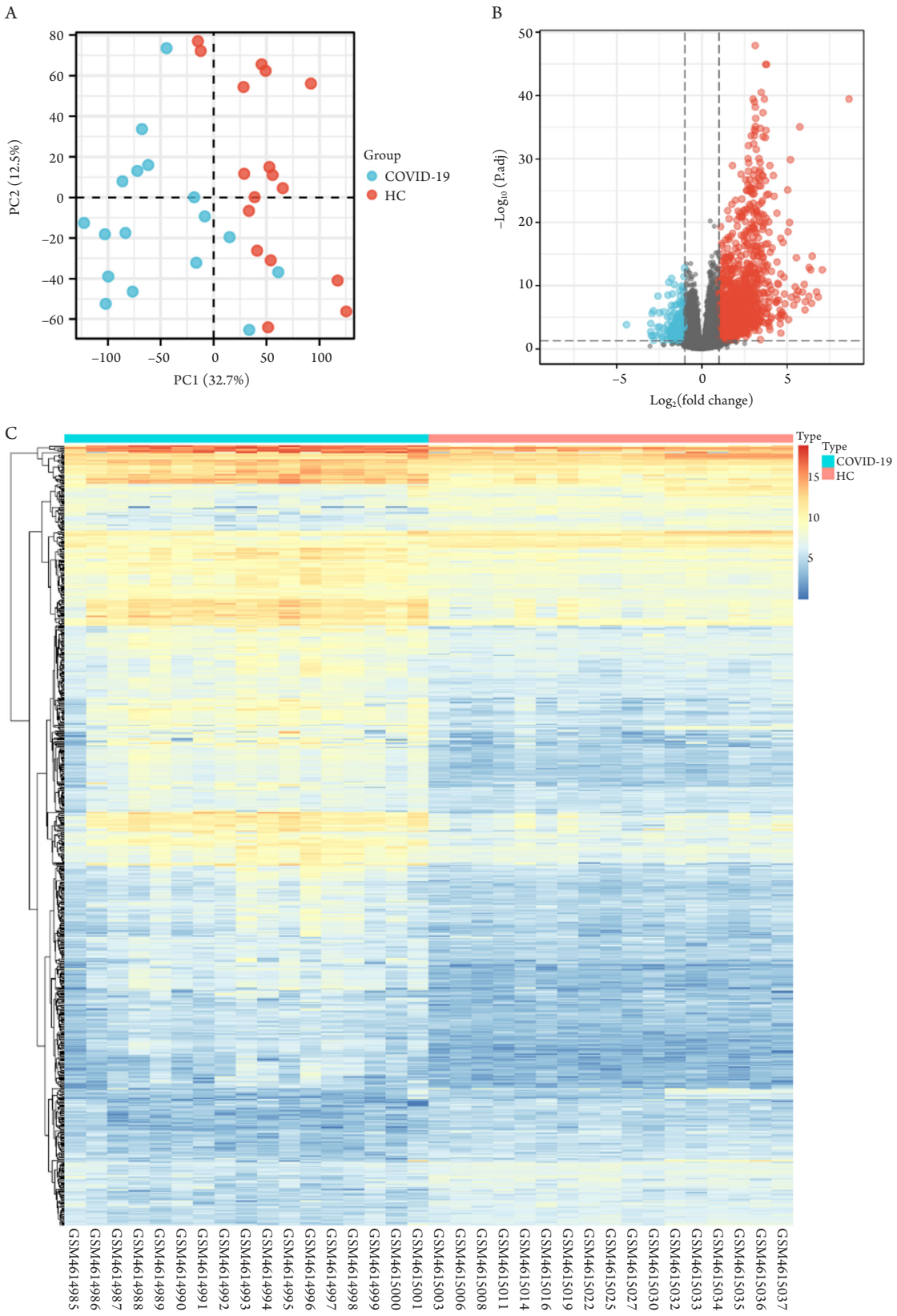

图1 样本预处理和基因差异表达分析

Figure 1 Sample preprocessing and gene differential expression analysis

(A)PCA；(B)火山图：红色，上调，蓝色，下调；(C)热图。HC，健康对照者。

(A) PCA; (B) Volcano plot: red, up-regulated, blue, down-regulated; (C) Heatmap. HC, healthy control.

