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# Construction for Long Non-Coding RNA (lncRNA)-Associated Competing Endogenous RNA (ceRNA) Network in Human Retinal Detachment (RD) with Proliferative Vitreoretinopathy (PVR)

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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**Background:** The aim of this study was to analyze the long non-coding RNA (lncRNA)-associated competing endogenous RNA (ceRNA) network in human retinal tissues following detachment with proliferative vitreoretinopathy (PVR).

**Material/Methods:** Expression data of 19 human detached retinas with PVR and 19 normal retinas from postmortem donors were downloaded from Gene Expression Omnibust (GEO) database (GSE28133). The R package "limma" was utilized to discriminate the dysregulated lncRNA and mRNA profiles. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses of differentially expressed mRNAs were performed using R packages "Clusterprofiler." The ceRNA network of dysregulated genes was constructed by using mircode, miRDB, miRTarBase and TargetScan databases, and was visualized by Cytoscape v3.6.1.

**Results:** A total of 23 lncRNAs and 994 mRNAs were identified significantly expressed between the human detached retinas with PVR and the normal retina tissues, with thresholds of $|\log_2 \text{FoldChange}| > 1.0$ and adjusted *P*-value <0.05. The constructed ceRNA network (lncRNA-miRNA-mRNA regulatory axis) included 9 PVR-specific lncRNAs, as well as 27 miRNAs and 73 mRNAs.

**Conclusions:** We demonstrated the differential lncRNA expression profile and constructed a lncRNA-associated ceRNA network in human detached retinas with PVR. This may ferret out an unknown ceRNA regulatory network in human retinal detachment with PVR.

**MeSH Keywords:** **Retinal Detachment • RNA, Long Noncoding • Vitreoretinopathy, Proliferative**

**Abbreviations:** **lncRNA** – long non-coding RNA; **ceRNA** – competing endogenous RNA; **miRNA** – microRNA; **RD** – retinal detachment; **PVR** – proliferative vitreoretinopathy; **GO** – Gene Ontology; **KEGG** – Kyoto Encyclopedia of Genes and Genomes; **GEO** – Gene Expression Omnibust; **PPI** – protein–protein interaction

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## Background

Retinal detachment (RD) occurs when the neurosensory retina layer separates from the retinal pigment epithelium. RD involving the foveal center can lead to profound loss of vision [1]. In most cases, RD occurs following a full thickness retinal break, allowing the ingress of fluid from the vitreous cavity to the subretinal space, which can result in retinal separation and is so-called "rhegmatogenous" [1,2]. A systematic review showed that rhegmatogenous RD incidence varied between 6.3 and 17.9 per 100 000 population, and was strongly associated with myopia, increasing age and certain vitreoretinal degenerations [3]. In addition to the loss of photoreceptors following RD, an inflammatory response also develops. RD triggers cell migration and proliferation and production of extracellular matrix proteins, which in turn results in the accumulation of vitreal and periretinal membranes, both hallmarks of proliferative vitreoretinopathy (PVR) [4]. In total, PVR occurs in 5–10% RD cases [5]. PVR can be detected during the late presentation of RD and complicate the post-operative period after a surgery for RD, leading to a re-detachment or limiting visual recovery [6,7]. Clinical studies using adjuvant therapy for the treatment of PVR have been conducted, including anti-inflammatory agents, anti-neoplastic/anti-proliferative agents, anti-growth factor pathway inhibitors and antioxidants, etc. [6,8–11]. However, the results are often contradictory or inconclusive with only limited success.

Long non-coding RNA (lncRNA) is a group of non-coding transcripts 200–10 000 bp in length but lacks significant protein-coding capacity. They interact in a regulatory manner before, during and after transcription [12]. Salmena et al. made a competing endogenous RNA (ceRNA) hypothesis that lncRNA, mRNA and other RNAs might act as microRNA (miRNA) sponges to inhibit miRNA function through sharing the miRNA response elements (MREs), which is a complicated post-transcriptional regulatory network [13]. There is much evidence to support this hypothesis [14–16]. On one hand, miRNAs can combine with their target mRNAs and inhibit their expression in the ceRNA network. On the other hand, lncRNA can regulate gene encoding protein level and following cell biology by competing with miRNAs [15,16]. Besides, each miRNA can influence up to hundreds of expressions of transcription, while each RNA transcription with different MREs can be targeted by multiple miRNAs [16,17].

In recent years, a growing number of studies have proven that the lncRNA-miRNA-mRNA regulation network is involved in the course of many diseases, including the tumors, Alzheimer disease and dermatitis etc. [12,16,18,19]. However, whether the ceRNA network takes part in human detached retinas with PVR has not been studied. Here, we downloaded expression data from Gene Expression Omnibust (GEO) database (GSE28133). It contained 19 specimens from patients for severe retinal detachment with PVR and 19 normal control retina specimens from postmortem donors. Bioinformatics analysis and the ceRNA network were conducted to explore the pathological mechanism and potential therapeutic targets of retinal detachment with PVR.

## Material and Methods

### Tissue samples from the GEO database and bioinformatics analysis

The RNA expression data of tissue samples were obtained from GEO database. The dataset (GSE28133) contained 19 specimens from patients for severe retinal detachment with PVR and 19 normal control retina specimens from postmortem donors. Clinical and pathological features of patients were described [2]. All the samples were analyzed using Affymetrix Human Genome U133 Plus 2.0 Array.

### Differentially expressed gene analysis

We used the "limma" package in R software to identify the differentially expressed genes (lncRNAs and mRNAs) between human detached retinas with PVR and the control group, with $|\log_2 \text{FoldChange (FC)}| > 1.0$ and adjusted *P*-value <0.05. Besides, mRNA and lncRNA annotation was performed using the Encyclopedia of DNA Elements (ENCODE) with ENSEMBL.

