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阿尔茨海默病ceRNA调控网络的生物信息学分析

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摘　要

阿尔茨海默病(Alzheimer's disease, AD)是痴呆症最常见的病因，它会导致衰老大脑中神经元的损伤和认知功能的退化。有证据表明非编码RNA (ncRNAs)参与AD相关的发病过程，并揭示了AD与竞争性内源性RNA (ceRNA)网络之间的潜在联系。鉴于此，本研究深入探讨ceRNA调控网络与AD疾病发病机制的潜在关系，为临床治疗提供基础。方法：通过GEO2R筛选差异mRNA和miRNA。利用DAVID对差异表达的mRNA作GO富集分析，筛选与神经系统疾病相关的通路中的差异性表达mRNA用作后续研究。选取top 10的miRNA (5个上调表达的miRNA和5个下调表达的miRNA)，使用miRWalk 3.0数据库预测其与目标差异表达mRNA之间的靶向关系，对预测的靶向mRNA和通路筛选到的目标差异表达mRNA取交集，此mRNA作为后续研究的目标差异表达mRNA，使用Cytoscape软件构建miRNA-mRNA靶向调控网络关系图。分别利用DIANA和ENCORI数据库做lncRNA-miRNA和circRNA-miRNA关系对的预测。最后构建circRNA-miRNA-mRNA和lncRNA-miRNA-mRNA ceRNA调控网络，并进行分析，这将有助于理解和阐明AD的发病机制，并为AD的临床诊断提供可能选择的生物标志物。

关键词

阿尔茨海默症，ceRNA，网络

Bioinformatics Analysis of ceRNA Regulatory Network in Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is the most common cause of dementia. It will lead to the damage of neurons in the aging brain and the degradation of cognitive function. There is evidence that non-coding RNAs (ncRNAs) are involved in the pathogenesis of AD and reveal the potential relationship between AD and competitive endogenous RNA (ceRNA) network. In view of this, this study deeply discusses the potential relationship between ceRNA regulatory network and the pathogenesis of AD disease, so as to provide a basis for clinical treatment. Differential mRNA and miRNA were screened by GEO2R. DAVID was used for go enrichment analysis of differentially expressed mRNA to screen differentially expressed mRNA in pathways related to nervous system diseases for follow-up study. The miRNAs of top 10 (5 up-regulated miRNAs and 5 down-regulated miRNAs) were selected, and the targeting relationship between them and the target differentially expressed mRNA was predicted using miRWalk 3.0 database. The intersection of the predicted target mRNA and the target differentially expressed mRNA screened by the pathway was taken as the target differentially expressed mRNA for subsequent research. The relationship diagram of miRNA-mRNA targeted regulation network was constructed by Cytoscape software. DIANA and ENCORI database was used to predict the relationship between lncRNA-miRNA and circRNA-miRNA. Finally, circRNA-miRNA-mRNA and lncRNA-miRNA-mRNA ceRNA regulatory networks were constructed and analyzed, which will help to understand and clarify the pathogenesis of AD and provide possible biomarkers for clinical diagnosis of AD.

Keywords

Alzheimer's Disease, ceRNA, Network

1. 引言

阿尔茨海默病(Alzheimer's disease, AD)是一种慢性进行性神经退行性疾病，是最常见的老年痴呆类型[1]。AD 的发病机制目前尚未完全阐明。

随着先进的全基因组和转录组等测序技术的发展，越来越清楚的是，人类基因组的 80%被转录为非编码 RNA (ncRNAs)，在细胞中行使必要的功能[2]。ncRNA 是一个庞大而多样的非蛋白编码转录物家族，通过许多不同的机制控制基因表达程序来调节细胞功能。实验证明与 AD 相关的 ncRNA 包括 circular RNA (circRNA)、long non-coding RNA (lncRNA)、microRNA (miRNA)等。

竞争性内源 RNA (competing endogenous, ceRNA)是一种能够竞争结合 RNA 的作用元件，通常 lncRNA 和 circRNA 会竞争结合 miRNA。ceRNA 调控网络中包括四种 RNA，即 mRNA、miRNA、circRNA 和 lncRNA，其中 miRNA 处于调控的核心地位。

MiRNA 是一类单链 RNA 分子，研究表明，miRNA 与突触功能和记忆形成过程中的特定信号密切相关[3] [4]。miRNA 作为一个转录后调控的重要因子，其活性可被 lncRNA 通过“海绵”吸附的方式调控。lncRNA 已被证实参与 AD 疾病中 β 淀粉酶的生成、突触损伤、神经营养因子的丢失、炎症、线粒体功能

障碍、氧化应激等多种过程[5] [6] [7]。

circRNA 可以通过吸附 miRNA、与 mRNA 结合蛋白相互作用或调节转录来调控基因的表达，甚至可以翻译产生多肽。最近的报告显示，circRNA 在 AD 等神经退行性疾病的发展中起着重要作用[8]。

越来越多的研究发现 ncRNAs 与 AD 发病机制有关[9]，尽管如此，与 AD 相关的 ceRNA 调控网络研究较少见，本文对 ceRNA 调控网络与 AD 发病机制的关系进行系统的挖掘，深入探讨 ceRNA 调控网络与 AD 疾病发病机制的潜在关系，为临床治疗提供基础。

2. 材料与方法

2.1. 材料

通过美国国立生物技术信息中心(NCBI)平台的基因表达综合数据库 GEO (Gene Expression Omnibus)，在人类物种下以“Alzheimer’s disease”为关键词寻找相关数据芯片，分别检索有关 mRNA 和 miRNA 的原始数据。本项目下载 mRNA 表达谱 GSE18309 (下载日期：2021/07/18)和 miRNA 表达谱 GSE120584 (下载日期：2021/07/18)进行后续数据分析。