表2 lncRNA差异表达分析

Table 2 lncRNA differential expression analysis

lncRNA	Log_2FC	*P*	校正后的*P*
AL954642.1	4.07	1.37E-06	1.69E-05
AC022730.4	3.88	7.99E-06	7.86E-05
LINC02128	3.72	8.28E-06	8.08E-05
AC112253.1	3.49	6.26E-07	8.68E-06
LINC02772	3.39	3.81E-06	4.09E-05
AC091173.1	3.38	1.59E-14	1.21E-12
LINC01606	3.32	1.11E-05	<0.01
LINC01482	3.05	1.12E-14	8.83E-13
LNCAROD	3.04	6.41E-12	3.22E-10
LINC01322	2.99	5.53E-07	7.80E-06
AC011379.1	−1.52	0.02	0.05
MMP2-AS1	−1.73	<0.01	0.01
AC084724.1	−1.75	4.76E-05	<0.01
AC104809.2	−1.80	<0.01	<0.01
AL034550.2	−1.82	4.53E-08	8.82E-07
AC016245.2	−1.94	<0.01	<0.01
AC083843.2	−2.05	<0.01	<0.01
FAM222A-AS1	−2.08	0.01	0.03
AL121899.4	−2.29	<0.01	0.01
AC009630.4	−4.42	1.68E-05	<0.01

表3 mRNA差异表达分析

Table 3 mRNA differential expression analysis

mRNA	Log_2FC	*P*	校正后的*P*
IFI27	8.64	1.15E-43	3.57E-40
CA1	7.06	3.69E-15	3.12E-13
GYPB	6.83	1.71E-10	6.24E-09
HBA2	6.73	2.11E-11	9.64E-10
HBM	6.44	2.18E-09	5.95E-08
HBD	6.39	3.90E-15	3.27E-13
ADAMTS2	6.29	1.48E-15	1.37E-13
ALAS2	6.29	1.33E-10	4.96E-09
IFIT1B	6.07	1.15E-09	3.41E-08
AHSP	6.05	3.32E-08	6.77E-07
ADAMTS5	−2.24	7.25E-08	1.34E-06
CCL20	−2.46	<0.01	<0.01
ADORA1	−2.49	1.11E-07	1.93E-06
SLC4A10	−2.58	1.18E-10	4.44E-09
PRSS35	−2.61	4.95E-05	0.000370024
OLR1	−2.66	<0.01	0.01
HSPA1B	−2.85	4.93E-08	9.45E-07
IL-1A	−2.87	<0.01	0.01
GJB2	−2.97	<0.01	0.01
BIRC7	−3.00	1.03E-05	9.68E-05

