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结节性甲状腺肿 circRNA-miRNA-mRNA 调控网络的构建

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摘 要:基于生物信息分析筛选结节性甲状腺肿中差异表达的环状 RNA(circRNA),并揭示 circRNA-miRNA-mRNA 调控网络在结节性甲状腺肿中的作用。从 GEO 数据库中检索结节性甲状腺肿组织基因芯片数据,利用 R 软件筛选出差异表达的 circRNA。联合多个生物信息数据库预测差异表达 circRNA 下游的 miRNA 及 mRNA,并对靶 mRNA 进行 GO 及 KEGG 富集分析。利用 STRING 在线数据库及 Cytoscape 软件筛选核心基因。确定了 2 个 circRNA,42 个 miRNA 及 546 个 mRNA。GO 及 KEGG 富集分析表明靶 mRNA 主要涉及细胞生长及基因表达调控过程。基于 Cytoscape 软件筛选出了 14 个核心基因(*SP*1、*IGF*1*R*、*RPS*6*KB*1、*SMAD*2、*SMAD*3、*SMAD*4、*VEGFA*、*CCND*1、*CDK*2、*HSPA*4、*HIF*1*A*、*CREB*1,*NR*3*C*1 和 *STAT*5*A*)。最终基于 2 个 circRNA、11 个 miRNA 和 14 个核心 mRNA 构建了 circRNA-miRNA-mRNA 调控网络。结节性甲状腺肿组织中异常表达的 circRNA 及相关的 circRNA-miRNA-mRNA 调控网络可能成为结节性甲状腺肿诊断与治疗的新靶点。

关键词:结节性甲状腺肿;环状 RNA;竞争性内源 RNA;GEO 数据库
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Construction of circRNA-miRNA-mRNA regulatory network in nodular goiter

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Abstract: To screen the differentially expressed circular RNA (circRNA) in nodular goiter based on bioinformatics analysis and reveal the role of circRNA-miRNA-mRNA regulatory network in nodular goiter. The microarray data of nodular goiter tissues are retrieved from GEO database, and the differentially expressed circRNA is screened by R software. Multiple bioinformatics databases are used to predict miRNAs and mRNA downstream, and target mRNA is enriched with GO and KEGG. The core genes are selected by STRING online database and Cytoscape software. Two circRNAs, 42 miRNAs and 546 mRNA are identified. GO and KEGG enrichment analysis show that the target mRNA is mainly involved in cell growth and gene expression regulation. 14 core genes (*SP*1, *IGF*1*R*, *RPS*6*KB*1, *SMAD*2, *SMAD*3, *SMAD*4, *VEGFA*, *CCND*1, *CDK*2, *HSPA*4, *HIF*1*A*, *CREB*1, *NR*3*C*1 *and STAT*5*A*) have been selected by the Cytoscape software. Finally, the circRNA-miRNA-mRNA regulatory network is constructed based on 2 circRNAs, 11 miRNAs and 14 core mRNA. The abnormal expression of circRNA and related circRNA-miRNA-mRNA regulatory network in nodular goiter may become a new target for diagnosis and treatment of nodular goiter.
Keywords: Nodular goiter; CircRNA; CeRNA; GEO database

结节性甲状腺肿(Nodular goiter,NG)是由于甲状腺内多个区域结构和功能的改变导致滤泡上皮细胞局灶性增生,随后在甲状腺内形成结节[1]。尽管在全民实施食盐加碘后,结节性甲状腺肿的发病率

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有所下降,但其仍是临床上最常见的甲状腺疾病之一[2]。据报道,结节性甲状腺肿在人群中的发病率约为 7%,其癌变率为 7%~14%[3]。多数结节性甲状腺肿患者无明显症状,常通过超声或体格检查发现结节,但结节大于 3 cm 时易产生压迫症状,如呼吸困难、吞咽困难等[4],且患者易合并焦虑、抑郁情绪,治疗意愿较强[5]。目前,西医对于此病尚无特效疗法,指南推荐以临床随访为主[6]。因此,亟需找到结节性甲状腺肿治疗的潜在靶点。