2.2. 差异表达分析

利用 GEO 数据库自带的交互式线上分析工具 GEO2R 对 GSE18309 (3 个 AD 样本和 3 个正常样本)和 GSE120584 (1021 个 AD 样本和 288 个正常样本)两个数据集进行分析，在 miRNA 和 mRNA 中比较出正常对照组和 AD 组中存在差异表达的信息，筛选出差异表达 miRNA 和 mRNA (P 值均≤0.05，其中 miRNA 数据集 FDR 也≤0.05)。

2.3. DAVID 做差异表达基因的本体论功能富集分析

使用 DAVID 对差异表达的 mRNA 进行 GO 通路富集分析，从生物学过程(BP, Biological Process)、细胞成分(CC, Cellular Component)及分子功能(MF, Molecular Function)三个角度，对这些基因的功能进行描述，以检测潜在的生物学功能和通路，筛选标准为 $P \leq 0.05$。

2.4. 目标基因筛选

分析生物学过程，通过注释信息，筛选与 AD 发病相关的信号通路基因进行后续分析。

2.5. 目标基因 ceRNA 调控网络分析

选取表达量高的 top 10 miRNA，使用 miRWalk 3.0 数据库预测 10 个 miRNA 的靶向 mRNA，并对预测的靶向 mRNA 和通过 AD 发病相关通路筛选到的目标差异表达 mRNA 取交集，利用 Cytoscape 3.8.2 构建 miRNA-mRNA 网络关系图。利用 DIANA 数据库对 top 10 miRNA 进行预测，获得 lnRNA-miRNA 关系对；利用 ENCORI 数据库预测 top 10 miRNA 的靶向 circRNA，获得 circRNA-miRNA 关系对，然后与文献中已报道的 AD 相关 circRNA 取交集，得到的重叠 circRNA 作为目标 circRNA。最后利用以上靶向关系，构建 circRNA-miRNA-mRNA 和 lncRNA-miRNA-mRNA ceRNA 调控网络，并探讨其与 AD 发病机制的关系。

3. 结果

3.1. 人类血液中差异性表达 mRNA 及 miRNA 的筛选

用 GEO2R 来筛选差异表达的 miRNA 和 mRNA，得到 1819 个差异表达的 mRNA，包括 769 个上调

基因和 1050 个下调基因；742 个差异表达的 miRNA，包括 642 个上调表达 miRNA 和 100 个下调表达 miRNA。

3.2. 差异表达基因的本体论功能富集分析

在 DAVID 软件中进行基因的本体论功能富集 GO 分析发现，差异表达基因中，ITGB1，SPARC，COL13A1 和 ITGB5 等 36 个基因在细胞外基质(extracellular matrix organization)的生物学过程中起作用，ITGB1，RAB1A，FOXE1 和 WWC1 等 26 个基因在细胞迁移(cell migration)的生物学过程中起作用，SPON1，NRP2，ITGB5 和 ITGB4 等 53 个基因在细胞粘附(cell adhesion)中发挥作用。根据 P 值大小排序，GO 分析中生物学过程富集结果前 20 名 P 值对数大小结果(见图 1)。

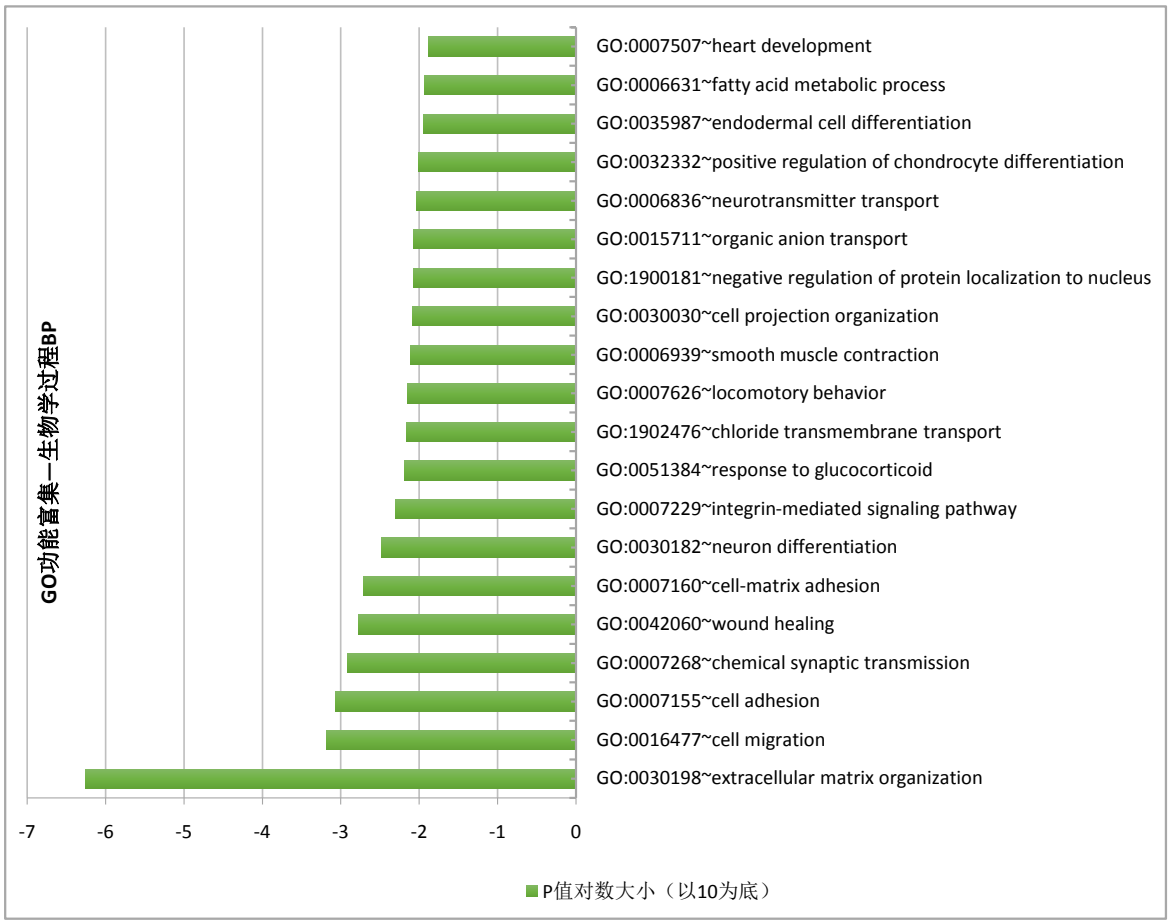


Figure 1. Functional enrichment of differentially expressed gene GO—BP results of biological process
图 1. 差异表达基因 GO 功能富集——生物学过程 BP 结果