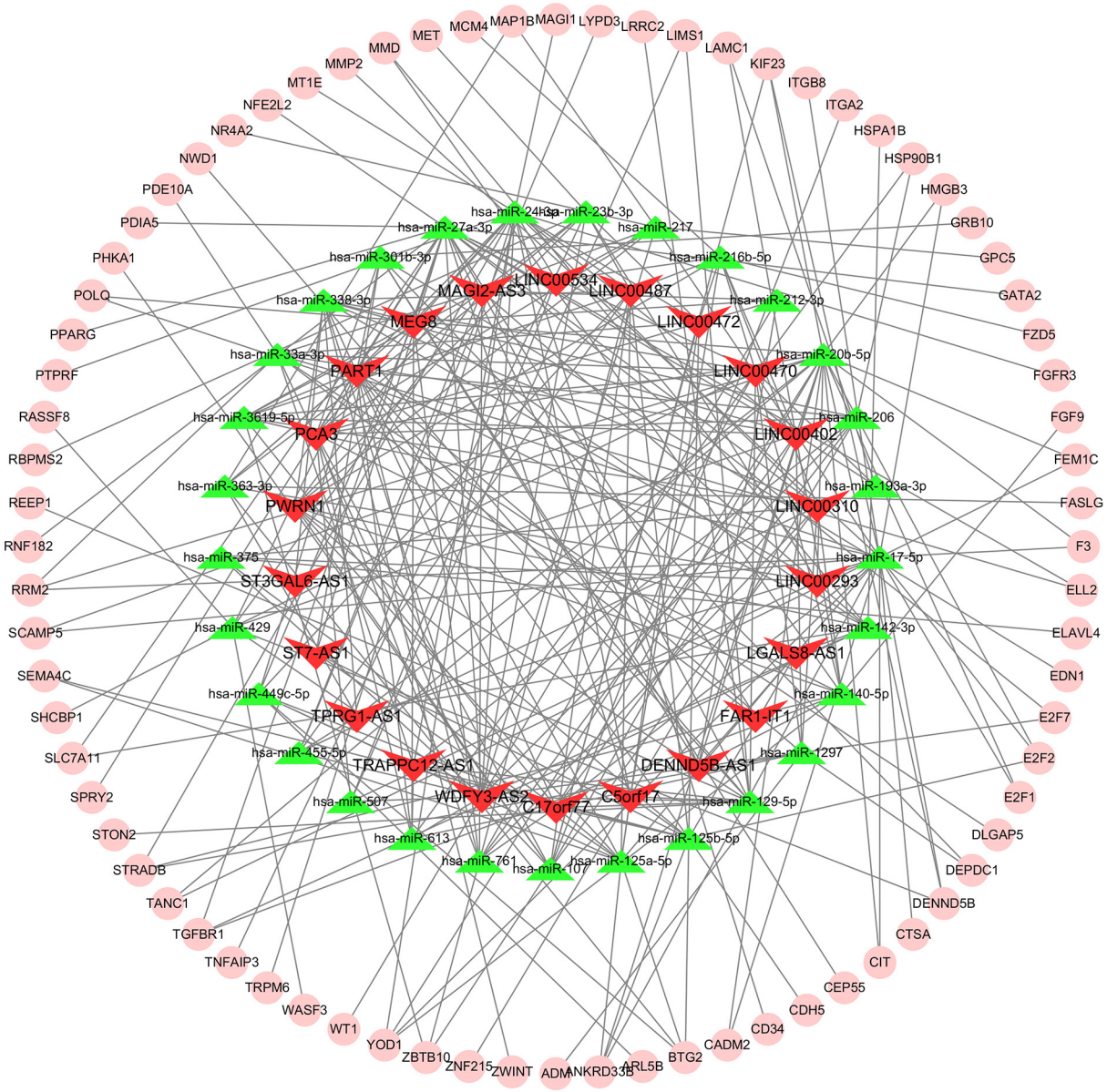

图2 ceRNA网络构建

Figure 2 ceRNA network construction

“V”形：lncRNA；三角形：miRNA；圆形：mRNA。

“V”: lncRNA; triangle: miRNA; circle: mRNA.

2.3 GO 富集分析

对ceRNA网络中的mRNA进行GO富集分析，发现其被富集到245个不同的GO子集中，包括BP和CC两个方面，主要有血管发育及生成的调控、(平滑)肌细胞增殖调控、上皮细胞凋亡过程、对缺氧的应答；黏着斑、细胞-基质黏附连接、紧密连接等(图3)。

2.4 KEGG 富集分析

对ceRNA网络中的mRNA进行KEGG通路富集分析，发现其显著富集到磷脂酰肌醇3激酶-蛋白激酶B (phosphatidylinositol 3 kinase-AKT，PI3K-AKT)信号通路、丝裂原活化蛋白激酶(mitogen-activated protein kinase，MAPK)信号通路、流体剪切应力与动脉粥样硬化、糖尿病并发症中的晚期糖基化终末产物-晚期糖基化终末产物受体(advanced glycation end-product and receptors of advanced glycation end-product，AGE-RAGE)信号通路等12个信号通路上(图4)。

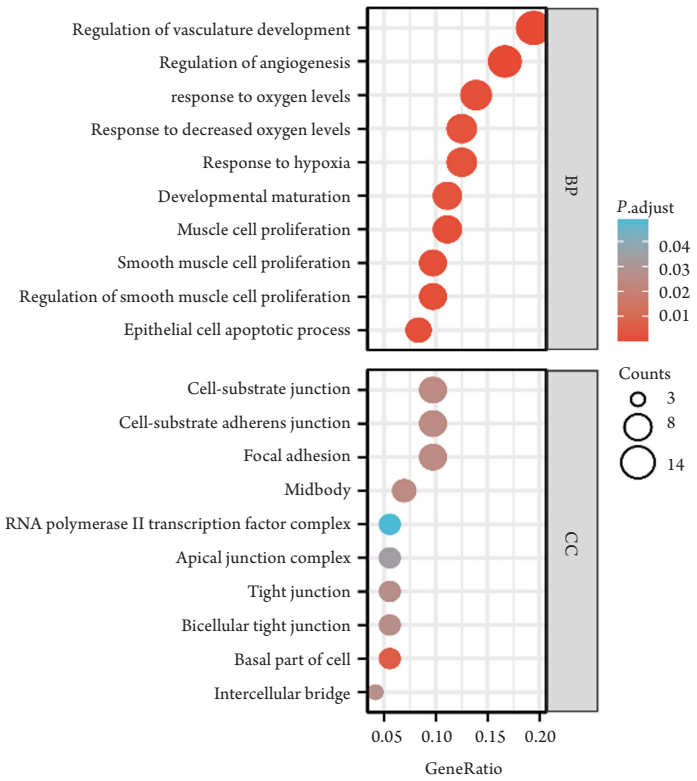

图3 mRNA GO富集分析部分可视化(气泡图)

Figure 3 Partial visualization for GO enrichment analysis of mRNA (bubble diagram)

GeneRatio：富集基因数目/背景基因数目；Counts：富集基因数目；*P*.adjust：校正后的*P*。

GeneRatio: Number of enriched genes/number of background genes; Counts: Number of enriched genes; *P*.adjust: Adjusted *P*.