### GO and KEGG functional enrichment analysis

To understand the potential biological functions and processes of differentially expressed genes, Gene Ontology database (GO, *http://www.geneontology.org*) and Kyoto Encyclopedia of Genes and Genomes (KEGG, *http://www.kegg.jp/*), as well as the "clusterProfilerGO" package and "clusterProfilerKEGG" in R software, were utilized to perform GO and KEGG pathway analysis. Records with *P*-value <0.05 and enrichment >2.0 were preserved.

### Construction of the lncRNA-miRNA-mRNA ceRNA and protein–protein interaction (PPI) networks

Based on the hypothesis that lncRNA could sponge the common miRNA and thus prevent miRNA from binding to their target genes [20], a ceRNA network was constructed. The mircode database (*http://www.mircode.org/*) was used for lncRNA to predict their targeted miRNAs. The miRNA-mRNA interactions were predicted by the miRTarBase (*http://mirtarbase.mbc.nctu.edu.tw/*), miRDB (*http://www.mirdb.org/*) and TargetScan (*http://www.targetscan.org/*). Finally, the predicted mRNAs were cross matched with the differentially expressed ones,





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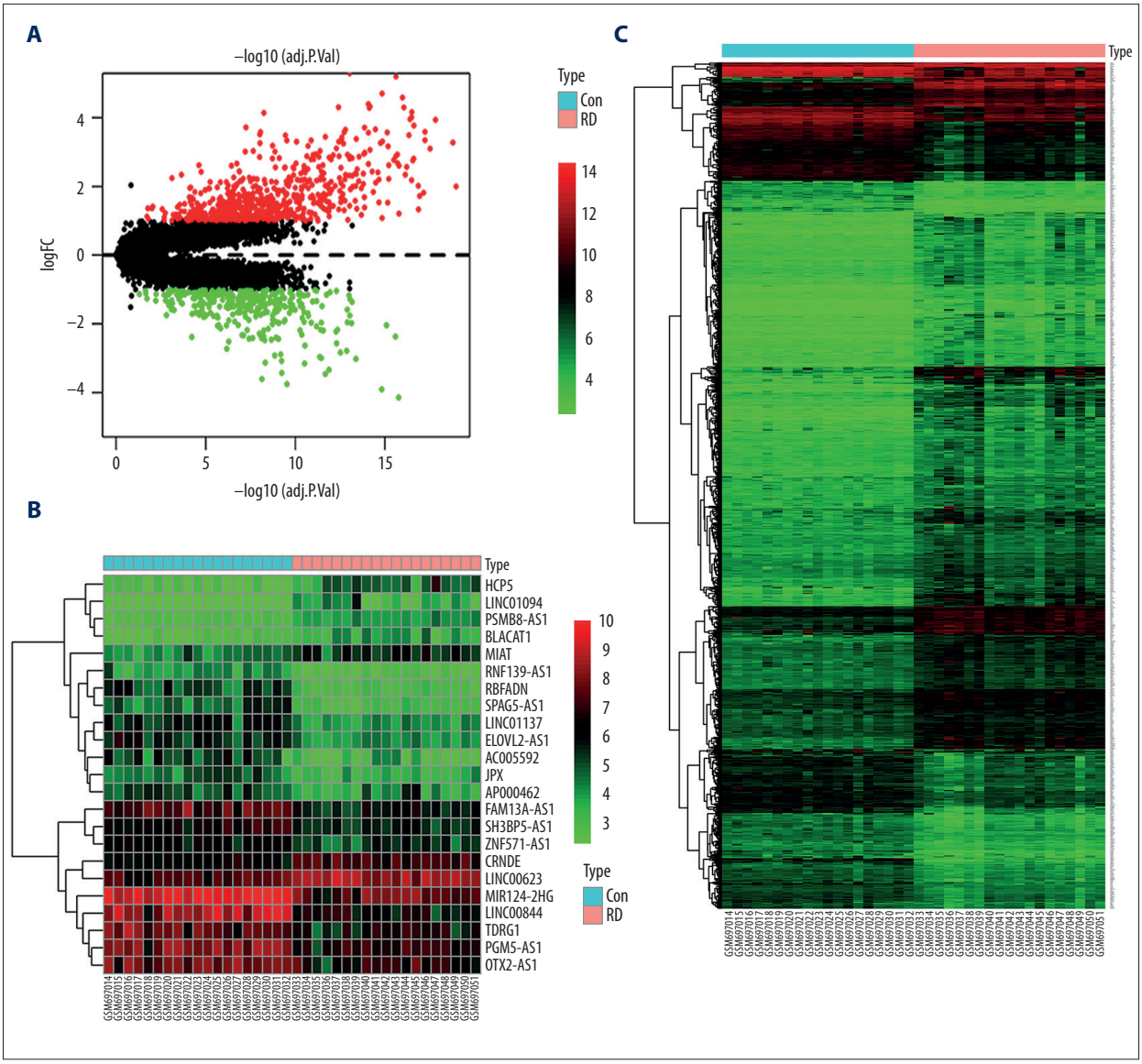


**Figure 1.** Differentially expressed genes between the human detached retinas with PVR and normal controls. (**A**) The volcano plot of the differently expressed genes. The x axis is -log10(adjusted *P*-value) while the y axis is $\log_2$FC. Black dots indicated non-differently expressed genes. The red dots indicate upregulated genes while the green dots indicate downregulated ones. (**B**) Heatmap of differently expressed lncRNAs. (**C**) Heatmap of differently expressed mRNAs. (**B, C**) The x axis is for the sample serial number and the y axis is for the differentially expressed gene. Red blocks represent upregulation and green blocks represent downregulation.

and the mRNA with no negatively regulated lncRNA or miRNA were abandoned. The lncRNA, miRNA and mRNA with $|\log_2 FC| > 1.0$ and adjusted *P*-value <0.05 were preserved. Construction and visualization of the lncRNA-miRNA-mRNA ceRNA network were conducted using Cytoscape v3.6.1. Besides, PPI network of mRNAs involved in the ceRNA network was set up with high confidence (0.700) by String (*https://string-db.org/*).