差异表达基因在细胞连接(cell junction)、基底膜(basement membrane)、蛋白质细胞外基质(proteinaceous extracellular matrix)等处发挥作用。根据 P 值大小排序，GO 分析中细胞成分富集结果前 20 名 P 值对数大小结果(见图 2)。

差异表达基因在肝素结合(heparin binding)、钙离子结合(calcium ion binding)、细胞外配体门控离子通道活性(extracellular ligand-gated ion channel activity)等分子功能中起作用。根据 P 值大小排序，GO 分析中分子功能富集结果前 20 名 P 值对数大小结果(见图 3)。

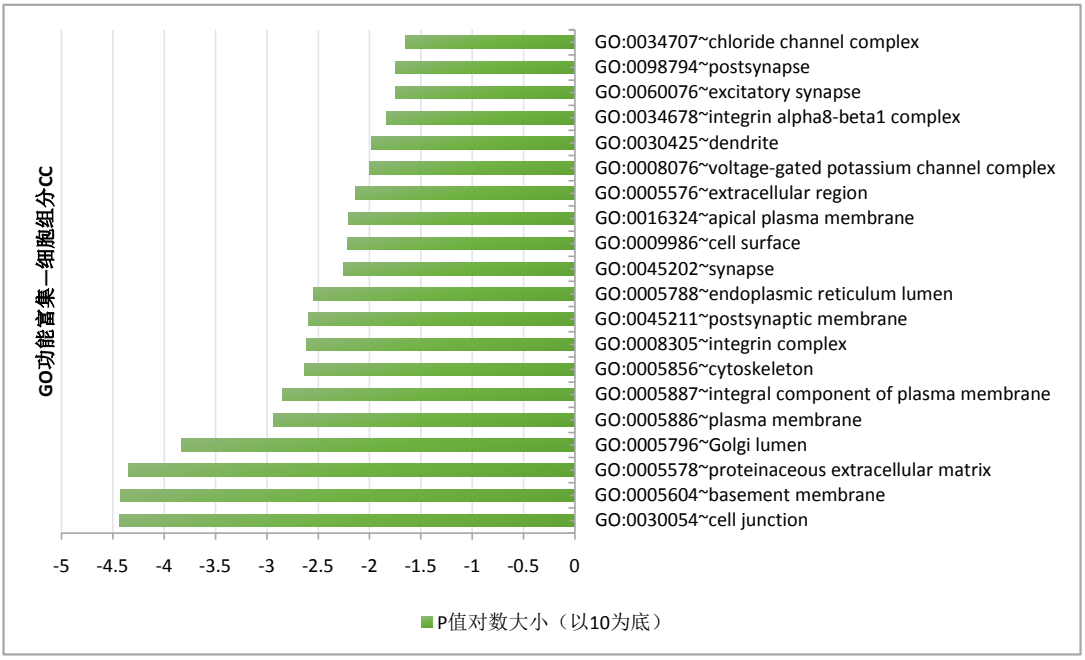


Figure 2. Functional enrichment of differentially expressed gene GO—cell component CC results
图 2. 差异表达基因 GO 功能富集——细胞组分 CC 结果

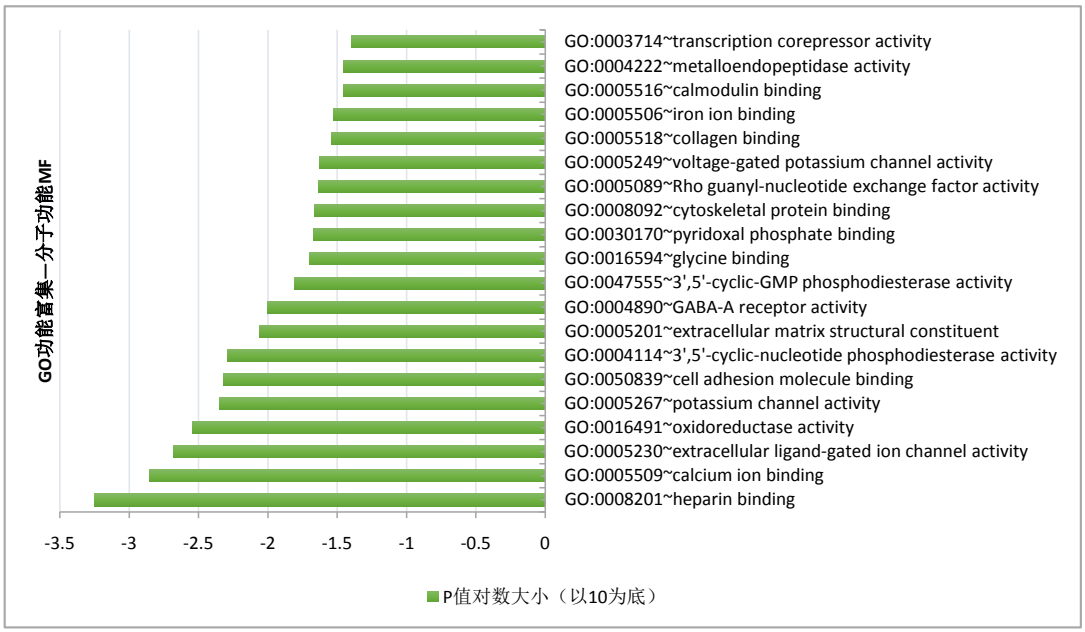


Figure 3. Functional enrichment of differentially expressed gene GO—results of molecular function MF
图 3. 差异表达基因 GO 功能富集——分子功能 MF 结果

