

Screening of key genes and miRNAs involved in age-related hearing loss

yin song (✉ songyin1994@qq.com)

shzdx <https://orcid.org/0000-0002-7023-7148>

xue zhang

Shihezi University

wei jia sun

Shihezi University

ling jin zhu

Shihezi University

jin huang

Shihezi University

Research article

Keywords: ARHL, GEO, Bioinformatics, gene, microRNA

Posted Date: August 28th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-64102/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Background

Age-related hearing loss (ARHL) is the most common sensory deficit and refers to the gradual loss of hearing function with age. We aimed to research the differential expression genes during the occur of ARHL and explore microRNAs that maybe regulate these genes.

Methods

Search the GEO data GSE6045, GSE35234, GSE62173, GSE45026 from NCBI's Gene Expression Omnibus database, and analyze in R. Normalize GEO data by RMA method in R, and use linear microarray data model (LIMMA) method in R to compare young and old mice to find differential genes and microRNAs.

Results

109 up-regulated and 121 down-regulated important genes were identified respectively. Functional enrichment shows that they are significantly enriched in protein digestion and absorption, neuroactive ligand-receptor interaction, and PI3K-Akt signaling pathway. Among the top 20 Hub genes, 7 down-regulated genes (Col3a1, Col1a2, Sparc, Col4a2, Col2a1, Lox, Sparc, Ctgf) have verified targeted miRNAs, which have interaction with differential miRNAs of GSE45026. Finally, we designated miR-29a-3p, miR-29b-3p, miR-29c-3p, and miR-124-3p as key miRNAs involved in the development of age-related hearing loss.

Conclusions

These low expression genes of Col3a1, Col1a2, Sparc, Col4a2, Col2a1, Lox, Sparc, and Ctgf maybe key genes of ARHL, and probably regulated by miR-29a-3p, miR-29b-3p, miR-29c-3p, and miR-124-3p.

Background

Age-related hearing loss (ARHL) is a complex disease caused by the cumulative effect of aging on the auditory system. It is defined as a progressive bilateral symmetry sensory neurological hearing loss (SNHL), which is characterized by Unable to convert sound information into usable neural signals [1, 2]. ARHL is most commonly caused by the damage, loss or death of sensory hearing cells in the cochlea[3], owing to the delicacy of many cochlear tissues, aging results in progressive damage to hair cells and the stria vascularis[4–6]. ARHL might also be caused by the damage in the neurons that relay auditory information from the inner ear to the central auditory circuitry[7]. Moreover, damaged cochlear hair cells are practically impossible to repair, so these types of cochlear disorders are currently permanent[6, 2, 8]. According to WHO, about one-third of the population aged 65 years or older experience difficulties with hearing[9], Although the disease is not life-threatening, it is still associated with severe psychological and medical morbidity, including social isolation, weakness, depression, and cognitive decline[10, 11].

Compared with congenital and early onset hearing loss, our understanding of the biochemical process and molecular biology in this case is more limited. In order to explore its molecular mechanism, some scholars have used age-related hearing loss model mice to conduct high-throughput microarray platform to detect gene expression changes during the occurrence of age-related hearing loss[12, 13].

High-throughput microarray platform is a new tool for detecting genetic changes and discovering biomarkers for diseases[14], in this way, we can test the change of gene and other little biomarkers. However, due to the insufficient number of samples, individual microarray studies often show a bias towards the identification of high-abundance molecules, so the results are often biased [15]. But by integrating multiple microarray data sets, sufficient samples can be provided to reduce this bias and produce more convincing results.

In this study, we obtained some abnormally expressed genes by mining three GEO datasets. Subsequently, we performed functional enrichment analysis on these abnormally expressed genes. Then the miRNA upstream of the hub gene was predicted and verified in the GSE45026 dataset. Finally, we found some differentially expressed genes and miRNAs that were never thought to be related to hearing loss. These markers may be used as potential targets for age-related hearing loss detection in the future.

Methods

Microarray research, data sets and sample characteristics in the GEO database

We query the GEO (<http://www.ncbi.nlm.nih.gov/geo/>) gene expression database for gene chips about age-related hearing loss, which collects submitted high-throughput gene expression data. According to the following standards, the chip is considered to meet our research requirements: (1) Research about age-related hearing loss. (2) The mouse model that used has been proved will occur age-related hearing loss. According to the above standards, 4 microarray data including GSE6045, GSE35234, GSE62173, and GSE45026 were collected from the GEO database. Among them, GSE6045, GSE35234, GSE62173 analysis results are differential genes, and GSE45026 analysis results are differential miRNAs. The datasets were subjected to principal component analysis (PCA) for dimensionality reduction and quality control. If the quality of a particular sample is not good enough, it is excluded for subsequent analysis. Details of each microarray chip are provided in Table 1.

Differential expression analysis

Since the datasets we collected from different platforms with different handling, so we used the RMA[16] method in R to standardize the data firstly, then the unnormalized original data was summarized in the form of a matrix. Next, the linear microarray data model (LIMMA) [17] method was used in R to compare the young and old mouse to find different genes and different miRNAs, and it is considered that there is a significant difference in genes when $P\text{-Value} < 0.05$ and $|\log\text{FC}| > 0.5$.

GO and KEGG pathway analysis

For the DEGs, we used clusterProfiler[18] package to perform GO and KEGG pathway enrichment analysis in R to find all the potential functions of DEGs in the network ($P < 0.05$).

Hub gene screening

Search for DEGs from the recognized STRING 10.0 (search tool for searching for interacting genes; <https://string-db.org/>) database and download PPI pairs, then import the PPI data into Cytoscap[19] to obtain the entire PPI network, and finally, use the CytoHubba[20] plug-in of Cytoscap software to screen out 20 hub genes.

Prediction and verification of miRNAs targeting hub genes

First, we used the default important parameters of the miRTarBase database (<http://mirtarbase.mbc.nctu.edu.tw/php/search.php>) to predict the miRNA targeting the hub genes and then verified the obtained miRNAs in the differential miRNAs of GSE45026.