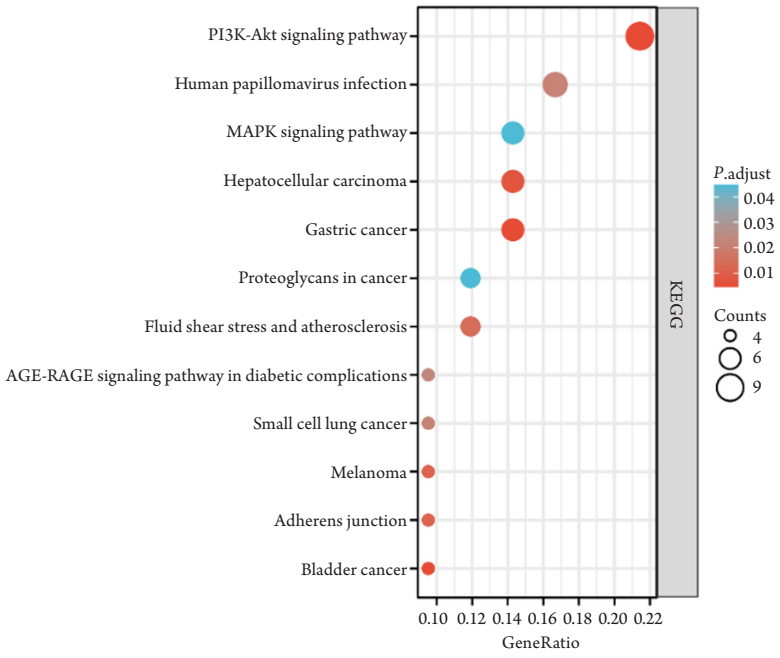

图4 mRNA KEGG富集分析部分可视化(气泡图)

Figure 4 Partial visualization for KEGG enrichment analysis of mRNA (bubble diagram)

GeneRatio：富集基因数目/背景基因数目；Counts：富集基因数目；*P*.adjust：校正后的*P*。

GeneRatio: Number of enriched genes/number of background genes; Counts: Number of enriched genes; *P*.adjust: Adjusted *P*.

3 讨论

SARS-CoV-2感染造成的多系统损害发病机制仍不清楚。而lncRNA和miRNA等ncRNA通过调控mRNA表达进而影响细胞功能的表观遗传学机制参与许多疾病的发生发展。在本研究中，通过对COVID-19患者PBMCs lncRNA表达谱及ceRNA调控网络的分析，探讨COVID-19的发病机制，为寻找新的治疗靶点提供理论依据。

首先，在本研究中，与对照组比较，轻-重型COVID-19患者的PBMCs中存在大量差异表达的lncRNA和mRNA。LINC01606位于8q12，由10个外显子编码。LINC01606主要参与肿瘤细胞的浸润和转移，如Luo等[9]发现在胃癌组织中LINC01606高表达，且将其水平降低后肿瘤细胞的转移和侵袭能力显著下降，这一效应可能是LINC01606通过降低miR-423-5p的水平从而激活Wnt/β-catenin信号通路来实现的。He等[10]在多发性骨髓瘤中也发现了类似效应，不同的是，他们认为LINC01606与miR-579-3p的相互作用是LINC01606导致肿瘤细胞侵袭和迁移的机制。对LNCAROD的研究目前也局限在肿瘤领域，Ban等[11]发现其在头颈部鳞状细胞癌中高表达，且与肿瘤进展及不良预后相关，其致病机制可能与通过甲基化增加自身稳定型、防止编码Y盒结合蛋白1(Y-box binding protein 1，YBX1)基因降解有关，从而促进肿瘤细胞浸润和迁移。Jia等[12]报道了在肝细胞肝癌中，LNCAROD能够通过发挥miR-145-5p“海绵”吸附作用，增加丙酮酸激酶表达水平，继而提高肿瘤细胞有氧糖酵解，参与肿瘤细胞恶化及抗药性的发生。在本研究中，LINC01606和LNCAROD的表达在COVID-19患者PBMCs中均是显著升高的。虽然目前尚未见这2个lncRNA参与COVID-19发生发展的直接依据，但考虑到它们均参与肿瘤细胞的浸润和迁移，提示其可能在SARS-CoV-2感染后促进肿瘤疾病恶化起关键作用[13]。它们在COVID-19病理生理过程中的作用需要进一步研究。