## Results

### Differentially expressed lncRNAs and mRNAs between the human detached retinas with PVR and normal controls

Clinical characteristics of patients were described in the previous study [2]. In total, 994 genes were significantly changed between human detached retinas with PVR and normal human retinas with thresholds of $|\log_2 FC| > 1.0$ and adjusted *P*-value <0.05, as is shown in the volcano map (Figure 1A). Among





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**Table 1.** Dysregulated lncRNAs between human detached retinas with PVR and normal controls.

| lncRNA | Gene ID | Expression change | logFC (PVR/N) | Adj. *P*-value |
|---|---|---|---|---|
| HCP5* | 10866 | Up-regulation | 1.947938297 | 1.53E-09 |
| LINC00623 | 728855 | Up-regulation | 1.230129889 | 5.99E-08 |
| LINC01094 | 100505702 | Up-regulation | 1.217325671 | 5.36E-06 |
| PSMB8-AS1 | 100507463 | Up-regulation | 1.210172661 | 1.66E-16 |
| MIAT* | 440823 | Up-regulation | 1.209215343 | 1.06E-06 |
| CRNDE* | 643911 | Up-regulation | 1.055524554 | 1.51E-08 |
| BLACAT1 | 101669762 | Up-regulation | 1.048582535 | 9.62E-07 |
| ZNF571-AS1 | 100507433 | Down-regulation | –1.031576283 | 1.01E-06 |
| AP000462* | --- | Down-regulation | –1.066771719 | 0.000173 |
| SH3BP5-AS1 | 100505696 | Down-regulation | –1.08329906 | 4.33E-07 |
| JPX* | 554203 | Down-regulation | –1.203614666 | 1.33E-07 |
| PGM5-AS1 | 572558 | Down-regulation | –1.248531249 | 6.67E-06 |
| OTX2-AS1 | 100309464 | Down-regulation | –1.26206851 | 1.89E-05 |
| RNF139-AS1 | 101927612 | Down-regulation | –1.383351463 | 1.96E-10 |
| TDRG1* | 732253 | Down-regulation | –1.399211813 | 2.00E-05 |
| ELOVL2-AS1 | 100506409 | Down-regulation | –1.472180825 | 6.24E-09 |
| LINC01137 | 728431 | Down-regulation | –1.613551017 | 1.38E-09 |
| FAM13A-AS1* | 285512 | Down-regulation | –1.689264402 | 5.73E-09 |
| SPAG5-AS1* | 100506436 | Down-regulation | –1.774851965 | 1.87E-11 |
| MIR124-2HG | 100130155 | Down-regulation | –1.894825134 | 3.78E-10 |
| AC005592* | 101926975 | Down-regulation | –2.035310985 | 1.55E-07 |
| RBFADN | 100506070 | Down-regulation | –2.175539179 | 4.42E-13 |
| LINC00844 | 100507008 | Down-regulation | –2.290846454 | 1.01E-08 |

PVR – proliferative vitreoretinopathy; N – normal human retinas. * Represents the lncRNA taking part in the ceRNA network.

them, 23 genes were lncRNA, with 16 genes downregulated and 7 genes upregulated. The aberrantly expressed lncRNAs can be viewed in the heatmap (Figure 1B) and their gene IDs and $\log_2$FC are listed (Table 1), in which the 9 lncRNAs taking part in the ceRNA network were marked with asterisk keys. Besides, 971 mRNAs were identified as differentially expressed in which 655 (67.46%) were upregulated and 316 (32.54%) were downregulated. The differentially expressed mRNA levels were also presented in the heatmap (Figure 1C).

### Functional enrichment analysis of differentially expressed mRNAs

GO and KEGG pathway analyses allow for the molecular function and signal pathway annotation of dysregulated mRNAs. Records with *P*-value <0.05 and enrichment >2.0 were preserved. The complete list of the 74 terms of GO analysis is presented in Supplementary Table 1. The top 20 GO terms were visualized in the bubble diagram and the most enriched pathways were the cell adhesion molecule binding (GO: 0050839), actin binding (GO: 0003779) and enzyme inhibitor activity (GO: 0004857), which contained 60, 49, and 44 genes respectively. As shown in Figure 2A), 13 GO terms of the top 20 took part in the binding function of the cell.

The KEGG pathway analysis indicated that 39 pathways were associated with differentially expressed mRNAs (Supplementary Table 2). The top 20 KEGG pathways enriched are visualized in the bubble diagram (Figure 2B). It shows that infection probably played an important role in retinal detachment with PVR, including human papillomavirus, Epstein-Barr virus, human cytomegalovirus, as well as tuberculosis, etc. In addition,