Results

Principal component analysis verifying independence of each group

To distinguish the significant differences between young and old GEO data samples, we performed PCA to reduce the dimensions and evaluate the independence of each group (Fig 1). The results show that there are significant differences between young and old samples in the data sets (GSE6045, GSE62173, GSE35234, GSE45026).

Differential expression analysis

Because there may be errors in the results of a single experiment, it is necessary to find several pieces of evidence to support, to increase the accuracy of the results. After standardizing the data using the RMA[16] method, the limma[17] package was used to identify differentially expressed genes according to thresholds of $P\text{-Value} < 0.05$ and $|\log\text{FC}| > 0.5$. The volcano graph in Fig 2 shows the number of differentially expressed genes identified from each of the 4 data sets. We identified 2091 genes in GSE6045, including 845 up-regulated genes and 1246 down-regulated genes, identified 481 genes in GSE62173 chip, including 240 up-regulated genes and 241 down-regulated genes, identified 1034 in GSE35234 chip Genes, including 528 up-regulated genes and 506 down-regulated genes, and identified 732 differential miRNAs in the GSE45026 chip, including 257 up-regulated miRNAs and 475 down-regulated miRNAs. The Venn diagram shows the intersection of genes between the three GEO datasets (Figure 2). In the last, we find 230 Co-expression genes (109 up-regulation DEGs and 121 down-regulation DEGs).

GO and KEGG pathway analysis

To explore the potential biological function of shared genes, GO, KEGG pathway and function enrichment analysis were performed. The GO annotation results contain three parts: biological processes, molecular functions, and cellular components.

For up-regulated important DEGs, such as Fig. 3A-C, rich GO functions include defense response to bacterium, positive regulation of leukocyte activation, and positive regulation of cell activation in the BP category; ligand-gated ion channel activity, ligand-gated channel activity and protease binding in the MF category; and secretory granule, receptor complex, intrinsic component of synaptic membrane, integral component of synaptic membrane in the CC category. Besides, Fig 4A reveals that these up-regulated DEGs are significantly enriched in certain pathways such as Neuroactive ligand-receptor interaction, Phagosome, Retrograde endocannabinoid signaling, Cell adhesion molecules, etc.

For down-regulated important DEGs, such as Fig 3D-F, rich GO functions include extracellular structure organization, extracellular matrix organization, ossification in the BP category; extracellular matrix structural constituent, growth factor binding, cell adhesion molecule binding, integrin binding in the MF category; and extracellular matrix, collagen-containing extracellular matrix in the CC category. In addition to these, Fig 4A reveals that these up-regulated DEGs are significantly enriched in certain pathways, such as PI3K-Akt signaling pathway, ECM-receptor interaction, Protein digestion and absorption, Focal adhesion, etc.

Hubgene screening

We enter 109 up-regulation DEGs and down-regulation DEGs 121 into the STRING database(<https://string-db.org/>), downloading the PPI pairs after querying the interaction. Then using the Cytoscap[19] software screen 10 up-regulation hub genes and 10 down-regulation hub genes by the cytoHubba[20] plug-in. The last we find 10 up-regulation hub genes including MPO, Hp, Cd52, Elane, Ii1b, H2-K1, Tyrobp, Cd74, Lcp1 and Ptprc, and 10 down-regulation hub genes including Col3a1, Col4a2, Acan, Postn, Col2a1, Col1a2, Sparc, Lox, Col5a2 and Ctgf (Fig 5).

Predict miRNAs targeting the hub genes and verify in GSE45026

We used miRTarbase (<http://mirtarbase.mbc.nctu.edu.tw/php/search.php>) to predict miRNAs targeting hub genes, and found that only 7 down-regulation hub genes (Col3a1, Col1a2, Sparc, Col4a2, Col2a1, Lox, Sparc, Ctgf) had results (figure 6A). Then we verified these miRNAs in the miRNAs differentially expressed by GSE45026 and found that miR-29a-3p, miR-29b-3p, miR-29c-3p and miR-124-3p were verified in GSE45026 (figure 5B). So we highly suspect that miR-29a-3p, miR-29b-3p, miR-29c-3p and miR-124-3p are important molecules involved in the regulation of age-related hearing loss.

Discuss

ARHL commonly occurs in old age and brings a bad experience to life. In addition to affecting hearing, some studies have shown a significant correlation between hearing sensitivity and the incidence of Alzheimer's disease[21, 22], as well as the difference between hearing sensitivity and cognitive function among non-demented individuals [23]. Due to the lack of good phenotyping methods and sufficient sample size, several published GWAS data on ARHL have not found reproducible results[24]. We are still unclear about the molecular mechanism of ARHL.

Because of the difficulties in human cohort studies, researchers began to use animal models to help determine the pathogenesis and genetics associated with ARHL. Mice are the main model organisms for studying the auditory function and aging of mammals[25]. For a long time, it has been reported that certain strains have good hearing for the elderly (e.g. CAST, CBA/CaJ, CBA/J, C3H/HeH), while others show a gradual decline in auditory function (e.g. BALB, C57BL/6, DBA/ 2J). The GSE datasets selected in this study are all mouse strains whose hearing loss occurs with age.

Integrating multiple data set displays can improve detection capabilities [26]. Compared with single array analysis, integrating multiple arrays is a better way to improve the reliability of the results. During the GEO search process, we found four chips that study ARHL including GSE45026, GSE6045, GSE62173, and GSE35234. Through analysis, we identified 230 genes (109 up-regulated and 121 down-regulated) co-expressed between GEO data, and screened out 10 up-regulated central genes (MPo, Hp, Cd52, Elane, Il1b, H2-K1, Tyrobp, Cd74, Lcp1 and Ptprc) and 10 down-regulated genes (Col3a1, Col4a2, Acan, Postn, Col2a1, Col1a2, Sparc, Lox, Col5a2 and Ctgf).

When conducting GO analysis on up-regulated genes, we found that they mainly focused on defense response to bacterium, ligand – gated ion channel activity, and s receptor complex. For down-gradulated genes, they mainly focused on extracellular structure organization, extracellular matrix structural constituent, cell adhesion molecule binding, etc. the up-regulated DEGs are significantly enriched in certain pathways such as Neuroactive ligand – receptor interaction, Phagosome, Retrograde endocannabinoid signaling, Cell adhesion molecules, etc. For down-regulated important DEGs, these down-regulated DEGs are significantly enriched in certain pathways, such as PI3K-Akt signaling pathway, ECM-receptor interaction, Protein digestion and absorption, Focal adhesion, etc.