circRNA 属于非编码 RNA 的一种,可作为 miRNA 海绵或竞争性内源 ceRNA,与其它 RNA 竞争 miRNA[7]。而 miRNA 可通过调节目标 mRNA 的翻译或稳定性(如细胞增殖、凋亡、分化、免疫等)来发挥细胞功能[8-9]。与其它 RNA 相比,circRNA 因其具有高度保守性及组织器官特异性赋予其具有诊断标志物和治疗靶点的巨大潜力[10]。目前,大量研究发现 circRNA 可调控不同细胞的增殖与凋亡,如甲状腺细胞、肝细胞、肺癌细胞、血管平滑肌细胞等[11-14]。而甲状腺细胞增殖与凋亡失衡是结节性甲状腺肿发病的主要原因[15]。本研究基于 GEO 数据库中结节性甲状腺肿组织基因芯片筛选出差异表达 circRNA 分子,并联合多个生物信息学数据库预测下游 miRNA 及 mRNA,构建 circRNA-miRNA-mRNA 调控网络,以揭示其发病的分子机制,并为临床诊断和治疗提供参考。

1 资料与方法

1.1 资料来源

以“Nodular goiter”,“circRNA”为关键词检索 GEO 数据库(http://www.ncbi.nlm.nih.gov/geo),筛选出 GSE93522 数据集。该数据集使用基因芯片技术检测了 3 例结节性甲状腺肿组织和 6 例正常组织中 circRNA 的表达量。

1.2 研究方法

1.2.1 差异表达 circRNA 的筛选

利用 R 4.0.2 软件 Limma 包寻找结节性甲状腺肿组织和正常组织中差异表达的 circRNA,筛选条件为结节性甲状腺肿组织相对正常组织 circRNA 表达量变化|logFC|≥1,且校正后 P 值(采用 Benjamini-Hochberg 方法对 P 值进行校正)小于0.05。

1.2.2 差异表达 circRNA 结合的 miRNA 预测

同时采用 4 个生物信息数据库对差异表达 circRNA 潜在结合的 miRNA 进行预测,数据库包括 circbank (http://www.circbank.cn/searchMiRNA.html)、circinteractome (https://circinteractome.irp.nia.nih.gov/)、miRDB (http://mirdb.org/cgi-bin/custom.cgi) 及 circatlas (http://circatlas.biols.ac.cn/),同时满足在 3 个或 3 个以上数据库中能预测到的视为候选 miRNA。

1.2.3 miRNA 下游靶基因预测

利用 TargetScan (http://www.targetscan.org/vert_72/), miRDB (http://mirdb.org/cgi-bin/custom.cgi), miRwalk (http://mirwalk.umm.uni-heidelberg.de/), miRTarBase (https://mirtarbase.cuhk.edu.cn/~miRTarBase/miRTarBase_2022/php/index.php), TargetMiner (http://www.isical.ac.in/~bioinfo_miu/targetminer20.htm), RNA22 (https://cm.jefferson.edu/rna22/), RNAInter (http://rnainter.org/) 等 7 大数据库预测 miRNA-mRNA 之间可能的靶向关系,至少在上述 5 个或 5 个以上数据库能预测到的视为候选 miRNA 下游 mRNA。

1.2.4 靶 mRNA GO 和 KEGG 富集分析

将预测到的靶 mRNA 输入在线分析工具(https://cloud.oebiotech.cn)进行基因本体论(Gene ontology,GO)和京都基因与基因组百科全书数据库(Kyoto encyclopedia of genes and genomes,KEGG)富集分析,筛选条件为 $P<0.01$。

1.2.5 靶 mRNA 蛋白互作 (Protein-protein interaction,PPI)网络及核心基因筛选

利用 STRING(https://string-db.org/)在线数据库获得预测的靶 mRNA 蛋白互作网络(截断值为信度评分>0.9),导入 Cytoscape 3.9.1 软件进一步绘图[16]。分别采用 Cytoscape 软件的 Cytohubba 及 MOCODE 插件(参数设置如下:degree cutoff = 2, k-core = 2, node score cutoff = 0.2, maximum depth = 100)[17]及根据 Degree 值中位数 2 倍及以上的方法筛选核心基因[18],以上 2 种方法获得的基因取交集视为最终筛选出的核心基因。