关于差异表达的mRNA，人β防御素2(human beta defensin 2，HBD-2)是一种上皮细胞来源的宿主防御肽，具有抗病毒的特性。已有研究[14-15]报道HBD-2能够通过特异性地与SARS-CoV-2的受体结合域结合，从而阻止其与血管紧张素转换酶2(angiotensin converting enzyme 2，ACE2)受体相互作用，进入细胞，发挥抗COVID-19的作用。在本研究中，HBD的表达在COVID-19患者PBMCs中显著升高，这可能是机体对病毒感染的一种保护性反应。因此，增加HBD-2的亲和力可能成为COVID-19的治疗靶点[16-17]。除HBD之外，在本研究中干扰素α诱导蛋白27(interferon alpha-inducible protein 27，IFI27)的表达在SARS-CoV-2感染的单核细胞中表达也是显著上调的。许多研究[18-21]已经报道IFI27是SARS-CoV-2感染的生物标志物，并与疾病的转归相关。一些生物信息学分析[22-23]也提示IFI27与COVID-19密切相关，这与我们的研究结果是一致的，提示IFI27可能成为COVID-19的治疗靶点。研究[24-27]报道：C-C基序趋化因子20(C-C motif chemokine 20，CCL20)是气道上皮和免疫细胞相互作用的标志，与COVID-19的严重程度相关。Xu等[28]发现基因70 kDa热休克蛋白1B[heat shock 70 kDa protein 1B，HSPA1B；即编码热休克蛋白72(heat shock protein 72，HSP72)]可能作为一个ACE2共表达基因参与COVID-19中炎症和心血管并发症的发生。一些生物信息学分析[29-34]发现白细胞介素1α(interleukin-1 alpha，IL-1A)是COVID-19参与免疫信号上调、细胞因子风暴、诱导心脏功能失调的核心分子，并可能是药物干预的重要靶点。并且IL-1A与重症COVID-19感染相关[35]。在本研究中，CCL20、HSPA1B和IL-1A的表达在患者PBMCs中显著下调。这些差异表达的mRNA可能成为COVID-19的治疗靶点，其作用机制需要基础和临床研究进一步证实。

其次，我们构建了ceRNA网络，并对其中差异表达的mRNA进行GO富集分析，探索其生物学功能。富集结果提示：SARS-CoV-2的感染会引起或加重肺血管内皮细胞的功能失调、溶解或死亡，造成血管通透性增加和肺水肿，促进急性呼吸窘迫综合征的发生和发展。另外，肺脏内皮屏障的破坏会造成病毒感染远隔器官，引起多系统受累和多脏器衰竭。值得注意的是，内皮屏障的破坏还会暴露组织因子，从而通过激活内源性凝血系统，造成体内高凝状态，这可能是COVID-19患者发生弥散性血管内凝血和急性冠脉综合征的机制之一[36]。

最后，我们对差异表达的mRNA进行了KEGG通路分析，进一步寻找参与COVID-19发病机制的信号通路。PI3K-AKT-哺乳动物雷帕霉素靶点(PI3K-AKT-mammalian target of rapamycin，PI3K-AKT-mTOR)信号通路通过激酶、磷酸酶等的参与调控磷酸化和去磷酸化过程，调节包括合成代谢、营养摄取、细胞生长、分化、增殖等在内的多种细胞功能。已有研究[37]发现SARS-CoV-2的感染会显著上调PI3K-AKT-mTOR通路，表现为大量