Previous studies have shown that hearing loss is mainly due to the loss of internal or external hair cells, which damages IHC ribbon synapses or spiral ganglion neurons [27]. It now appears that the biological processes of gene enrichment, cell composition, and molecular function are consistent with the occurrence of ARHL.

To systematically analyze the relationship and function of the important DEG in ARHL, we mapped DEG to STRING database and obtained PPI network. As we all know, genes with higher node degree in PPI network usually play more roles. To further identify the key genes in ARHL, we selected the top ten up-regulated and down-regulated hub genes for further analysis. The analysis results showed that seven down-regulated genes (Col3a1, Col1a2, Sparc, Col4a2, Col2a1, Lox, Sparc, Ctgf) may be the key genes of ARHL.

We predicted the miRNA targeting the hub gene through miRTarBase, and verified in the difference results of the GSE45026 chip that miR-29a-3p, miR-29b-3p, miR-29c-3p, miR-124-3p were verified, they may play a regulatory role in the process of hearing loss, so we highly suspect that miR-29a-3p, miR-29b-3p, miR-29c-3p, miR-124-3p may be key molecules involved in the occurrence of hearing loss. In the analysis of GSE45026, we only selected C57 mice for analysis, because C57 mice have a clear phenomenon of senile hearing loss. In previous studies, Mun found that the expression of miR-29b-3p was elevated in the cochlea of aged C57BL/6 mice[28], which was consistent with our analysis. For others, miR-29a-3p, miR-29c-3p and

miR-124-3p were mainly related to cancer[29–34], AD[35, 36], and miR-124-3p also related to neurogenesis[37, 38] and depression[39], with no research related to hearing loss.

All in all, our study analyzed the GEO database data and found differential genes for age-related hearing loss, and found some key miRNA molecules, which may be potential biological targets for predicting age-related hearing loss.

Conclusion

As age increases, miR-29a-3p, miR-29b-3p, miR-29c-3p and miR-124-3p expression increases, which in turn regulates gene Col3a1, Col1a2, Sparc, Col4a2, Col2a1, Lox, Sparc and Ctgf expression decreases, and mediates hearing loss.

Abbreviations

ARHL age-related hearing loss

Declarations

Ethics approval and consent to participate

This article does not contain any studies with human participants performed by any of the authors.

Consent for publication

Not Applicable

Availability of data and material

The datasets generated and/or analysed during the current study are available in the [Gene Expression Omnibus] repository, [<https://www.ncbi.nlm.nih.gov/geo/>]

Competing interests

The authors declare that they have no Competing interests.

Funding

This study was funded by discovery and verification of the interaction between Neuritin and membrane protein EphA2 (grant number ZZZC201806A). The funder participated in the revision of the article.

Authors' contributions

Data curation, SY and ZX; formal analysis, SY; project administration, SJW; writing—original draft, SY; writing—review & editing, ZJL and HuangJin. All authors have read and approved the manuscript.

Acknowledgements

Thanks to Professor Huang Jin and Wang Haiyan for the analysis ideas and Zhang Shuai for the technical support.

References

1. Smith RJ, Bale JF Jr, White KR. Sensorineural hearing loss in children. *Lancet*. 2005;365(9462):879–90. doi:10.1016/S0140-6736(05)71047-3.
2. Wong AC, Ryan AF. Mechanisms of sensorineural cell damage, death and survival in the cochlea. *Front Aging Neurosci*. 2015;7:58. doi:10.3389/fnagi.2015.00058.
3. Schuknecht HF, Kimura RS, Naufal PM. The pathology of sudden deafness. *Acta Otolaryngol*. 1973;76(2):75–97. doi:10.3109/00016487309121486.
4. Schuknecht HF. The Pathology of Several Disorders of the Inner Ear Which Cause Vertigo. *South Med J*. 1964;57:1161–7. doi:10.1097/00007611-196410000-00008.
5. Fetoni AR, Picciotti PM, Paludetti G, Troiani D. Pathogenesis of presbycusis in animal models: a review. *Exp Gerontol*. 2011;46(6):413–25. doi:10.1016/j.exger.2010.12.003.
6. Yamasoba T, Lin FR, Someya S, Kashio A, Sakamoto T, Kondo K. Current concepts in age-related hearing loss: epidemiology and mechanistic pathways. *Hear Res*. 2013;303:30–8. doi:10.1016/j.heares.2013.01.021.
7. Wilson C, Roberts A, Stephens D. Aetiological investigation of sensorineural hearing loss in children. *Arch Dis Child*. 2005;90(3):307–9. doi:10.1136/adc.2004.049197.
8. Park YH. Stem Cell Therapy for Sensorineural Hearing Loss, Still Alive? *J Audiol Otol*. 2015;19(2):63–7. doi:10.7874/jao.2015.19.2.63.
9. WHO. (2019) Deafness and hearing loss.
10. Rutherford BR, Brewster K, Golub JS, Kim AH, Roose SP. Sensation and Psychiatry: Linking Age-Related Hearing Loss to Late-Life Depression and Cognitive Decline. *Am J Psychiatry*. 2018;175(3):215–24. doi:10.1176/appi.ajp.2017.17040423.
11. Kamil RJ, Betz J, Powers BB, Pratt S, Kritchevsky S, Ayonayon HN, Harris TB, Helzner E, Deal JA, Martin K, Peterson M, Satterfield S, Simonsick EM, Lin FR, Health ABCs. Association of Hearing Impairment With Incident Frailty and Falls in Older Adults. *J Aging Health*. 2016;28(4):644–60. doi:10.1177/0898264315608730.
12. Someya S, Yamasoba T, Prolla TA, Tanokura M. Genes encoding mitochondrial respiratory chain components are profoundly down-regulated with aging in the cochlea of DBA/2J mice. *Brain Res*. 2007;1182:26–33. doi:10.1016/j.brainres.2007.08.090.