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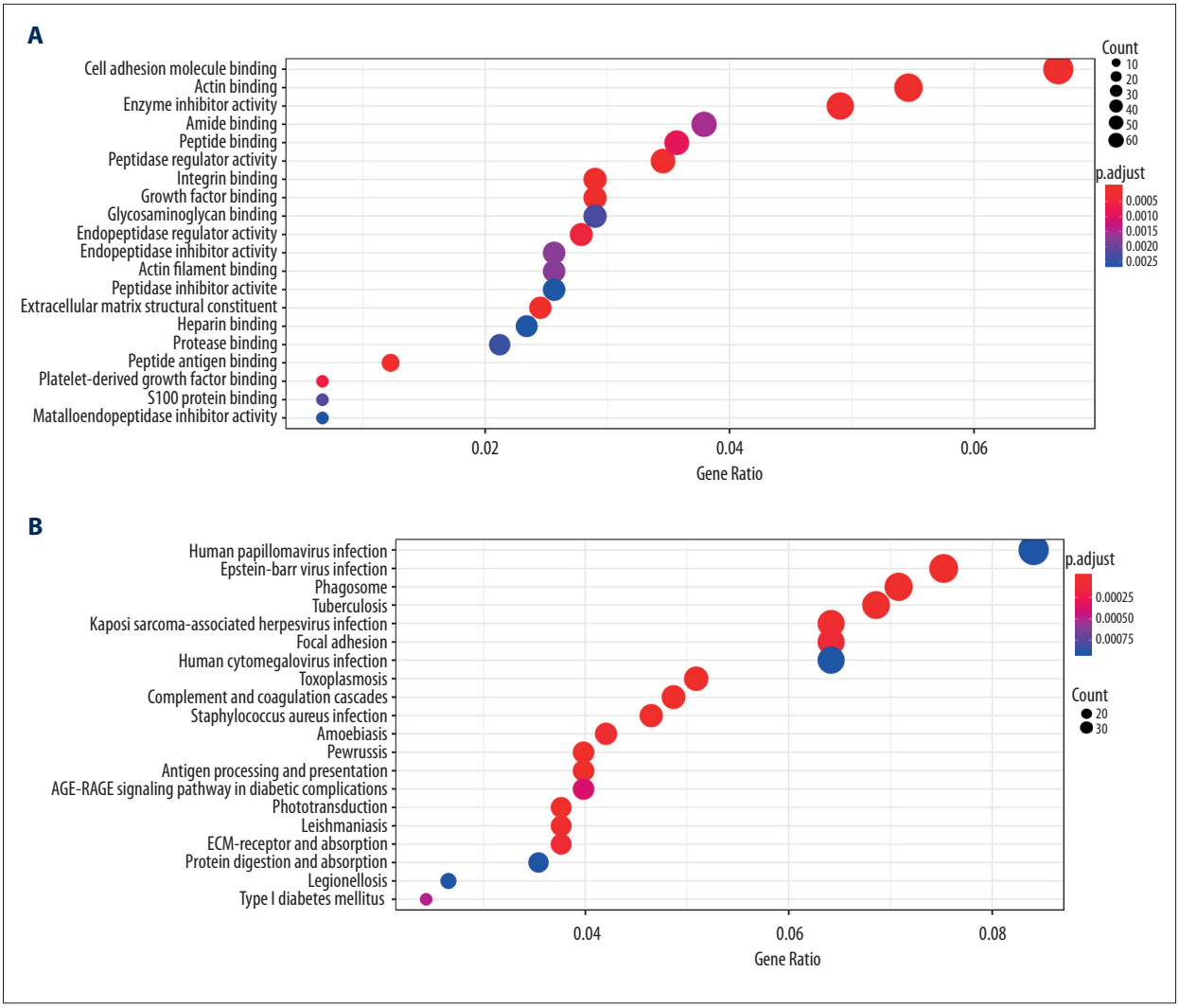


**Figure 2.** Functional enrichment analysis of differentially expressed mRNAs in the human detached retinas with PVR. (**A**) Top 20 most significant biological processes in GO analysis. (**B**) Top 20 most significant KEGG pathways.

phagosome, focal adhesion, and complement and coagulation cascade were also suggested as participate in PVR progress, containing 32, 29, and 22 genes respectively.

### Construction of a ceRNA network and PPI network in the human detached retinas with PVR

To further understand how lncRNA regulates mRNA through combining with miRNA in human detached retinas with PVR, a lncRNA-miRNA-mRNA (ceRNA) network was constructed. Consequently, we found that 9 lncRNAs interacted with the 27 miRNAs in the ceRNA network using the miRcode database (Table 2). A total of 1269 miRNA-targeted mRNAs based on the 27 miRNAs was predicted through miRDB, miRTarBase, and TargetScan databases. MiRNA-targeted mRNAs which were not contained in the differentially expressed mRNAs, which were discarded, and the 73 mutual mRNAs which were preserved (Figure 3A, Table 3). Finally, 9 lncRNAs, 27 miRNAs, and 73 mRNAs constituted the ceRNA network (Figure 4). Besides, KEEG pathways enriched by mRNAs in ceRNA network showed that most of them were bound with the PI3K-Akt signaling pathway and human cytomegalovirus infection (Figure 3B). The PPI network constructed for the 73 mRNAs furnished 21 genes with high confidence 0.700 (Figure 3C).

## Discussion

PVR is known to complicate the post-operative period after a surgery for RD, leading to a re-detachment or limiting visual recovery. Traditional adjuvant therapy included anti-proliferative agents, anti-inflammatory agents, and anti-growth factor pathway inhibitors. But so far, studies for the treatment of PVR report limited success [6,8–11]. The previous study detected





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**Table 2.** lncRNAs and specific targeted miRNAs in ceRNA network.

| lncRNA | miRNA |
|---|---|
| TDRG1 | miR-17-5p, miR-20b-5p, miR-27a-3p, miR-125a-5p, miR-125b-5p |
| HCP5 | miR-137, miR-139-5p, miR-140-5p, miR-17-5p, miR-20b-5p, miR-216b-5p, miR-22-3p, miR-23b-3p, miR-24-3p, miR-363-3p, miR-1297, miR-27a-3p, miR-107, miR-425-5p, miR-125a-5p, miR-125b-5p, miR-10a-5p |
| JPX | miR-301b-3p, miR-140-5p, miR-193a-3p, miR-216b-5p, miR-23b-3p, miR-24-3p, miR-363-3p, miR-449c-5p, miR-129-5p |
| MIAT | miR-301b-3p, miR-139-5p, miR-140-5p, miR-17-5p, miR-20b-5p, miR-206, miR-613, miR-216b-5p, miR-22-3p, miR-23b-3p, miR-24-3p, miR-363-3p, miR-27a-3p, miR-107, miR-449c-5p, miR-125a-5p, miR-125b-5p, miR-10a-5p,miR-129-5p |
| SPAG5-AS1 | miR-17-5p, miR-20b-5p, miR-429, miR-217, miR-24-3p, miR-107, miR-425-5p |
| AC005592 | miR-139-5p, miR-17-5p, miR-20b-5p, miR-206, miR-613, miR-216b-5p, miR-107, miR-425-5p, miR-125a-5p, miR-125b-5p, miR-137, miR-140-5p, miR-429, miR-23b-3p, miR-363-3p, miR-33a-3p, miR-10a-5p, miR-129-5p |
| CRNDE | miR-140-5p, miR-142-3p, miR-193a-3p, miR-216b-5p, miR-217, miR-22-3p, miR-23b-3p, miR-363-3p, miR-1297, miR-27a-3p, miR-129-5p |
| FAM13A-AS1 | miR-137, miR-139-5p, miR-142-3p, miR-17-5p, miR-20b-5p, miR-217, miR-22-3p, miR-23b-3p, miR-24-3p, miR-363-3p, miR-107, miR-449c-5p, miR-125a-5p, miR-125b-5p, miR-129-5p |
| AP000462 | miR-17-5p, miR-20b-5p, miR-1297, miR-206, miR-613, miR-22-3p, miR-24-3p, miR-449c-5p, miR-1297 |