蛋白质磷酸化位点的修饰。相反地，已有在体研究[38-39]发现二甲双胍作为mTOR的抑制剂，能够显著改善COVID-19患者的预后。Zhou等[40]通过生物信息学方法也提示另一种mTOR的抑制剂—雷帕霉素也可能是COVID-19的治疗选择。因此，抑制PI3K-AKT-mTOR通路可能成为COVID-19的治疗靶点[37]。MAPK信号通路被认为能够介导血小板聚集，研究发现COVID-19患者MAPK的下游信号分子，如细胞外调节蛋白激酶1/2(extracellular regulated protein kinases 1/2，ERK1/2)、p38等的磷酸化水平是显著上调的，提示MAPK通路显著激活[41]；而MAPK还能够通过激活磷脂酶A2从而增加血栓素的产生[42]。MAPK信号通路的激活以及血栓素的产生可能是COVID-19患者血小板激活和聚集的原因之一。因此抑制MAPK信号通路可能也是治疗SARS-CoV-2感染引起的血栓并发症的策略之一[41]。最新的研究[43]提示COVID-19患者的血液黏度会大幅升高。此外，一项基于GSE152075的COVID-19患者的生物信息学分析[44]也提示流体剪切应力与动脉粥样硬化通路在SARS-CoV-2感染的分子机制中发挥重要作用。南非醉茄既往被认为有免疫增强、抗病毒等多种药理作用，最近有研究报道其能够改变Ras相关C3肉毒素底物1(Ras-related C3 botulinum toxin substrate 1，RAC1)在内的多种蛋白质表达水平的变化，从而调节流体剪切应力与动脉粥样硬化等通路，最终发挥抗COVID-19感染的作用[45]。这些结果提示我们通过补液等方式降低血液黏度可能对COVID-19患者起到治疗作用。AGE介导了炎症蛋白和结缔组织之间的相互作用，使其容易受到免疫失调的损伤。而AGE受体(receptor for AGE，RAGE)在多种炎症细胞上表达，其被AGE激活后引发许多下游信号通路的激活，最终导致炎症反应的组织损伤[46]。已有研究[47]报道AGE-RAGE信号通路可能与COVID-19的严重程度有关。多项研究[48-52]发现AGE-RAGE信号通路可能参与多种中药对COVID-19的治疗作用。这些结果提示AGE-RAGE信号通路与SARS-CoV-2的致病作用密切相关。

总而言之，差异表达的lncRNA和mRNA以及lncRNA相关的ceRNA网络参与的多种生物学功能及信号通路可能在COVID-19多器官多系统损害的发生发展中发挥关键作用，可能成为治疗SARS-CoV-2感染的精准靶点。但是，本文也存在一定局限性。比如，本研究是基于公共数据库分析所得，尚缺乏临床人群样本的佐证。另外，新冠病毒可侵袭多系统致病，本研究分析PBMC DEGs在疾病机制中可能的作用，可能也涉及多种疾病，故尚缺乏针对单一疾病或单一通路的深入挖掘，仍需进一步研究。

参考文献

1. Zaim S, Chong JH, Sankaranarayanan V, et al. COVID-19 and multiorgan response[J]. Curr Probl Cardiol, 2020, 45(8): 100618.
2. Weidle UH, Birzele F, Kollmorgen G, et al. Long non-coding RNAs and their role in metastasis[J]. Cancer Genomics Proteomics, 2017, 14(3): 143-160.
3. Akhade VS, Pal D, Kanduri C. Long noncoding RNA: genome organization and mechanism of action[J]. Adv Exp Med Biol, 2017, 1008: 47-74.
4. Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository[J]. Nucleic Acids Res, 2002, 30(1): 207-210.
5. Barrett T, Wilhite SE, Ledoux P, et al. NCBI GEO: archive for functional genomics data sets--update[J]. Nucleic Acids Res, 2013, 41(Database issue): D991-D995.
6. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks[J]. Genome Res, 2003, 13(11): 2498-2504.
7. Ashburner M, Ball CA, Blake JA, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium[J]. Nat Genet, 2000, 25(1): 25-29.
8. Kanehisa M, Goto S. KEGG: Kyoto encyclopedia of genes and genomes[J]. Nucleic Acids Res, 2000, 28(1): 27-30.
9. Luo Y, Tan W, Jia W, et al. The long non-coding RNA LINC01606 contributes to the metastasis and invasion of human gastric cancer and is associated with Wnt/β-catenin signaling[J]. Int J Biochem Cell Biol, 2018, 103: 125-134.
10. He X, Fan X, Zhang B, et al. Expression of LINC01606 in multiple myeloma and its effect on cell invasion and migration[J]. Am J Transl Res, 2021, 13(8): 8777-8786.
11. Ban Y, Tan P, Cai J, et al. LNCAROD is stabilized by m6A methylation and promotes cancer progression via forming a ternary complex with HSPA1A and YBX1 in head and neck squamous cell carcinoma[J]. Mol Oncol, 2020, 14(6): 1282-1296.
12. Jia G, Wang Y, Lin C, et al. LNCAROD enhances hepatocellular carcinoma malignancy by activating glycolysis through induction of pyruvate kinase isoform PKM2[J]. J Exp Clin Cancer Res, 2021, 40(1): 299.
13. Au L, Boos LA, Swerdlow A, et al. Cancer, COVID-19, and antiviral immunity: the CAPTURE study[J]. Cell, 2020, 183(1): 4-10.