13. Mutai H, Miya F, Fujii M, Tsunoda T, Matsunaga T. Attenuation of progressive hearing loss in DBA/2J mice by reagents that affect epigenetic modifications is associated with up-regulation of the zinc importer Zip4. *PLoS One*. 2015;10(4):e0124301. doi:10.1371/journal.pone.0124301.
14. Loffredo LF, Abdala-Valencia H, Anekalla KR, Cuervo-Pardo L, Gottardi CJ, Berdnikovs S. Beyond epithelial-to-mesenchymal transition: Common suppression of differentiation programs underlies epithelial barrier dysfunction in mild, moderate, and severe asthma. *Allergy*. 2017;72(12):1988–2004. doi:10.1111/all.13222.
15. Kulasingam V, Diamandis EP. Strategies for discovering novel cancer biomarkers through utilization of emerging technologies. *Nat Clin Pract Oncol*. 2008;5(10):588–99. doi:10.1038/ncponc1187.
16. Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics*. 2003;4(2):249–64. doi:10.1093/biostatistics/4.2.249.
17. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*. 2015;43(7):e47. doi:10.1093/nar/gkv007.
18. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS*. 2012;16(5):284–7. doi:10.1089/omi.2011.0118.
19. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. 2003;13(11):2498–504. doi:10.1101/gr.1239303.
20. Chin CH, Chen SH, Wu HH, Ho CW, Ko MT, Lin CY. (2014) cytoHubba: identifying hub objects and sub-networks from complex interactome. *BMC Syst Biol* 8 Suppl 4:11. doi:10.1186/1752-0509-8-S4-S11.
21. Lin FR, Metter EJ, O'Brien RJ, Resnick SM, Zonderman AB, Ferrucci L. Hearing loss and incident dementia. *Arch Neurol*. 2011;68(2):214–20. doi:10.1001/archneurol.2010.362.
22. Gates GA, Anderson ML, McCurry SM, Feeney MP, Larson EB. Central auditory dysfunction as a harbinger of Alzheimer dementia. *Arch Otolaryngol Head Neck Surg*. 2011;137(4):390–5. doi:10.1001/archoto.2011.28.
23. Humes LE, Busey TA, Craig J, Kewley-Port D. Are age-related changes in cognitive function driven by age-related changes in sensory processing? *Atten Percept Psychophys*. 2013;75(3):508–24. doi:10.3758/s13414-012-0406-9.
24. Fransen E, Bonneux S, Corneveaux JJ, Schrauwen I, Di Berardino F, White CH, Ohmen JD, Van de Heyning P, Ambrosetti U, Huentelman MJ, Van Camp G, Friedman RA. Genome-wide association analysis demonstrates the highly polygenic character of age-related hearing impairment. *Eur J Hum Genet*. 2015;23(1):110–5. doi:10.1038/ejhg.2014.56.
25. Vanhooren V, Libert C. The mouse as a model organism in aging research: usefulness, pitfalls and possibilities. *Ageing Res Rev*. 2013;12(1):8–21. doi:10.1016/j.arr.2012.03.010.
26. Ma T, Liang F, Oesterreich S, Tseng GC. A Joint Bayesian Model for Integrating Microarray and RNA Sequencing Transcriptomic Data. *J Comput Biol*. 2017;24(7):647–62. doi:10.1089/cmb.2017.0056.

27. Moser T, Starr A. Auditory neuropathy–neural and synaptic mechanisms. *Nat Rev Neurol*. 2016;12(3):135–49. doi:10.1038/nrneurol.2016.10.
28. Mun SK, Chae H, Piao XY, Lee HJ, Kim YK, Oh SH, Chang M. MicroRNAs Related to Cognitive Impairment After Hearing Loss. *Clin Exp Otorhinolaryngol*. 2020. doi:10.21053/ceo.2019.01382.
29. Chen X, Zhu H, Ye W, Cui Y, Chen M. MicroRNA29a enhances cisplatin sensitivity in nonsmall cell lung cancer through the regulation of REV3L. *Mol Med Rep*. 2019;19(2):831–40. doi:10.3892/mmr.2018.9723.
30. Liang Y, Li E, Min J, Gong C, Gao J, Ai J, Liao W, Wu L. miR29a suppresses the growth and metastasis of hepatocellular carcinoma through IFITM3. *Oncol Rep*. 2018;40(6):3261–72. doi:10.3892/or.2018.6745.
31. Gong HL, Tao Y, Mao XZ, Song DY, You D, Ni JD. MicroRNA-29a suppresses the invasion and migration of osteosarcoma cells by regulating the SOCS1/NF-kappaB signalling pathway through negatively targeting DNMT3B. *Int J Mol Med*. 2019;44(4):1219–32. doi:10.3892/ijmm.2019.4287.
32. Li W, Yi J, Zheng X, Liu S, Fu W, Ren L, Li L, Hoon DSB, Wang J, Du G. miR-29c plays a suppressive role in breast cancer by targeting the TIMP3/STAT1/FOXO1 pathway. *Clin Epigenetics*. 2018;10:64. doi:10.1186/s13148-018-0495-y.
33. Wang JY, Zhang Q, Wang DD, Yan W, Sha HH, Zhao JH, Yang SJ, Zhang HD, Hou JC, Xu HZ, He YJ, Hu JH, Zhong SL, Tang JH. (2018) MiR-29a: a potential therapeutic target and promising biomarker in tumors. *Biosci Rep* 38 (1). doi:10.1042/BSR20171265.
34. Fu W, Wu X, Yang Z, Mi H. The effect of miR-124-3p on cell proliferation and apoptosis in bladder cancer by targeting EDNRB. *Arch Med Sci*. 2019;15(5):1154–62. doi:10.5114/aoms.2018.78743.
35. Wu Y, Xu J, Xu J, Cheng J, Jiao D, Zhou C, Dai Y, Chen Q. Lower Serum Levels of miR-29c-3p and miR-19b-3p as Biomarkers for Alzheimer's Disease. *Tohoku J Exp Med*. 2017;242(2):129–36. doi:10.1620/tjem.242.129.
36. Angelopoulou E, Paudel YN, Piperi C. miR-124 and Parkinson's disease: A biomarker with therapeutic potential. *Pharmacol Res*. 2019;150:104515. doi:10.1016/j.phrs.2019.104515.
37. Yang Y, Ye Y, Kong C, Su X, Zhang X, Bai W, He X. MiR-124 Enriched Exosomes Promoted the M2 Polarization of Microglia and Enhanced Hippocampus Neurogenesis After Traumatic Brain Injury by Inhibiting TLR4 Pathway. *Neurochem Res*. 2019;44(4):811–28. doi:10.1007/s11064-018-02714-z.
38. Jiao S, Liu Y, Yao Y, Teng J. miR-124 promotes proliferation and neural differentiation of neural stem cells through targeting DACT1 and activating Wnt/beta-catenin pathways. *Mol Cell Biochem*. 2018;449(1–2):305–14. doi:10.1007/s11010-018-3367-z.
39. Gu Z, Pan J, Chen L. (2019) MiR-124 suppression in the prefrontal cortex reduces depression-like behavior in mice. *Biosci Rep* 39 (9). doi:10.1042/BSR20190186.