1.2.6 circRNA-miRNA-mRNA 调控网络的建立

将筛选出的核心基因与 miRNA 预测靶基因取交集,得到 miRNA-核心 mRNA 靶向关系,构建 circRNA-miRNA-核心 mRNA 调控网络,基于 Cytoscape 3.9.1 软件进行网络调控的可视化分析。

2 结 果

2.1 结节性甲状腺肿组织差异表达 circRNA

GSE93522 数据集共筛选到 2 个符合条件的差异表达 circRNA,2 个 circRNA 均表达上调,分别为 hsa_circ_0100181 和 hsa_circ_0104916,详见表 1。对差异表达 circRNA 进行后续分析。

表 1　差异表达 circRNA 基本信息

Table 1　Basic information on differentially expressed circRNA

circRNA	表达水平	logFC	Adjusted P	染色体定位	宿主基因
*hsa_circ*_0100181	上调	2.631	0.032	chr13: 28830428-28841538	*PAN3*
*hsa_circ*_0104916	上调	2.962	0.032	chr15: 93467550-93528903	*CHD2*

2.2　预测差异表达 circRNA-miRNA 和 miRNA-mRNA 靶向关系

通过 circbank、circinteractome、miRDB 及 circatlas 4 个生物信息数据库预测差异表达 circRNA 结合的 miRNA 共 42 个。基于 TargetScan,miRDB,miRwalk,miRTarBase,TargetMiner,RNA22,RNAInter 7 大数据库预测靶 miRNA 下游 mRNA,共获得 611 对 miRNA-mRNA,546 个下游靶 mRNA,详见表 2。

表 2　circRNA-miRNA 及 miRNA-mRNA 靶向关系预测结果

Table 2　Predicted results of circRNA-miRNA and miRNA-mRNA

circRNA	miRNA	mRNA 数目
	hsa-miR-767-3*p*	5
	hsa-miR-1287-5*p*	5
	hsa-miR-1294	20
	hsa-miR-3185	14
	hsa-miR-3192-5*p*	39
	hsa-miR-3713	0
*hsa_circ*_0100181	*hsa-miR*-4314	1
	hsa-miR-4722-5*p*	39
	hsa-miR-576-3*p*	3
	hsa-miR-605-3*p*	22
	hsa-miR-6736-3*p*	24
	hsa-miR-6873-5*p*	44
	hsa-miR-6894-5*p*	9
	hsa-miR-103*a*-2-5*p*	5
	hsa-miR-1200	10
	hsa-miR-1229-3*p*	10
	hsa-miR-145-5*p*	28
	hsa-miR-3126-5*p*	5
	hsa-miR-3157-5*p*	5
	hsa-miR-3944-3*p*	0
	hsa-miR-4326	2
	hsa-miR-4436*b*-5*p*	0
	hsa-miR-452-3*p*	0
	hsa-miR-4639-3*p*	0
	hsa-miR-4684-3*p*	6
	hsa-miR-4695-3*p*	0
	hsa-miR-4778-3*p*	13
*hsa_circ*_0104916	*hsa-miR*-4786-3*p*	9
	hsa-miR-5002-3*p*	2
	hsa-miR-5195-3*p*	10
	hsa-miR-5196-3*p*	0
	hsa-miR-629-3*p*	5
	hsa-miR-6748-3*p*	6
	hsa-miR-6804-5*p*	21
	hsa-miR-6812-3*p*	1
	hsa-miR-6815-3*p*	16
	hsa-miR-6834-5*p*	14
	hsa-miR-6868-3*p*	48
	hsa-miR-6873-3*p*	149
	hsa-miR-6882-3*p*	14
	hsa-miR-767-3*p*	5
	hsa-miR-877-3*p*	2

2.3　miRNA 下游靶基因 GO 和 KEGG 富集分析

将预测到的 546 个靶基因导入在线分析工具进行 GO 和 KEGG 富集分析,结果见图 1 和图 2。GO 富集分析显示,细胞成分注释方面,靶基因最常见的细胞定位是核质,其次为基底膜等;生物过程注释方面,靶基因主要参与 RNA 聚合酶 II 启动子转录调控、模板 DNA 转录、细胞增殖调控、器官生长等;分子功能方面,靶基因主要参与肌动蛋白、离子通道、β 连环蛋白、蛋白磷酸酶 2A 的结合等。KEGG 富集结果表明,上述靶基因主要富集于癌症相关信号通路、AGE-RAGE in diabetic complications 信号通路、Endocytosis, PI3K-Akt 信号通路, MicroRNAs in cancer,TGF-beta 信号通路等。