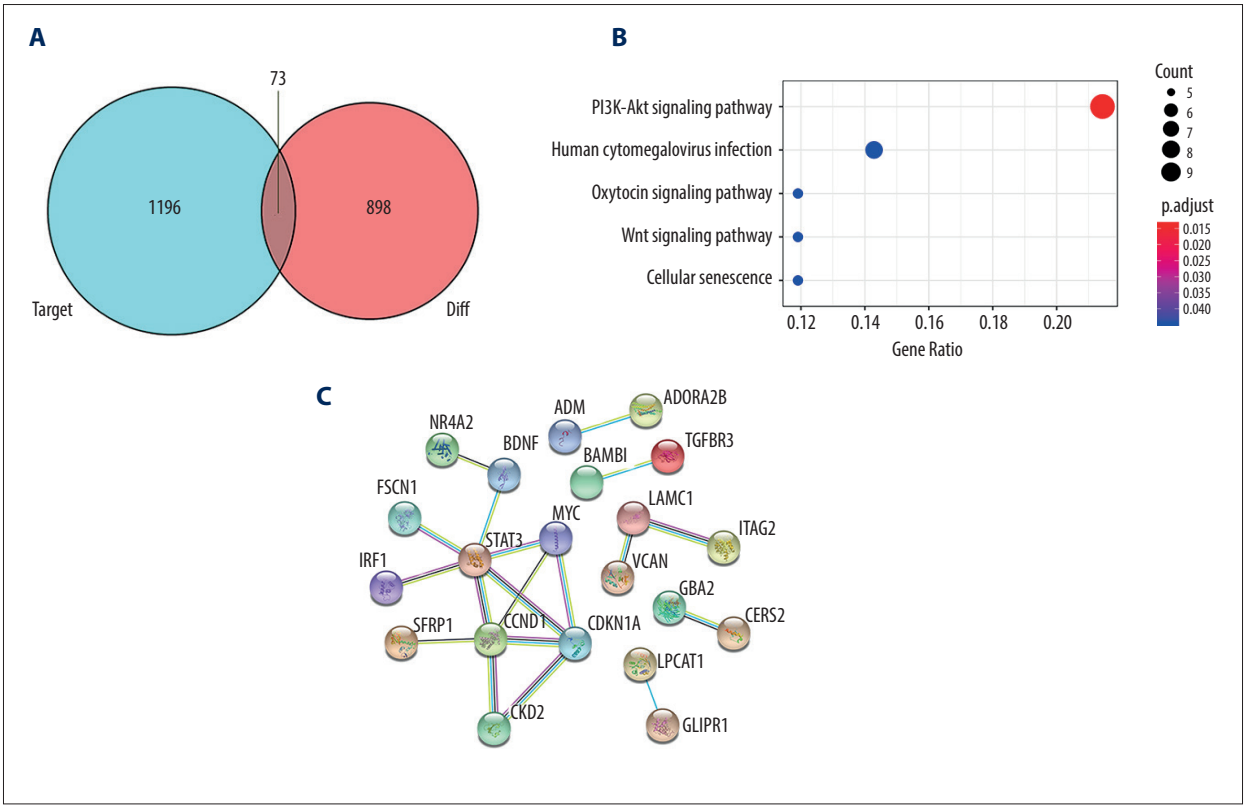


**Figure 3.** mRNAs in ceRNA network. (**A**) Venn diagram of differentially expressed mRNAs in ceRNA network. (**B**) KEEG pathways enriched by mRNAs in ceRNA network. (**C**) The protein-protein interaction network constructed for mRNA involved in ceRNA network.





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**Table 3.** miRNAs and specific targeted mRNAs in ceRNA network.

| miRNA | mRNA |
|---|---|
| miR-107 | VCAN, TGFBR3, GABRB1, ITGA2, LCOR, PLEKHF2,PCSK5 |
| miR-10a-5p | ELOVL2 |
| miR-125a-5p | TMEM136, EIF4EBP1, MTUS1, TMEM136, STAT3, LIPA, EIF4EBP1 |
| miR-129-5p | SPRY4, C1S |
| miR-1297 | ADM, CKS2, MAN2A1 |
| miR-137 | GLIPR1 |
| miR-139-5p | LCOR, ZBTB34 |
| miR-140-5p | LAMC1 |
| miR-142-3p | EGR2, ZNF217, LCOR, MTUS1 |
| miR-17-5p | TMEM123, CDKN1A, PLS1, NETO2, FJX1, CCND1, FAM57A, FAM129A, RAPGEF4, SLC16A9, CYBRD1, PPP3R1, PLEKHO2, CADM2, BTN3A1, STAT3, WEE1, TXNIP, SSX2IP, LCOR, TMEM138 |
| miR-193a-3p | LAMC1 |
| miR-206 | GJA1, KCNJ2, CERS2, BDNF, SFRP1, WEE1 |
| miR-20b-5p | TXNIP, CADM2, RAPGEF4, CCND1, TMEM123, SLC16A9, PLEKHO2, FJX1, SSX2IP, STAT3, CYBRD1, PPP3R1, CDKN1A, PLS1, BAMBI, NETO2, FAM129A |
| miR-216b-5p | SMAD1 |
| miR-217 | NR4A2 |
| miR-22-3p | RGS2, CSF1R |
| miR-23b-3p | GJA1 |
| miR-24-3p | GBA2, SCML1, MLEC, ZNF217, ADD1, FSCN1 |
| miR-27a-3p | PPIF, TGFBR3, LPCAT1, PHLPP2, PLXND1, ADD1, WEE1, ADORA2B |
| miR-301b-3p | GRB10, PRUNE2, TRPC3, IRF1 |
| miR-33a-3p | PPP3R1 |
| miR-363-3p | CNNM4, PDPN, GFPT2, PHLPP2, LHFPL2 |
| miR-425-5p | LCOR, RAB31 |
| miR-429 | TPD52L1 |
| miR-449c-5p | MYC |
| miR-613 | CERS2, WEE1 |