Tables

Table 1 Information of each microarray chip collected from GEO datasets.

GSE	Publication	Total differentially expressed genes	Up-regulated	Down-regulated	Technology/Platform	Age
GSE6045	Brain Res	2091	845	1246	Affymetrix Mouse Expression 430A Array	Control☒ 7weeks Old☒ 36weeks
GSE35234	None	1034	528	506	Illumina MouseWG-6 v2.0 expression beadchip	Control☒ 4weeks Old☒ 45weeks
GSE62173	PLoS One	481	240	241	Affymetrix Mouse Genome 430 2.0 Array	Control☒ 4weeks Old☒ 12weeks
GSE45026	PLoS One	732	257	475	Affymetrix Multispecies miRNA-1 Array	Control☒ 21days Old☒ 9months

Figures

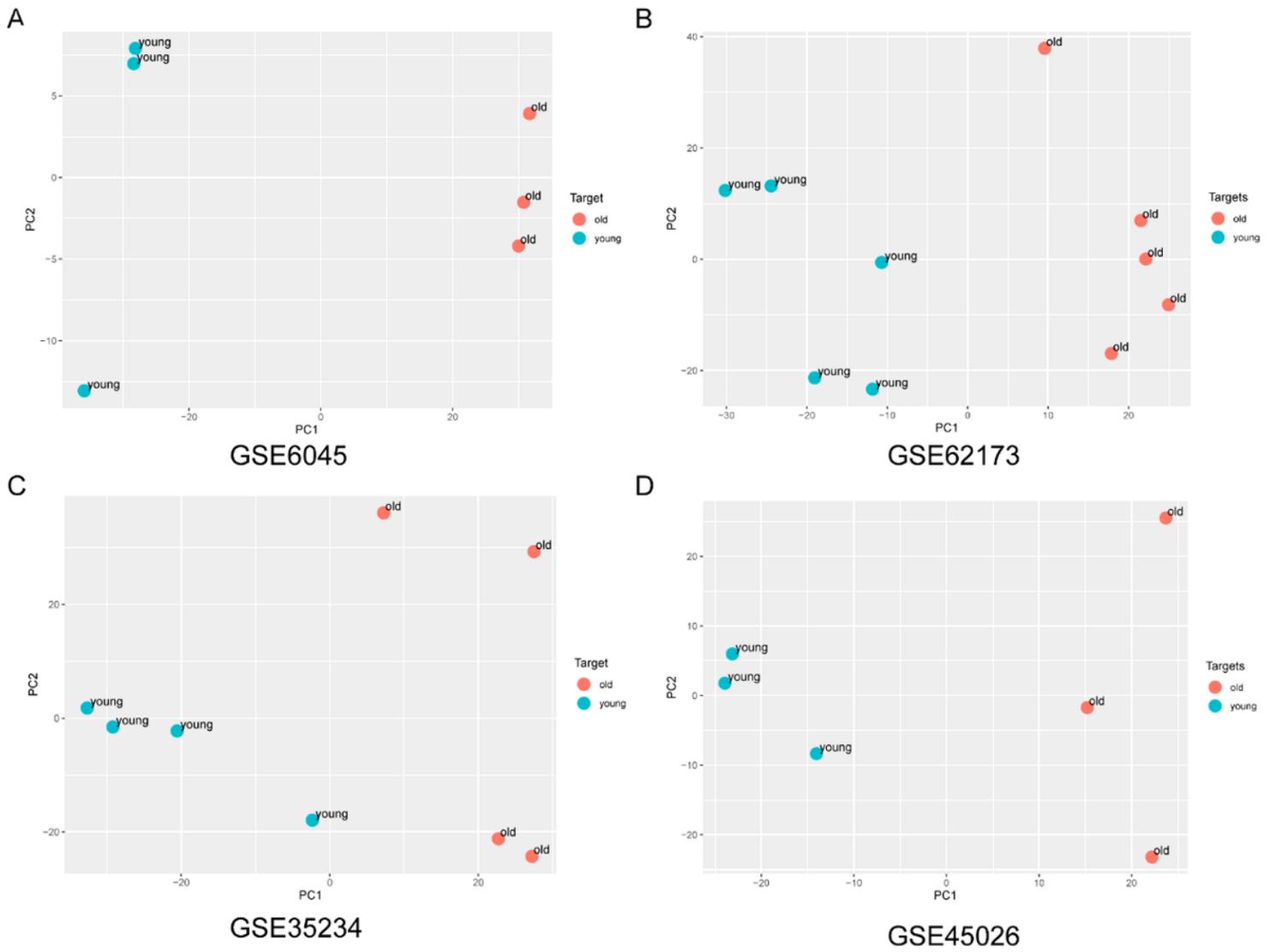


Figure 1

Results from the principal component analysis for microarray studies downloaded from the GEO database. (A-D) The principal component analysis of GSE6045, GSE62173, GSE35234 and GSE45026.