2.4　PPI 网络分析及核心基因筛选

通过 STRING 在线工具获得靶基因蛋白互作网络图,进一步导入 Cytoscape 软件绘图,见图 3。基于 Cytoscape 软件筛选出 14 个核心基因,分别是 *SP*1, *IGF*1*R*, *RPS*6*KB*1, *SMAD*2, *SMAD*3, *SMAD*4, *VEGFA*, *CCND*1, *CDK*2, *HSPA*4, *HIF*1*A*, *CREB*1, *NR*3*C*1 和 *STAT*5*A*。

2.5　circRNA-miRNA-mRNA/核心基因调控网络的构建

根据上述结果最终确定了 2 个 circRNA,11 个 miRNA 和 14 个核心 mRNA,建立了 circRNA-miRNA-mRNA 调控网络,展示 ceRNA 在结节性甲状腺肿中可能的调控机制,见图 4。

3　讨　论

大多数非蛋白质编码 RNA 被认为具有参与细胞稳态的多样化功能。circRNA 作为一种非编码 RNA,除了直接影响 mRNA 转录外,还可以通过 miRNA 海绵或 RNA 结合蛋白来调控靶基因的表达,对许多生理和病理状态的启动和发展起着关键作用[19]。近年来,大量研究揭示了 circRNA-miRNA-mRNA 轴在细胞增殖[11-12]、细胞凋亡[13]、胰岛素抵抗[19]、血管生成[20]等中的作用机制。相似的是,这些病理过程同时促进了结节的发生和发展[15,21]。