14. Zhang L, Ghosh SK, Basavarajappa SC, et al. HBD-2 binds SARS-CoV-2 RBD and blocks viral entry: strategy to combat COVID-19[J]. iScience, 2022, 25(3): 103856.
15. Zhang L, Ghosh SK, Basavarajappa SC, et al. Molecular dynamics simulations and functional studies reveal that hBD-2 binds SARS-CoV-2 spike RBD and blocks viral entry into ACE2 expressing cells[J]. bioRxiv, 2021. doi: 10.1101/2021.01.07.425621.
16. Nugraha AP, Rahmadhani D, Puspitaningrum MS, et al. Molecular docking of anthocyanins and ternatin in Clitoria ternatea as coronavirus disease oral manifestation therapy[J]. J Adv Pharm Technol Res, 2021, 12(4): 362-367.
17. Hariono M, Hariyono P, Dwiastuti R, et al. Potential SARS-CoV-2 3CLpro inhibitors from chromene, flavonoid and hydroxamic acid compound based on FRET assay, docking and pharmacophore studies[J]. Results Chem, 2021, 3: 100195.
18. Gupta RK, Rosenheim J, Bell LC, et al. Blood transcriptional biomarkers of acute viral infection for detection of pre-symptomatic SARS-CoV-2 infection: a nested, case-control diagnostic accuracy study[J]. Lancet Microbe, 2021, 2(10): e508-e517.
19. Won SY, Seol IC, Yoo HR, et al. Antiviral effect of Hyunggaeyungyo-Tang on A549 cells infected with human coronavirus[J]. Evid Based Complement Alternat Med, 2021, 2021: 4494389.
20. Kulasinghe A, Tan CW, Ribeiro Dos Santos Miggiolaro AF, et al. Profiling of lung SARS-CoV-2 and influenza virus infection dissects virus-specific host responses and gene signatures[J]. Eur Respir J, 2022, 59(6): 2101881.
21. Wang Y, Li J, Zhang L, et al. Plasma cell-free RNA characteristics in COVID-19 patients[J]. Genome Res, 2022, 32(2): 228-241.
22. Hu RW, Liu C, Yan YY, et al. Identification of hub genes and molecular subtypes in COVID-19 based on WGCNA[J]. Eur Rev Med Pharmacol Sci, 2021, 25(20): 6411-6424.
23. Gao X, Liu Y, Zou S, et al. Genome-wide screening of SARS-CoV-2 infection-related genes based on the blood leukocytes sequencing data set of patients with COVID-19[J]. J Med Virol, 2021, 93(9): 5544-5554.
24. Chua RL, Lukassen S, Trump S, et al. COVID-19 severity correlates with airway epithelium-immune cell interactions identified by single-cell analysis[J]. Nat Biotechnol, 2020, 38(8): 970-979.
25. Hue S, Beldi-Ferchiou A, Bendib I, et al. Uncontrolled innate and impaired adaptive immune responses in patients with COVID-19 acute respiratory distress syndrome[J]. Am J Respir Crit Care Med, 2020, 202(11): 1509-1519.
26. Gruber CN, Patel RS, Trachtman R, et al. Mapping systemic inflammation and antibody responses in multisystem inflammatory syndrome in children (MIS-C)[J]. Cell, 2020, 183(4): 982-995.e14.
27. Saris A, Reijnders TDY, Reijm M, et al. Enrichment of CCR6(+) CD8(+) T cells and CCL20 in the lungs of mechanically ventilated patients with COVID-19[J]. Eur J Immunol, 2021, 51(6): 1535-1538.
28. Xu F, Gao J, Munkhsaikhan U, et al. The genetic dissection of Ace2 expression variation in the heart of murine genetic reference population[J]. Front Cardiovasc Med, 2020, 7: 582949.
29. Islam ABMMK, Khan MA, Ahmed R, et al. Transcriptome of nasopharyngeal samples from COVID-19 patients and a comparative analysis with other SARS-CoV-2 infection models reveal disparate host responses against SARS-CoV-2[J]. J Transl Med, 2021, 19(1): 32.
30. Wang J, Kaplan N, Wysocki J, et al. The ACE2-deficient mouse: a model for a cytokine storm-driven inflammation[J]. FASEB J, 2020, 34(8): 10505-10515.
31. He DD, Zhang XK, Zhu XY, et al. Network pharmacology and RNA-sequencing reveal the molecular mechanism of Xuebijing injection on COVID-19-induced cardiac dysfunction[J]. Comput Biol Med, 2021, 131: 104293.
32. Qin J, Guo C, Yang L, et al. Bioinformatics and in-silico findings reveal medical features and pharmacological targets of biochanin A against colorectal cancer and COVID-19[J]. Bioengineered, 2021, 12(2): 12461-12469.
33. Badary OA. Pharmacogenomics and COVID-19: clinical implications of human genome interactions with repurposed drugs[J]. Pharmacogenomics J, 2021, 21(3): 275-284.
34. Pandey SR, Adhikari Yadav S, Gautam S, et al. Effectiveness of low-dose radiation therapy in COVID-19 patients globally: a systematic review[J]. F1000Res, 2022, 11: 62.
35. Shaath H, Vishnubalaji R, Elkord E, et al. Single-cell transcriptome analysis highlights a role for neutrophils and inflammatory macrophages in the pathogenesis of severe COVID-19[J]. Cells, 2020.
36. Galán M, Jiménez-Altayó F. Small resistance artery disease and ACE2 in hypertension: a new paradigm in the context of COVID-19[J]. Front Cardiovasc Med, 2020, 7: 588692.
37. Basile MS, Cavalli E, McCubrey J, et al. The PI3K/Akt/mTOR pathway: a potential pharmacological target in COVID-19[J]. Drug Discov Today, 2022, 27(3): 848-856.
38. Luo P, Qiu L, Liu Y, et al. Metformin treatment was associated with decreased mortality in COVID-19 patients with diabetes in a retrospective analysis[J]. Am J Trop Med Hyg, 2020, 103(1): 69-72.
39. Lally MA, Tsoukas P, Halladay CW, et al. Metformin is associated with decreased 30-day mortality among nursing home residents infected with SARS-CoV2[J]. J Am Med Dir Assoc, 2021, 22(1): 193-198.
40. Zhou Y, Hou Y, Shen J, et al. Network-based drug repurposing for novel coronavirus 2019-nCoV/SARS-CoV-2[J]. Cell Discov, 2020, 6: 14.
41. Manne BK, Denorme F, Middleton EA, et al. Platelet gene expression and function in patients with COVID-19[J]. Blood, 2020, 136(11): 1317-1329.