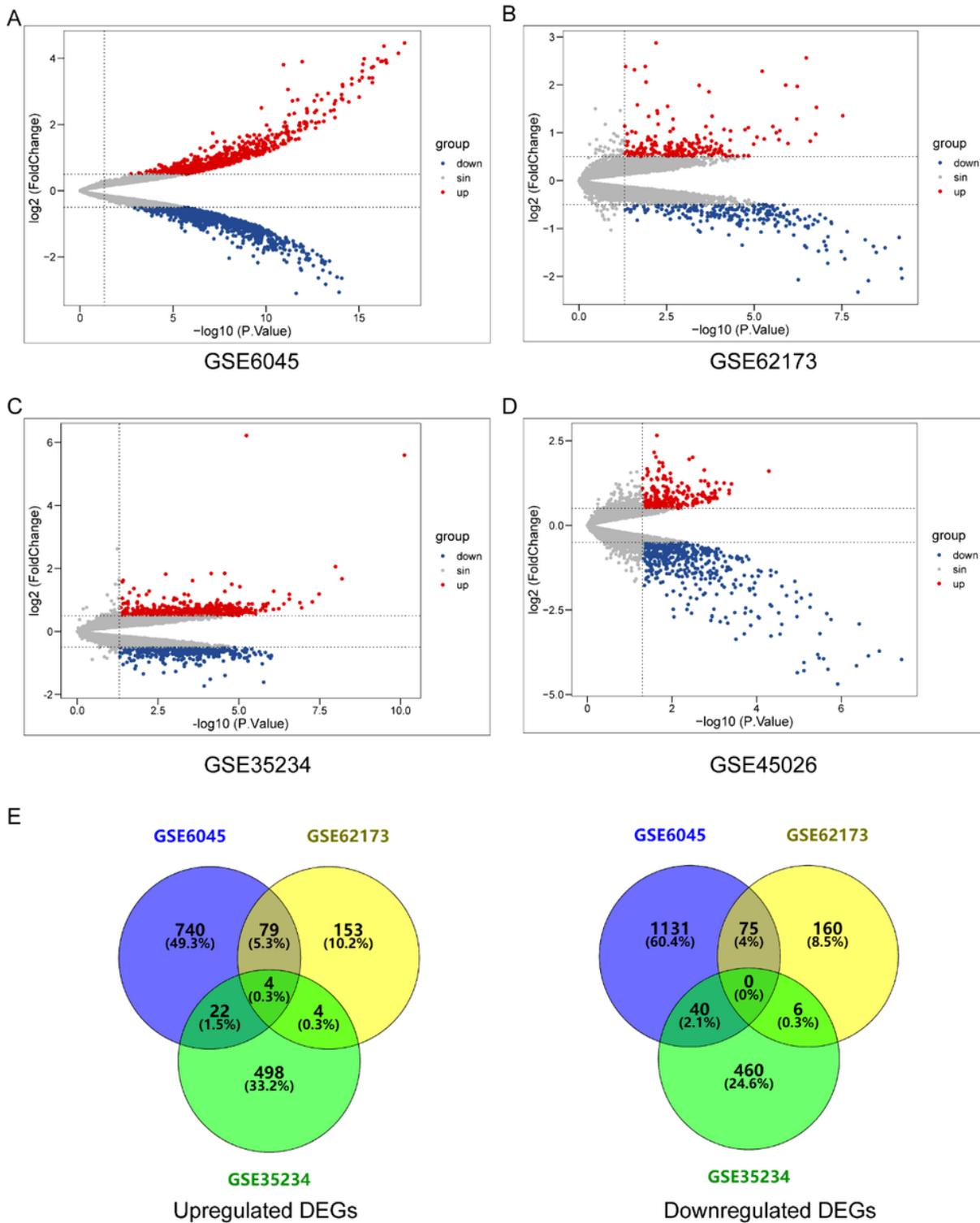


Figure 2

Identification of significant differentially expressed genes (DEGs) in ARHL. (A-C) Volcano plots showing the DEGs identified from GSE6045, GSE62173 and GSE35234. (D) Volcano plots showing the difference miRNAs identified from GSE45026. X axis represents log transformed Pvalue, and Y axis indicates the mean expression differences of genes between old samples and young samples. Note: The four volcano plots showed all of the DEGs and miRNAs; the black dots represent genes that are not differentially expressed between young samples and old samples, and the blue dots and red dots represent the downregulated and

upregulated genes in old samples, respectively. $|\log_2FC| > 0.05$ and $Pvalue < 0.05$ were set as the cut-off criteria. (F) The intersection of upregulated DEGs of GSE6045, GSE62173 and GSE35234 datasets. (F) The intersection of downregulated DEGs of GSE6045, GSE62173 and GSE35234 datasets. The intersected DEGs were defined as the significant DEGs.