3.3. 功能分析

使用 DAVID 对差异表达的 mRNA 进行 GO 通路富集分析，依据生物学过程，筛选了 18 条与神经系统疾病相关的通路(见表 1)，选择这些通路所对应的基因，经过对这些通路包含的基因进行重复项等的筛选，共得到 252 个基因。

Table 1. 18 pathways related to AD
表 1. 与 AD 相关的 18 条通路

Term	Count	P Value	Genes
GO:0007268~chemical synaptic transmission	32	0.001211904	OPRD1, CHRNA4, KCNA1, NRXN1, CACNA1B, GRIK2, GJC1, GLRA3, HTR7, KCNMB2, DLGAP1, MBP, HCRT, NTSR1, UNC13C, HTR1D, PMCHL2, GAD2, SLC6A11, OPRM1, SSTR2, SYN2, GABRG1, GRIN1, LRFN5, DLG4, SST, KCNQ2, KCNQ5, FGF12, RAPSN, SCN2B
GO:0030182~neuron differentiation	16	0.003318537	BTG4, WNT10A, TRPC6, WNT2B, NLGN4X, WNT5B, DAPK3, WNT5A, ATP2B2, FZD10, SMARCA1, WNT16, POU3F2, HIPK2, WNT6, NNAT
GO:1902476~chloride transmembrane transport	15	0.006875807	GABRB3, GABRA2, GABRP, CLIC5, GABRB1, SLC12A1, SLC4A1, ANO4, GABRG2, CLCN1, GABRG1, CLCN7, GLRA3, FXYD1, SLC17A7
GO:0006836~neurotransmitter transport	7	0.009331592	GABRA2, SLC6A8, SLC6A16, SLC6A15, SLC17A7, SLC6A11, SLC17A8
GO:0007165~signal transduction	105	0.013442611	DGKG, CHRM3, SPARC, RAPH1, ARR3, ANTXR1, ARHGAP44, GRB14, ADGRA1, AKT2, RASSF6, SOX9, HLA-DOA, CHRNB4, TLE1, KCNK10, IGFBP2, ANK2, TOM1L1, CLDN4, BCAM, S100A6, PLA2R1, PDE9A, PDE1C, CHRNA4, CABP5, CABP2, SAV1, C3, CD79B, CASKIN1, OR51B5, CGA, MAP2K7, HMGA2, NR2F2, ESR1, GNG11, ARHGAP33, CXCL11, FGF14, RHEB, DLG4, DLG5, FGF18, PPP1R1B, FGF12, LRP12, NOX1, GABRB3, ACVRL1, BCAR3, GABRB1, TRADD, LIMD1, ARHGAP5, ADRA1A, WISP1, CXCL5, SMPD1, PDE4A, PRKG1, TMED4, RPS6KL1, ANXA1, SMAD5-AS1, PDE4D, IL19, TRAF4, CEACAM6, RARA, PDE5A, PLCB1, DOCK1, DEPDC4, ROCK1, PLA2G1B, NRXN1, NEDD9, ACVR1B, PDE11A, ERBB3, WIF1, SPOCK3, PDE6B, PAK5, PAK4, GABRP, RCVRN, EGF, AMFR, KCNIP3, LIMK1, NR1H4, ACVR2B, SMOC2, P2RX6, KITLG, CDKN2AIPNL, DLC1, SARM1, RGS12, SIGLEC8, FCGR2C
GO:0043524~negative regulation of neuron apoptotic process	18	0.014571801	GABRB3, DLX1, JUN, ROCK1, CITED1, ANGPT1, ITSN1, NDNF, AMBRA1, GRIK2, HIPK2, GRIN1, TOX3, ERBB3, STAR, BIRC5, LGMN, SNCA
GO:0019934~cGMP-mediated signaling	4	0.016093962	EDNRB, PDE3A, PDE9A, PRKG1
GO:0035024~negative regulation of Rho protein signal transduction	5	0.019592119	ITGB1, DLC1, TNFAIP1, ADRA1A, ARHGAP35
GO:0007200~phospholipase C-activating G-protein coupled receptor signaling pathway	11	0.019716351	OPRD1, CCKAR, EDNRB, HTR1D, AGTR1, PLCE1, OPRM1, HCRT, ESR1, ADRA1A, GNG13
GO:0043547~positive regulation of GTPase activity	55	0.021952213	BCAR3, DOCK6, ITGB1, TRIO, DENND5B, RGSL1, ITSN1, KNDC1, FZD10, ARHGAP5, ARHGAP35, RCBTB2, ARHGAP44, PREX2, RASGEF1C, PLCE1, AGFG2, GARNL3, PDGFRA, ARHGEF12, FRS2, AXIN2, CDC42EP4, IL3RA, PKP4, DENND6B, RAPGEF5, PLCB1, DOCK1, RGS18, ARHGEF28, RGS16, NPRL3, AGAP11, ASAP3, ARHGAP18, AGAP1, ERBB3, EPS8L2, FARP1, JUN, ANGPT1, EGF, ARHGEF39, GRIN1, ARHGAP33, KITLG, DNMBP, DLG4, DLC1, FGF18, GNAS, RGL3, RGS12, FGFR4