expression profile of 19 PVR patients and controls, but only focused on part of differentially expressed mRNAs [2]. Neither GO and KEGG functional analysis nor construction of ceRNA network were established. Thus, we re-analyzed the published microarray data of GEO database (GSE28133).

In our analysis, we found 971 mRNAs significantly differentially expressed in the human detached retinas with PVR compared to normal human retinas. The GO pathways enriched by the differentially expressed mRNA showed that main pathways of the top 20 took part in the binding function of the cell, such as cell adhesion molecule binding which contained CLIC1 (logFC=2.76), S100A11 (logFC=4.31) and ICAM1 (logFC=3.06), etc. Chloride intracellular channel 1 (CLIC1), and S100 calcium-binding protein A11 (S100A11) were positively correlated with cell proliferation, invasion, and migration and angiogenesis [21,22]. Intercellular adhesion molecule-1 (ICAM1) is involved in the adhesion of leukocytes to the blood vessel wall [23]. Such significantly dysregulated genes involved in cell adhesion and binding function may have an important role in PVR pathogenesis and deserve





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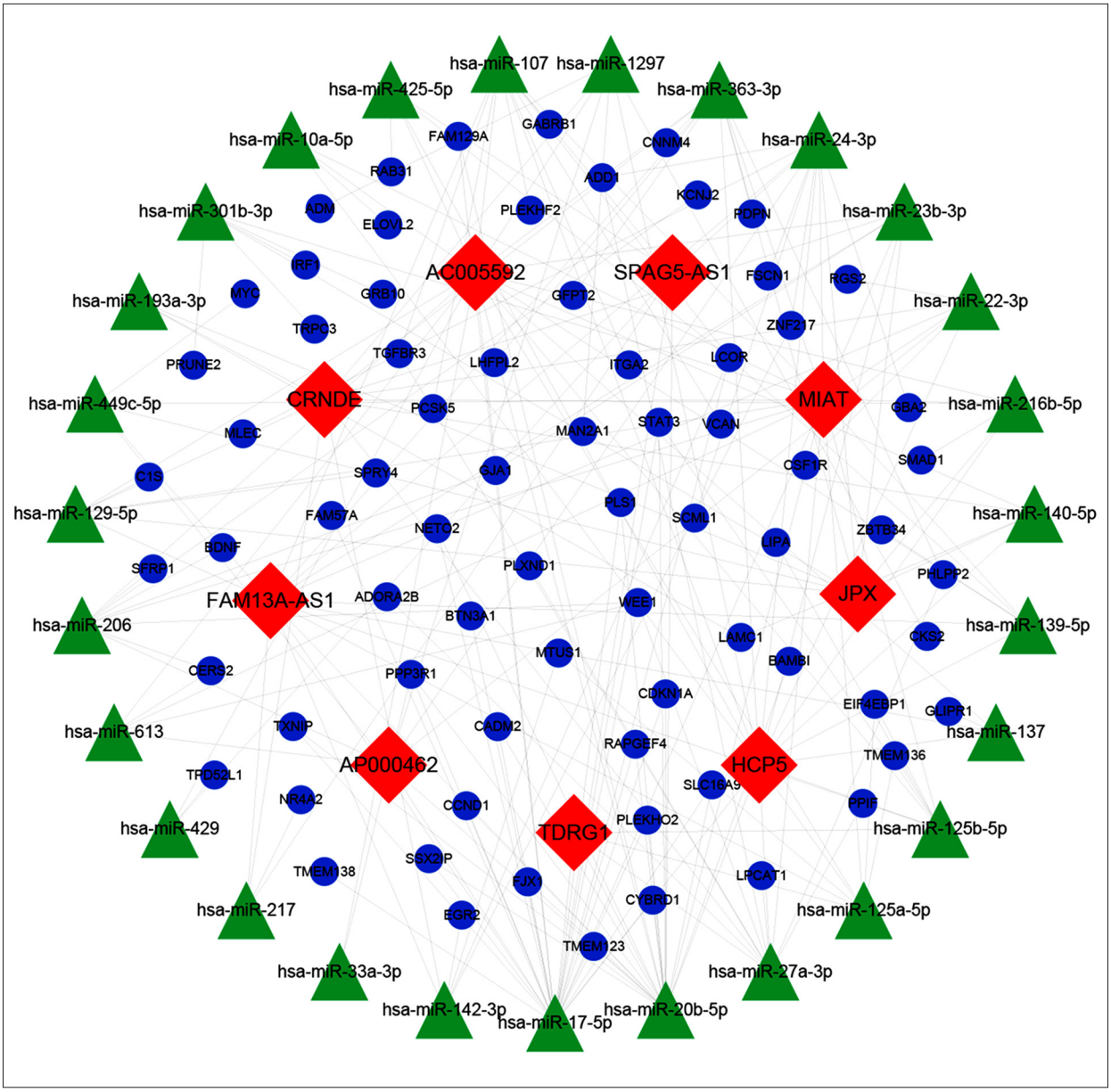