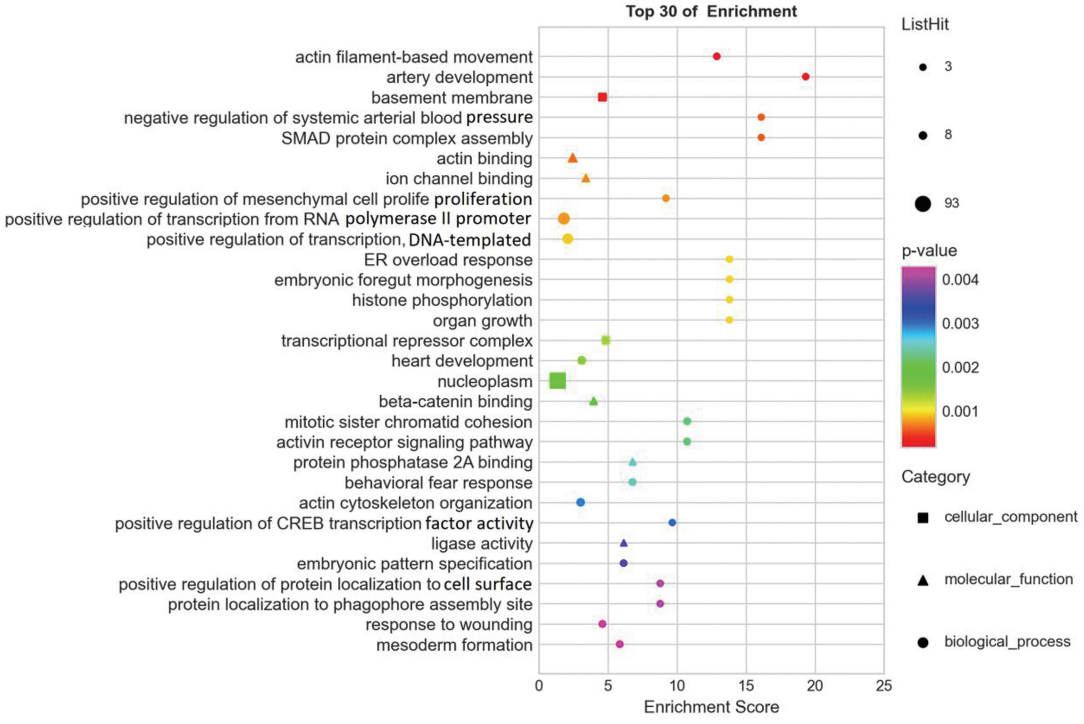

图 1 靶基因 GO 分析

Fig.1 GO analyses of target gene

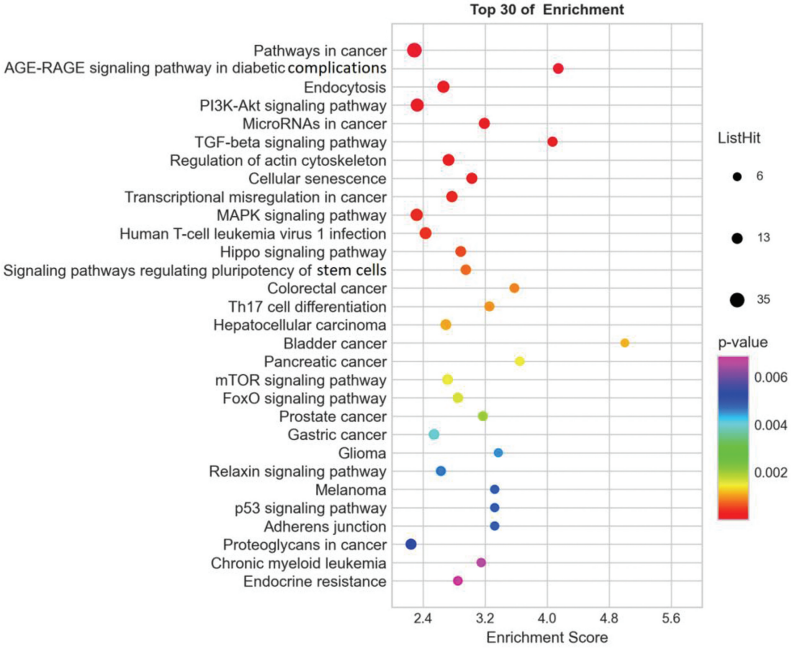

图 2 靶基因 KEGG 富集分析

Fig.2 KEGG analyses of target gene

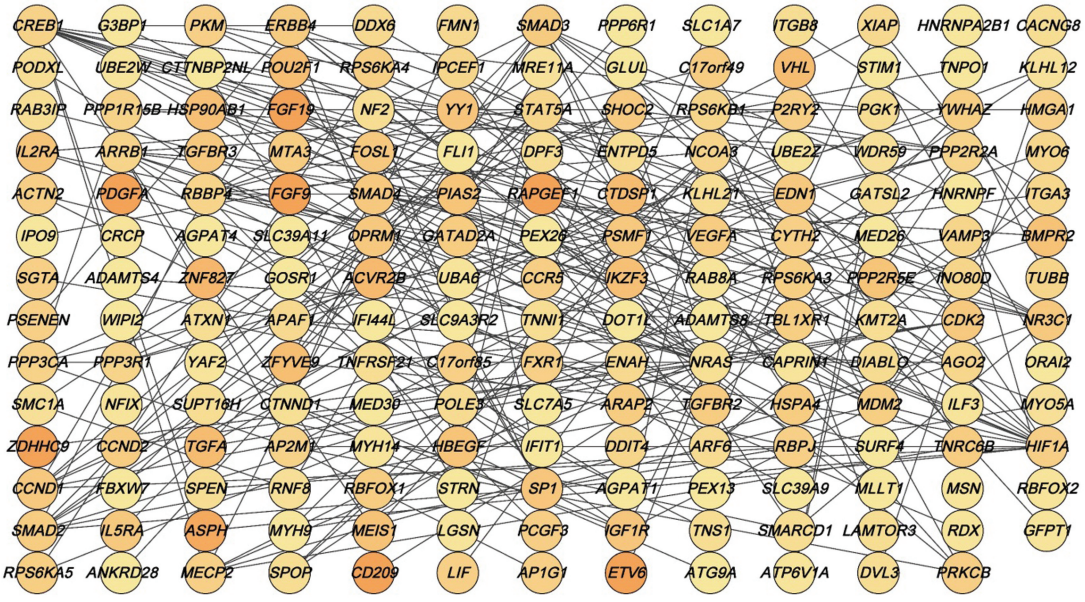

图 3　靶基因 PPI 网络

Fig.3　PPI network of target gene

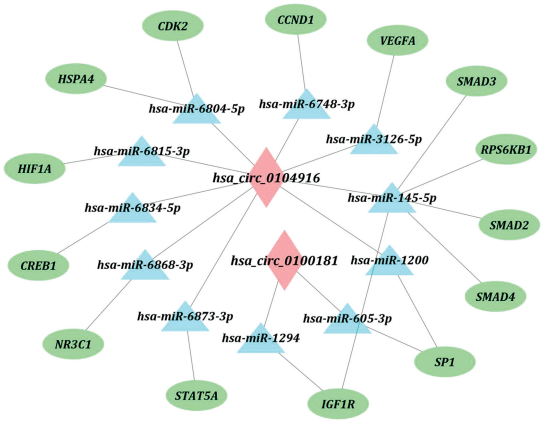

图 4　circRNA-miRNA-mRNA 调控网络

Fig.4　circRNA-miRNA-mRNA regulatory network

注:菱形代表 circRNA,三角形代表 miRNA,圆形代表 mRNA/核心基因.

目前关于 circRNA 在结节性甲状腺肿中的作用鲜有报道。本研究基于 GEO 数据库发现,与正常组织相比,结节性甲状腺肿组织中 hsa_circ_0100181 和 hsa_circ_0104916 表达上调,基于生物信息学分析确定了匹配度最高的 circRNA-miRNA 及 miRNA-mRNA。对靶基因进行 KEGG 富集分析发现它们在 PI3K-Akt 信号通路富集明显,这与文献报道结果一致[22]。hsa_circ_0100181 在人类和小鼠的肠道干细胞中高度表达,并且以肽非依赖性方式调节肠道干细胞的自我更新[23]。而在甲状腺结节患者中发现肠道微生物种类和基因家族数量的减少[24],这提示 hsa_circ_0100181 可能通过调节肠道功能影响甲状腺代谢。前期研究发现 has-miR-1294 可直接靶向抑制 IGF1R 的表达[25]。