42. Manne BK, Münzer P, Badolia R, et al. PDK1 governs thromboxane generation and thrombosis in platelets by regulating activation of Raf1 in the MAPK pathway[J]. J Thromb Haemost, 2018, 16(6): 1211-1225.
43. S SN, Saha S, Bhattacharjee A. A 2D FSI mathematical model of blood flow to analyze the hyper-viscous effects in atherosclerotic COVID patients[J]. Results Eng, 2021, 12: 100275.
44. Vastrad B, Vastrad C, Tengli A. Bioinformatics analyses of significant genes, related pathways, and candidate diagnostic biomarkers and molecular targets in SARS-CoV-2/COVID-19[J]. Gene Rep, 2020, 21: 100956.
45. Khanal P, Chikhale R, Dey YN, et al. Withanolides from Withania somnifera as an immunity booster and their therapeutic options against COVID-19[J]. J Biomol Struct Dyn, 2022, 40(12): 5295-5308.
46. Ahirwar AK, Kaim K, Ahirwar P, et al. The second wave of COVID-19 results in outbreak of mucormycosis: diabetes and immunological perspective[J]. Horm Mol Biol Clin Investig, 2022, 43(3): 353-355.
47. Tang B, Yan C, Shen X, et al. The bidirectional biological interplay between microbiome and viruses in periodontitis and type-2 diabetes mellitus[J]. Front Immunol, 2022, 13: 885029.
48. Xie R, Lin Z, Zhong C, et al. Deciphering the potential anti-COVID-19 active ingredients in Andrographis paniculata (Burm. F.) Nees by combination of network pharmacology, molecular docking, and molecular dynamics[J]. RSC Adv, 2021, 11(58): 36511-36517.
49. Cao LH, Jia XY, He HJ, et al. Using a system pharmacology method to search for the potential targets and pathways of Yinqiaosan against COVID-19[J]. J Healthc Eng, 2022, 2022: 9248674.
50. Xie YZ, Peng CW, Su ZQ, et al. A practical strategy for exploring the pharmacological mechanism of luteolin against COVID-19/asthma comorbidity: findings of system pharmacology and bioinformatics analysis[J]. Front Immunol, 2021, 12: 769011.
51. Tianyu Z, Xiaoli C, Yaru W, et al. New tale on LianHuaQingWen: IL6R/IL6/IL6ST complex is a potential target for COVID-19 treatment[J]. Aging (Albany NY), 2021, 13(21): 23913-23935.
52. Du HX, Zhu JQ, Chen J, et al. Revealing the therapeutic targets and molecular mechanisms of emodin-treated coronavirus disease 2019 via a systematic study of network pharmacology[J]. Aging (Albany NY), 2021, 13(11): 14571-14589.

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