**Figure 4.** CeRNA network in human detached retinas with PVR. Diamonds represent lncRNAs, triangles represent miRNAs and rounds represent protein-coding genes. Gray edges indicate lncRNA-miRNA-mRNA interactions.

more exploration. The KEGG pathways showed that infection and Phosphoinositide 3-kinase (PI3K)-Akt signaling pathway come out in front in RD tissues with PVR. Cytomegalovirus, chlamydia trachomatis, and human immunodeficiency virus have been confirmed in many RD cases [24–27]. These are in conformance with our analysis and remind us of necessary etiological detection for tissues in vitreous cavity and possible targeted treatment. PI3K plays a crucial role as a mediator of growth factor signaling, cell proliferation, cell survival, and apoptotic inhibition. As the major component of the extracellular matrix in PVR, type I collagen was found to be regulated by the PI3K/Akt pathway in human retinal pigment epithelial cells [28]. Treatment targeting PI3K/Akt pathway to prevent PVR after surgery is worth being studied.

The miRNA is an extensive class of endogenous, noncoding and single-strand RNAs with 18–24 nucleotides that negatively regulates gene expression through interacting with the 30-untranslated regions (30UTR) of their target mRNAs [29]. Thus, miRNAs have essential roles in homeostasis and pathogenesis [29]. In the eye, various miRNAs could act on the retina and have an important role in neuroprotection and angiogenesis [30–32]. In our analysis, 27 miRNAs were predicted by the 9 lncRNAs in the ceRNA network using the miRcode database.





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MiR-107, miR-125a-5p, miR-17-5p, miR-20b-5p, and miR-27a-3p interrelated with 7 or more protein-coding genes, which may play a more important role in ceRNA network. These aberrantly expressed miRNAs also played key roles in multiple biological processes of various diseases [33–35]. For example, miR-107 was reported to inhibit cell migration and invasion by modulating Notch2 expression and regulate autophagy and apoptosis by targeting TRAF3 [36,37]. However, the influence of these miRNAs on PVR is rarely explained.

Compared with protein-coding genes and miRNAs, lncRNAs have significant advantages as prognostic biomarkers or therapeutic targets [38,39]. LncRNA regulates gene encoding protein level and participates in the regulation of cell biology through competing with miRNAs in the ceRNA network. There were 23 lncRNAs that were detected to be significantly differentially expressed in the human detached retinas with PVR and 9 of them were involved in the ceRNA network. The most upregulated lncRNA in the ceRNA network is reported to be HCP5. HCP5 was reported to express mainly in the immune system and often considered to be associated with herpes zoster and cancers [40,41]. HCP5 can promote cancer via the PI3K/AKT pathway, which was found to be the most enriched in the KEGG pathways by mRNAs in the ceRNA network [40]. In our analysis, HCP5 was also predicted to interact with 16 miRNAs. Other notable lncRNAs in the ceRNA network reported have included TDRG1 and MIAT [42,43]. TDRG was reported to promote the proliferation and progression of cells through PI3K/Akt/mTOR signaling [42]. Targeting MIAT was found to protect against myocardial hypoxia/reoxygenation injury, but its function in RD and PVR has not been studied [43]. In addition, MALAT1 was also found to be significantly upregulated in the fibrovascular membranes and the peripheral blood samples of PVR patients. *In vitro* studies revealed a critical role of MALAT1 in RPE proliferation and migration [44]. However, studies that focus on the function of lncRNA as miRNA sponges in human RD and PVR are in deficiency.

Our analysis revealed how specific lncRNAs interact with miRNAs and mRNAs through the successful construction of a lncRNA-miRNA-mRNA network in human detached retinas with PVR. This may reveal unknown pathological mechanisms of RD with PVR, which may help to provide potential therapeutic targets. However, we did not conduct quantitative real-time polymerase chain reaction analysis to validate the results of our bioinformatics analysis. The results would be more convincing with such verification experiments.

## Conclusions

We demonstrated the differential expression profiles of lncRNAs, and we constructed a lncRNA-associated ceRNA network in human retinal detachment with PVR. Our analysis may contribute to increased understanding of the pathogenesis of human retinal detachment with PVR and provide novel lncRNAs as potential therapeutic targets.

### Conflicts of interest

None.

## Supplementary Data

**Supplementary Table 1.** GO pathways enriched by the differentially expressed coding genes.

| ID | Description | Count | P-value |
|---|---|---|---|
| GO: 0005201 | Extracellular matrix structural constituent | 22 | 2.10E-11 |
| GO: 0005178 | Integrin binding | 26 | 1.92E-10 |
| GO: 0050839 | Cell adhesion molecule binding | 60 | 2.62E-10 |
| GO: 0019838 | Growth factor binding | 26 | 2.98E-09 |
| GO: 0003779 | Actin binding | 49 | 2.78E-08 |
| GO: 0042605 | Peptide antigen binding | 11 | 5.64E-08 |
| GO: 0061134 | Peptidase regulator activity | 31 | 2.34E-07 |
| GO: 0004857 | Enzyme inhibitor activity | 44 | 1.17E-06 |
| GO: 0061135 | Endopeptidase regulator activity | 25 | 4.70E-06 |
| GO: 0048407 | Platelet-derived growth factor binding | 6 | 6.50E-06 |
| GO: 0042277 | Peptide binding | 32 | 1.06E-05 |
| GO: 0033218 | Amide binding | 34 | 2.05E-05 |
| GO: 0004866 | Endopeptidase inhibitor activity | 23 | 2.51E-05 |