而在甲状腺中,IGF-1 由甲状腺滤泡上皮细胞合成,可通过自分泌及旁分泌的方式调节甲状腺细胞增殖[26],因此 hsa_circ_0100181/has-miR-1294/IGF1R 调控通路也可能成为治疗结节性甲状腺肿的潜在通路。VEGF 能促进血管内皮细胞增殖,改变毛细血管通透性,并通过旁分泌的方式作用于 VEGFR 从而促进甲状腺内血管生成[27]。VEGF 在正常甲状腺组织中呈阴性表达,而在结节性甲状腺肿患者中表达升高[28],本研究结果发现 hsa_circ_0104916 可通过 hsa-miR-3126-5p 影响 VEGFR 表达,这可能促进了结节性甲状腺肿的发生与发展。CREB 是一类转录因子,能特异性结合基因启动上游 cAMP 反应元件[29]。结节性甲状腺肿多由于缺碘引起甲状腺激素合成减少,促甲状腺激素反馈性升高,而促甲状腺激素可通过 TSHR-cAMP-PKA 途径磷酸化 CREB,从而调节 NIS 和 TPO,促进甲状腺组织增生[21]。因此 hsa_circ_0104916/has-miR-6834-5p/CREB1 调控轴可能是结节性甲状腺肿发病的重要机制。TGF-β1/SMADS 信号通路可调控细胞的增殖、凋亡、分化等生物过程[30],而 has-miR145-5p 负反馈调控 TGF-β1/SMADS 通路[31],这与本研究的结果吻合,提示 hsa_circ_0104916/ has-miR145-5p/SMADS 可能在结节性甲状腺肿发生发展中发挥一定的作用。此外,前期研究发现 has-miR-605-3p,hsa-miR-1200 在细胞增殖及凋亡中发挥重要作用[32-33],但其在甲状腺细胞中的作用尚未被揭示。未来可进一步深入研究上述 miRNA 对甲状腺细胞的作用。

综上所述,本研究确定了 16 个 circRNA-miRNA-

mRNA 相关信号轴,这些调控轴可能在结节性甲状腺肿的发生发展中占据着重要的位置。由于该结果仅基于生物信息学分析,未来还需要细胞和动物实验进一步验证。

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