Continued

GO:0007266~Rho protein signal transduction	9	0.026142736	ROPN1B, COL1A2, ROCK1, CDC42EP4, LIMK1, RHOJ, AGTR1, EPS8L2, ARHGAP5
GO:0051924~regulation of calcium ion transport	6	0.026410095	OPRD1, RCVRN, SLN, CACNA2D1, CACNA1B, ANK2
GO:0050808~synapse organization	7	0.029193173	NFASC, NLGN4X, LRRTM1, LRP4, TNR, ATP2B2, SNCA
GO:0035023~regulation of Rho protein signal transduction	12	0.031283484	PREX2, FARP1, PLEKHG4, ARHGEF10, DNMBP, TRIO, ARHGEF12, ARHGEF39, DLC1, ARHGEF28, ITSN1, EPS8L2
GO:0007413~axonal fasciculation	5	0.036960688	SEMA3A, NRCAM, CNTN4, EPHB2, ARHGAP35
GO:0035725~sodium ion transmembrane transport	11	0.036961832	SLC13A1, SCNN1G, SLC34A2, SLC4A9, SLC6A8, SLC24A3, SLC4A11, SLC17A7, KCNK1, SLC17A4, SCN2B
GO:0007411~axon guidance	19	0.039233935	MEG3, NRP2, NRXN1, SEMA3A, WNT5A, NTN4, CRMP1, EFNA5, ARHGAP35, EFNA1, NFASC, RPS6KA5, EFNA2, FLRT3, FEZ1, BOC, TNR, CNTN4, EPHB2
GO:0060079~excitatory postsynaptic potential	6	0.048161299	SLC17A7, GRIK2, OPRM1, HCRT, GRIN1, SNCA

3.4. 差异表达 miRNA 的靶基因预测

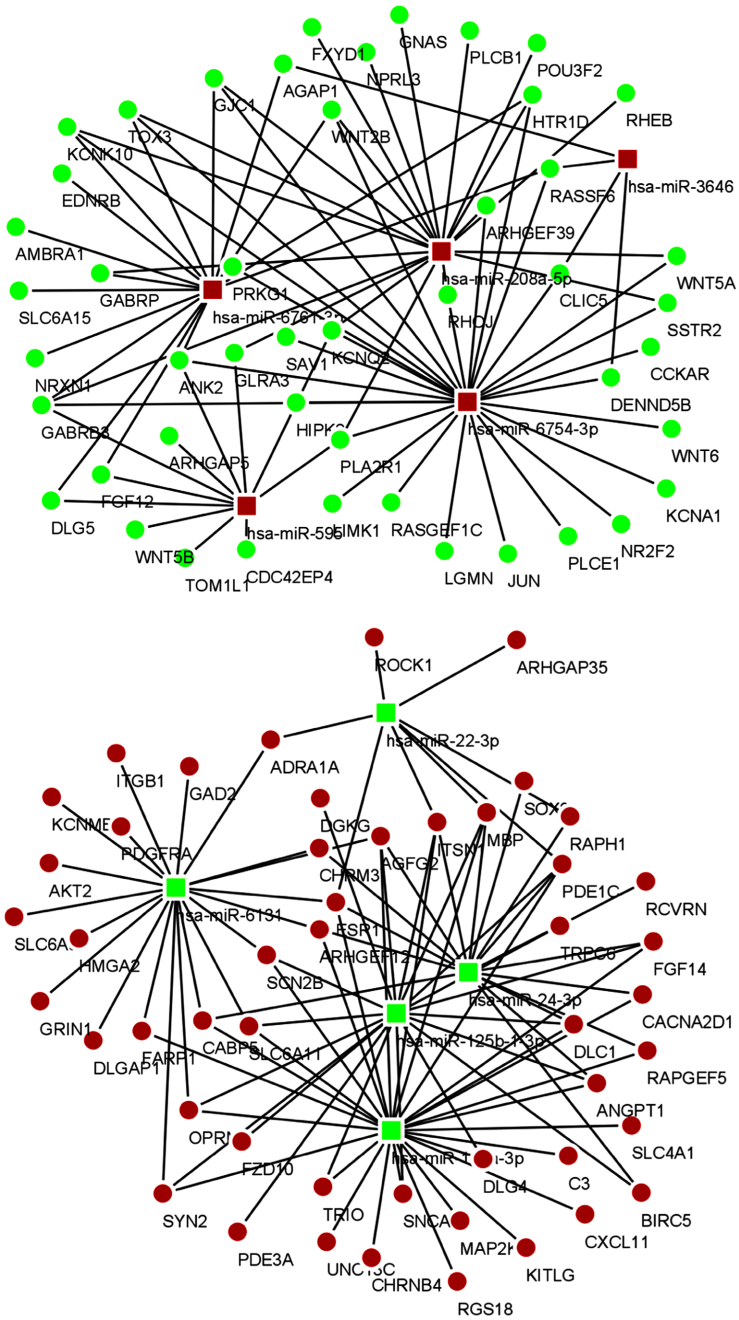
利用 miRWalk 3.0 数据库预测 10 个 miRNA (见表 2)的靶向 mRNA，对预测的靶向 mRNA 和通路筛选到的目标差异表达 mRNA 取交集，得到 181 个重合的差异表达基因。

Table 2. 10 highly expressed miRNAs
表 2. 10 个高表达的 miRNA

miRNA 名称	miRNA 表达变化
has-miR-208a-5p	上调
hsa-miR-6761-3p	上调
hsa-miR-3646	上调
hsa-miR-595	上调
hsa-miR-6754-3p	上调
hsa-miR-125a-3p	下调
hsa-miR-6131	下调
hsa-miR-24-3p	下调
hsa-miR-125b-1-3p	下调
hsa-miR-22-3p	下调

3.5. miRNA-Gene 靶向调控网络的构建

利用 miRWalk 3.0 数据库预测 10 个高表达 miRNA 的靶向 mRNA，并对预测的靶向 mRNA 和通路筛选到的目标差异表达 mRNA 取交集，得到相对应的 miRNA-mRNA 关系对，选取有特定对应关系的 miRNA 和 mRNA (上调 miRNA 对应下调的 mRNA，下调 miRNA 对应上调的 mRNA)，使用 Cytoscape 3.8.2 构建 miRNA-mRNA 靶向调控网络，如下(见图 4)，并可以借此图讨论其生物学意义。