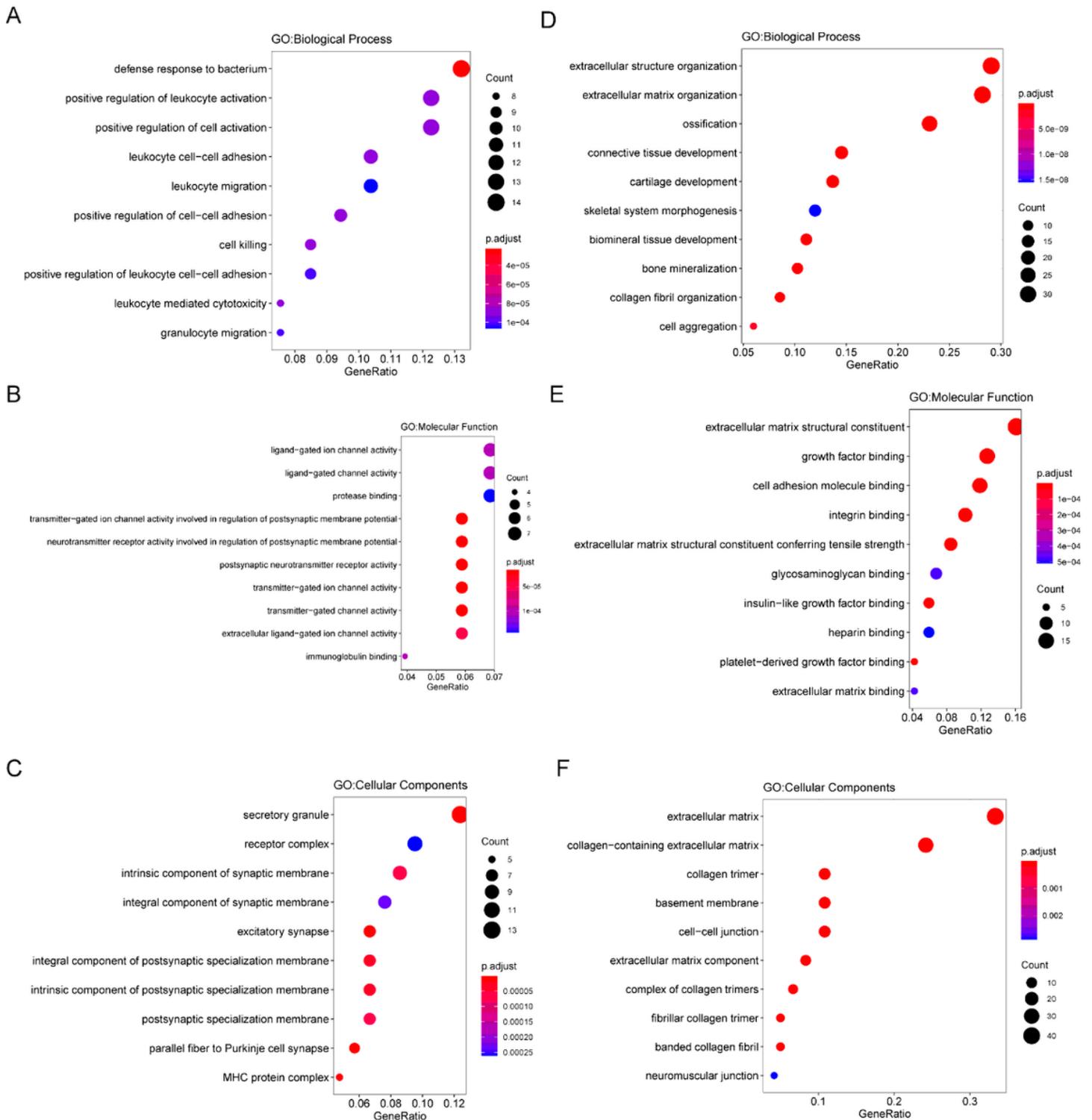


Figure 3

GO functional annotation for the significant DEGs. (A) The top ten enriched biological process (BP) of the upregulated significant DEGs. (B) The top ten enriched cellular component (CC) of the upregulated

significant DEGs. (C) The top ten enriched molecular function (MF) of the upregulated significant DEGs. (D) The top ten enriched biological process (BP) of the downregulated significant DEGs. (E) The top ten enriched cellular component (CC) of the downregulated significant DEGs. (F) The top ten enriched molecular function (MF) of the downregulated significant DEGs.

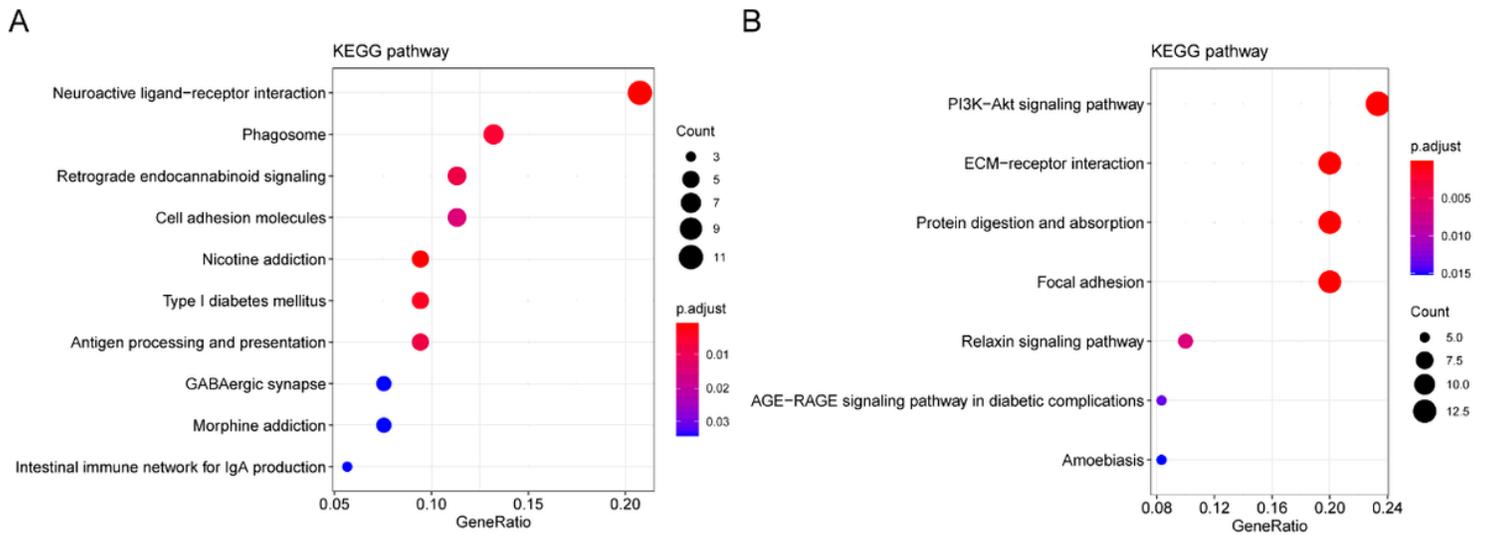


Figure 4

KEGG pathway enrichment analysis for the significant DEGs. (A) The top ten enriched KEGG pathways of the upregulated significant DEGs. (B) The top ten enriched KEGG pathways of the downregulated significant DEGs.