注：红色代表上调，绿色代表下调；方形代表 miRNA，圆形代表 mRNA (上调 miRNA 对应下调 mRNA，下调 miRNA 对应上调 mRNA)。

Figure 4. miRNA-Gene targeting regulatory network
图 4. miRNA-Gene 靶向调控网络

3.6. lnRNA-miRNA 关系对和 circRNA-miRNA 关系对的预测

利用 DIANA 数据库获得 lnRNA-miRNA 关系对，选取 Score = 1 的 lncRNA (见表 3)；利用 ENCORI 数据库中预测 circRNA-miRNA 关系对，然后与文献中和 AD 相关的 circRNA 取交集，得到重叠的 circRNA (见表 4)。

Table 3. Relationship between lncRNA-miRNA-mRNA
表 3. lncRNA-miRNA-mRNA 关系表

miRNAs	mRNA	lncRNAs targeting the miRNAs
hsa-miR-208a-5p	ARHGEF39; FXYD1; GABRB3; GABRP; GJC1; GLRA3; GNAS; HTR1D; KCNK10; KCNQ2; NPRL3; PLA2R1; PLCB1; POU3F2; RHEB; RHOJ; SSTR2; TOX3; WNT2B; WNT5A	chr22-38_28785274-29006793.1; KCNQ1OT1
hsa-miR-6761-3p	AGAP1; AMBRA1; ANK2; DLG5; EDNRB; FGF12; GABRB3; GABRP; GJC1; HTR1D; KCNK10; NRXN1; PRKG1; RASSF6; SLC6A15; TOX3; WNT2B	
hsa-miR-3646	AGAP1; CLIC5; DENND5B; RASSF6	XLOC_011696; chr22-38_28785274-29006793.1; XLOC_002204; AC006548.28; XLOC_012784; CTB-83J4.2; NUTM2B-AS1; CTB-92J24.2; TSIX; MIR6818; LINC00665; KCNQ1OT1; RP11-96K19.4
hsa-miR-595	ANK2; ARHGAP5; CDC42EP4; DLG5; FGF12; GABRB3; GLRA3; KCNQ2; PLA2R1; TOM1L1; WNT5B	chr22-38_28785274-29006793.1; XLOC_001711; XLOC_013708
hsa-miR-6754-3p	ANK2; ARHGEF39; CCKAR; CLIC5; DENND5B; GABRB3; GJC1; HIPK2; HTR1D; JUN; KCNA1; KCNK10; KCNQ2; LGMN; LIMK1; NR2F2; PLA2R1; PLCE1; PRKG1; RASGEF1C; RASSF6; RHOJ; SAV1; SSTR2; TOX3; WNT2B; WNT5A; WNT6	RP11-780O24.2; chr22-38_28785274-29006793.1; CTD-2291D10.2; RP11-274B21.9; XLOC_013272; RP11-47L3.1; TTTY10; LINC01002; XLOC_003171; RP11-586K12.10; AF127936.7; RP11-1437A8.4; XLOC_002461; RP11-34P13.13; RP11-98O2.1; KCNQ1OT1; XLOC_013276; AC018717.1; XLOC_007621; XLOC_004058
hsa-miR-125a-3p	AGFG2; ANGPT1; ARHGEF12; C3; CABP5; CACNA2D1; CHRNB4; CXCL11; DLC1; DLG4; ESR1; FARP1; FGF14; ITSN1; KITLG; MAP2K7; MBP; OPRM1; PDE1C; RAPGEF5; RGS18; SCN2B; SLC4A1; SLC6A11; SNCA; SYN2; TRIO; UNC13C	chr22-38_28785274-29006793.1; KCNQ1OT1
hsa-miR-6131	ADRA1A; AGFG2; AKT2; ARHGEF12; CABP5; CHRM3; DLGAP1; ESR1; FARP1; GAD2; GRIN1; HMGA2; ITGB1; KCNMB2; OPRM1; PDGFRA; SCN2B; SLC6A11; SLC6A8; SYN2	chr22-38_28785274-29006793.1; LOC100190986; KCNQ1OT1; XLOC_009222; AC114776.3; MIR6818
hsa-miR-24-3p	AGFG2; ANGPT1; ARHGEF12; BIRC5; CABP5; CACNA2D1; CHRM3; DLC1; ESR1; FGF14; ITSN1; MBP; PDE1C; RAPGEF5; RAPH1; RCVRN; SOX9	chr22-38_28785274-29006793.1; XLOC_013272; XLOC_007432; XLOC_006331; RP11-856M7.6; KCNQ1OT1; MIR4534; RP11-856M7.6; XLOC_009043; XLOC_007663; RP11-402G3.5
hsa-miR-125b-1-3p	AGFG2; ANGPT1; BIRC5; DGKG; DLC1; DLG4; FGF14; FZD10; ITSN1; MBP; OPRM1; PDE1C; PDE3A; SCN2B; SLC6A11; SNCA; SYN2; TRIO; TRPC6	
hsa-miR-22-3p	MBP; ADRA1A; ARHGAP35; ESR1; ROCK1; ITSN1; RAPH1; PDE1C	chr22-38_28785274-29006793.1; XLOC_006684; XLOC_003973; XLOC_010383

Table 4. Relationship between circRNA-miRNA-mRNA
表 4. circRNA-miRNA-mRNA 关系表

miRNAs	mRNA	circRNAs targeting the miRNAs
hsa-miR-24-3p	AGFG2; ANGPT1; ARHGEF12; BIRC5; CABP5; CACNA2D1; CHRM3; DLC1; ESR1; FGF14; ITSN1; MBP; PDE1C; RAPGEF5; RAPH1; RCVRN; SOX9	LRRC47; ACOT7; MTOR; DNAJC16; NECAP2; USP48; ID3; WASF2; YTHDF2; ZMYM4; PPCS; AKR1A1; FAF1; EPS15; DHCR24; JAK1; VCAM1; NOTCH2; NOTCH2NL; PEX11B; PBXIP1; ASH1L; UCK2; CREG1; DCAF6; CEP350; STX6; ELK4; BIRC6; FEZ2; PRKCE; TTC7A; RTN4; USP34; GGCX; SAP130; UGGT1; MGAT5; ITGA6; GLS; IGFBP5; TNS1; CTDSP1; CUL3; PSMD1; ARMC9; CMTM6; MYD88; CTNNB1; MAPKAPK3; ROBO1; ZBTB11; BBX; CDV3; TOPBP1; WWTR1; GFM1; NMD3; LPP; OPA1; HES1; PPP1R2; CTBP1; FGFR3; WHSC1; TMEM165; GRSF1; ANKRD17; G3BP2; WDFY3; PDLIM5; TBC1D9; SLC12A7; NSUN2; VCAN; UQCRQ; HSPA4; DDX46; MATR3; PDGFRB; WWC1; MAML1; C6orf62; BTN3A3; CDKN1A; TFEB; CNPY3; EEF1A1; SENP6; SNX3; GJA1; HIVEP2; SCAF8; TMEM181; EIF2AK1; TNS3; GBAS; BAZ1B; BCL7B; HIP1; CDK14; CDK6; LMTK2; CUX1; TMEM209; NUP205; ZC3HAV1; UBN2; HIPK2; MTMR9; TNFRSF10B; SCARA3; PROSC; BAG4; PCMTD1; YWHAZ; UBR5; PHF20L1; KANK1; AGTPBP1; FAM120A; IKBKAP; ZBTB34; TSC1; CAMSAP1; NACC2; NOTCH1; LRRC20; ADK; SMC3; FAM53B; ZRANB1; CTBP2; TEAD1; NAV2; ARHGAP1; MTCH2; SDHAF2; AHNAK; POLR2G; CDC42EP2; FCHSD2; DYNC2H1; TRAPPC4; TNFRSF1A; CHD4; HDAC7; SP1; CTDSP2; ATP2B1; ATP2A2; MED13L; PAN3p; UBL3; KPNA3; TUBGCP3; SUPT16H; LRP10; AMD4A; FBXO34; PPM1A; SLC39A9; YLPM1; GALC; C14orf2; PACS2; NIPA2; TJP1; MGA; AP2K1; ARIH1; ADPGK; IQGAP1; SRRM2; CREBBP; ABCC1; CHD9; OGFOD1; NUP93; FAM192A; NAE1; CYB5B; AARS; AP1G1; ZFHX3; SLC7A5; TUBB3; MYO1C; MYH10; TAOK1; CPD; ATAD5; SUZ12; PTRF; LSM12; CDC27; LRRC59; RNF213; RNMT; RIOK3; ATP8B1; PIGN; CTDP1; DAZAP1; LMNB2; SAFB2; INSR; ZNF780B; CIC; PPP1R13L; VASP; RPL13A; RPL28; ATRN; CDS2; GSS; C20orf24; RBL1; ZMYND8; ARFGEF2; CSE1L; BACH1; IFNGR2; RRP1B; PCNT; TRIOBP; GTSE1; MBTPS2; MED14; HUWE1
hsa-miR-22-3p	MBP; ADRA1A; ARHGAP35; ESR1; ROCK1; ITSN1; RAPH1; PDE1C	LRRC47; MTOR; NECAP2; MRTO4; ID3; WASF2; STX12; ATPIF1; YTHDF2; PUM1; HDAC1YARS; PPT1; UQCRH; RAB3B; DHCR24; GNG12; VCAM1; GPSM2; NOTCH2; PBXIP1; ASH1L; UBQLN4; CEP350; STX6; KIF14; CNIH4; HEATR1; PDIA6; ZFP36L2; PRKCE; USP34; ACTR2; GGCX; IMMT; TXNDC9; AFF3; MAP4K46; IL1R1; POLR1B; WDR33; UGGT1; MGAT5; ITGA6; IGFBP5; TNS1; CTDSP1; ACSL3; PTMA; MKRN2; STT3B; FYCO1; SCAP; MAP4; ATRIP; PCNP; ATP6V1A; PIK3R4; SRPRB; NMD3; PDCD10; MFN1; GNB4; TRA2B; LPP; MAEA; FGFR3; WHSC1; PDS5A; TMEM165; ANKRD17; SEC31A; HSD17B11; HERC5; KIAA0922; NSUN2; RAI14; PPWD1; HMGCR; VCAN; CHD1; UBE2D2; CANX; MAML1; EXOC2; CDKN1A; TMEM14A; RWDD1; UTRN; ARID1B; EZR; WTAP; FAM120B; CDK13; TNS3; GBAS; VKORC1L1; RABGEF1; BAZ1B; HIP1; ARPC1A; POP7; CUX1; LAMB1; NUP205; CREB3L2; NDUFB2; SCARA3; MCM4; ARFGEF1; UBR5; KIAA0196; PHF20L1; KANK1; VLDLR; RCL1; SMU1; FAM120A; NIPSNAP3A; ZBTB34; ABL1; TSC1; NOTCH1; SFMBT2; ZEB1; ZWINT; DDIT4; KIF11; CUTC; MGEA5; NOLC1; CTBP2; NAV2; EXT2; RTN3; PPFIA1; RPS3; RSF1; NARS2; MTMR2; ATM; WNK1; CCND2; TNFRSF1A; CHD4; EPS8; HDAC7; SP1; ITGA5; CTDSP2; PPP1R12A; TXNRD1; CORO1C; MED13L; RAN; AKAP11; LCP1; DAD1; LRP10; PSME1; BAZ1A; PRPF39; SAMD4A; PLEKHG3; EIF2B2; SEL1L; GALC; ATG2B; TJP1; MGA; HERC1; ZNF609; MAP2K1; ARIH1; EDC3; ETFA; SLCO3A1; IGF1R; TM2D3; SRRM2; CLUAP1; ABCC1; ORAI3; TGFB1I1; CHD9; FAM192A; ZFHX3; KARS; CRISPLD2; MYO1C; PITPNA; KDM6B; MYH10; SUZ12; ATAD5; LRRC59; MMD; VEZF1; MTMR4; TRIM37; CLTC; USP32; MED13; BPTF; RNF213; PIGN; PTBP1; LMNB2; INSR; NOTCH3; KCTD15; HNRNPUL1; CIC; MARK4; PPP1R13L; VASP; ARHGAP35; ZNF544; ATRN; ITCH; RALGAPB; SDC4; TAF4; YTHDF1; RRP1B; PCNT; DIP2A; TRIOBP; NUP50; PDK3; FTSJ1; HUWE1; RPS4X