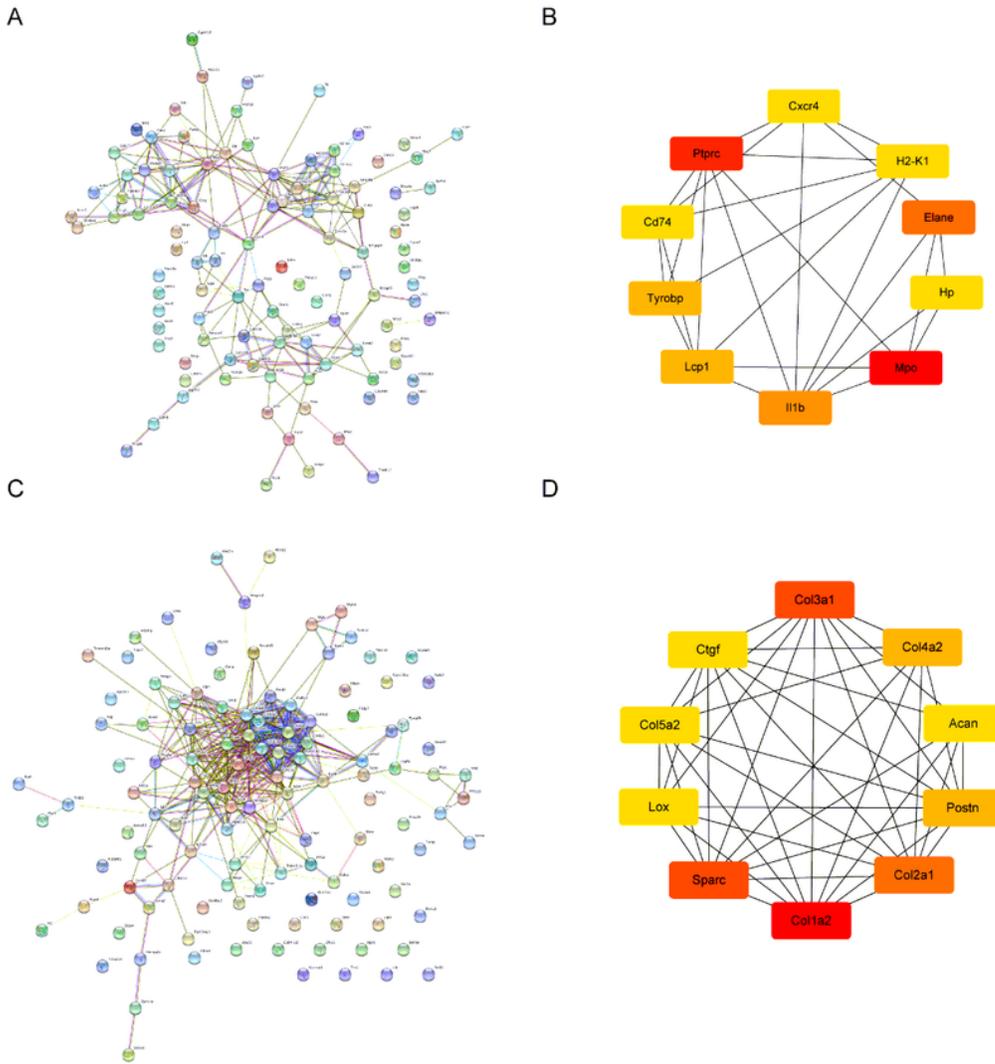
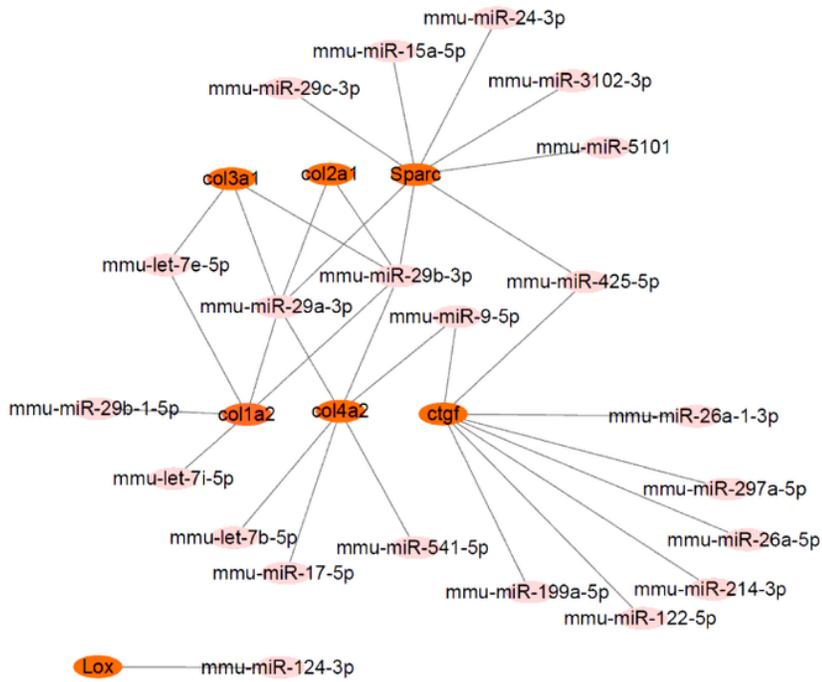


Figure 5

The top 10 hub genes identified in protein-protein interaction (PPI) networks. (A) The PPI network of the significant upregulated DEGs. (B) The top 10 hub genes of the significant upregulated DEGs. (C) The PPI network of the significant downregulated DEGs. (D) The 10 hub genes of the significant downregulated DEGs.

A



B

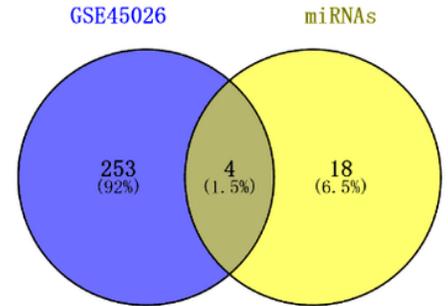


Figure 6

Construction of miRNA-gene network using Cytoscape software. (A) The results of miRNAs predicted by miRTarbase. (B) The intersection of upregulated miRNAs of predicted and GSE45026 datasets.