4. 讨论

AD 是一种进行性神经退行性疾病，是全世界老年人痴呆的主要原因。尽管在研发关于预防和治疗 AD 的药物方面付出了巨大努力，但目前还没有有效的成果，这在个人、医疗和社会经济层面都造成了越来越大的负担。

通过对靶 mRNA 的作用，miRNA 参与了许多细胞过程，包括增殖、凋亡、分化、衰老、应激和免疫刺激反应，以及癌症、心血管、糖尿病、AD 和其他神经退行性过程等人类疾病[10]。本研究共选取了 10 个高表达的 miRNA (5 个上调，5 个下调)。

在本研究中发现，与对照组相比，AD 患者的 miR-125b 显著下调。此外，在 APP/PS1 转基因小鼠模型中也发现循环 miR-125b 减少[11]。在另一项研究中，Galimberti 等人使用 miRNA PCR 阵列分析，他们发现 miR-125b 显著下调[12]。在 βamyloid (Aβ)病理状态下，miR-125b 表达的降低是皮质神经元神经毒性效应的关键事件。miR-125b 的异常表达可能是 AD 脑内神经功能障碍的原因之一。

且已有文献报道 hsa-miR-22 与 hsa-miR-24 在 AD 病人中低表达，与本研究中的发现一致：Jovicic 等[13]指出 miR-22 在阿尔茨海默症患者中低表达，增强 miR-22 表达可能是治疗神经退行性疾病的合理治疗策略。Hu 等[14]通过荟萃分析发现 miR-24 在 AD 患者的脑脊液中低表达。

在差异表达基因 GO 功能富集—生物学过程 BP 分析结果中，我们选取了 18 条与神经系统疾病相关的通路(如表 1)，对预测的靶向 mRNA 和通路筛选到的目标差异表达 mRNA 取交集，得到重合的差异表达基因共 181 个。

通过表 3 可发现，Estrogen Receptor 1 (ESR1)、Intersectin 1 (ITSN1)、Myelin Basic Protein (MBP)、Phosphodiesterase 1C (PDE1C)、Ras association and pleckstrin homology domains 1 (RAPH1)等上调基因均与 hsa-miR-22 和 hsa-miR-24 有对应关系，其中 Bertram 等报道，ESR1 PvuII 和 XbaI 多态性与 AD 风险相关，通过系统荟萃分析，ESR1 基因可能是 AD 发病的候选基因[15]。

在基因中值得注意的还有 DiscsLarge Homolog (DLG4)。DLG4 与将谷氨酸受体锚定到突触后膜有关。DLG4 的异常表达可能导致谷氨酸能神经传递的紊乱。此外，DLG4 参与调节淀粉样蛋白 b 沉积[16]。在我们的研究中，在 AD 患者中检测到上调的 DLG4，DLG4 在化学性突触传递、信号转导和三磷酸鸟苷环水解酶(GTPase)活性的正调控等方面发挥作用，且 DLG4 与两个下调 miRNA hsa-miR-125b-1-3p 和 hsa-miR-125a-3p 均有对应关系，表明其在 AD 中的重要作用，已有文献报道上调的 DLG4 可能作为 AD 的生物标志物[17]。

此外，已有研究发现一个可以调节特定 miRNA 和突触功能的 circRNA-CDR1-AS 在 AD 大脑中表达下调[8]。还有研究发现在人脑和视网膜中一种高度表达的 circRNA ciRS-7 (cerebellar degeneration-related protein 1 antisense, CDR1as)，它作为一种内源性、抗补体的 miRNA 抑制剂或“海绵”来抑制 miRNA-7 的正常功能[18]。然而，在 AD 发病机制中通过其他机制发挥作用的 circRNA (例如，作为蛋白质翻译模板、与蛋白质相互作用形成 circ Ribonucleoprotein 或作为 mRNA 陷阱的 circRNA)仍需要进一步探索。

在过去的几年里，ceRNA 假说已经被大量的实验所证实。然而，ceRNA 机制及其相互作用网络的研究主要在癌症研究中进行[19] [20]。近年来，研究人员开始系统地探讨 ceRNA 对特定神经退行性疾病的调控机制，由于 ceRNA 相互作用网络是多因素的，它们可能在这些复杂的神经退行性疾病的研究中具有优势；ceRNA network 的构建也有助于识别新的基因调控分子机制，从而更好地理解各种 AD 的发病机制，并揭示新的治疗靶点和获得有关疾病病理过程的相关信息。

我们希望基于 ceRNA 的分析将成为预防、延迟发病、诊断和治疗阿尔茨海默病的可行思路。然而，这项研究有一些局限性。首先，需要对更大样本的患者进行全面和详细地分析，以进一步验证我们的发

现。此外，还需要后续的相关实验研究来验证我们的结果。

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