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| ID | Description | Count | P-value |
|---|---|---|---|
| GO: 0051015 | Actin filament binding | 23 | 2.76E-05 |
| GO: 0044548 | S100 protein binding | 6 | 3.70E-05 |
| GO: 0005539 | glycosaminoglycan binding | 26 | 4.10E-05 |
| GO: 0002020 | Protease binding | 19 | 4.63E-05 |
| GO: 0008201 | Heparin binding | 21 | 5.60E-05 |
| GO: 0030414 | Peptidase inhibitor activity | 23 | 5.65E-05 |
| GO: 0008191 | Metalloendopeptidase inhibitor activity | 6 | 5.90E-05 |
| GO: 0005518 | Collagen binding | 12 | 7.07E-05 |
| GO: 0023026 | MHC class II protein complex binding | 6 | 9.03E-05 |
| GO: 0031406 | Carboxylic acid binding | 23 | 0.000101 |
| GO: 0043177 | Organic acid binding | 23 | 0.000119 |
| GO: 0019864 | IgG binding | 5 | 0.000123 |
| GO: 0016641 | Oxidoreductase activity, acting on the CH-NH2 group of donors, oxygen as acceptor | 6 | 0.000133 |
| GO: 0004859 | Phospholipase inhibitor activity | 5 | 0.000202 |
| GO: 0019955 | Cytokine binding | 15 | 0.000229 |
| GO: 0001968 | Fibronectin binding | 7 | 0.000249 |
| GO: 0030507 | Spectrin binding | 7 | 0.000249 |
| GO: 0050840 | Extracellular matrix binding | 10 | 0.000259 |
| GO: 0008020 | G-protein coupled photoreceptor activity | 5 | 0.000315 |
| GO: 0023023 | MHC protein complex binding | 6 | 0.000366 |
| GO: 0005504 | Fatty acid binding | 7 | 0.00041 |
| GO: 0030674 | Protein binding, bridging | 20 | 0.000439 |
| GO: 0016504 | Peptidase activator activity | 8 | 0.000464 |
| GO: 0005520 | Insulin-like growth factor binding | 7 | 0.000517 |
| GO: 0005546 | Phosphatidylinositol-4,5-bisphosphate binding | 11 | 0.000544 |
| GO: 0030246 | Carbohydrate binding | 27 | 0.000547 |
| GO: 0016638 | Oxidoreductase activity, acting on the CH-NH2 group of donors | 6 | 0.000646 |
| GO: 0019865 | Immunoglobulin binding | 6 | 0.000646 |
| GO: 0009881 | Photoreceptor activity | 5 | 0.000674 |
| GO: 0070492 | Oligosaccharide binding | 5 | 0.00094 |
| GO: 0033293 | Monocarboxylic acid binding | 10 | 0.000973 |
| GO: 0042578 | Phosphoric ester hydrolase activity | 33 | 0.001054 |
| GO: 0046982 | Protein heterodimerization activity | 42 | 0.001109 |
| GO: 0019203 | Carbohydrate phosphatase activity | 4 | 0.001111 |
| GO: 0035325 | Toll-like receptor binding | 4 | 0.001111 |
| GO: 0050308 | Sugar-phosphatase activity | 4 | 0.001111 |
| GO: 0008329 | Signaling pattern recognition receptor activity | 5 | 0.001276 |
| GO: 0038187 | Pattern recognition receptor activity | 5 | 0.001276 |
| GO: 0055102 | Lipase inhibitor activity | 5 | 0.001276 |
| GO: 0019956 | Chemokine binding | 6 | 0.001345 |
| GO: 0015298 | Solute: cation antiporter activity | 7 | 0.001425 |





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| ID | Description | Count | P-value |
|---|---|---|---|
| GO: 0004197 | Cysteine-type endopeptidase activity | 12 | 0.001529 |
| GO: 0071949 | FAD binding | 6 | 0.001673 |
| GO: 0050786 | RAGE receptor binding | 4 | 0.001676 |
| GO: 0043531 | ADP binding | 7 | 0.001704 |
| GO: 0060090 | Molecular adaptor activity | 20 | 0.001883 |
| GO: 0005507 | Copper ion binding | 9 | 0.00199 |
| GO: 0003785 | Actin monomer binding | 6 | 0.00206 |
| GO: 1901681 | Sulfur compound binding | 23 | 0.002196 |
| GO: 0098641 | Cadherin binding involved in cell-cell adhesion | 5 | 0.002201 |
| GO: 0001540 | Amyloid-beta binding | 9 | 0.002258 |
| GO: 0036041 | Long-chain fatty acid binding | 4 | 0.002413 |
| GO: 0045296 | Cadherin binding | 29 | 0.002488 |
| GO: 0043394 | Proteoglycan binding | 7 | 0.002799 |
| GO: 0031994 | Insulin-like growth factor i binding | 4 | 0.003346 |
| GO: 0032561 | Guanyl ribonucleotide binding | 33 | 0.003348 |
| GO: 0019001 | Guanyl nucleotide binding | 33 | 0.003483 |
| GO: 0001848 | Complement binding | 5 | 0.003539 |
| GO: 0098631 | Cell adhesion mediator activity | 7 | 0.003784 |
| GO: 0015297 | Antiporter activity | 11 | 0.003925 |
| GO: 0008236 | Serine-type peptidase activity | 25 | 0.004086 |

**Supplementary Table 2.** KEGG pathways enriched by the differentially expressed coding genes.

| ID | Description | Count | *P*-value |
|---|---|---|---|
| hsa04744 | Phototransduction | 17 | 1.47E-14 |
| hsa05150 | Staphylococcus aureus infection | 21 | 1.87E-10 |
| hsa04145 | Phagosome | 32 | 2.46E-10 |
| hsa04610 | Complement and coagulation cascades | 22 | 6.39E-10 |
| hsa05169 | Epstein-Barr virus infection | 34 | 2.67E-08 |
| hsa05152 | Tuberculosis | 31 | 6.42E-08 |
| hsa05145 | Toxoplasmosis | 23 | 1.69E-07 |
| hsa05133 | Pertussis | 18 | 3.54E-07 |
| hsa04612 | Antigen processing and presentation | 18 | 4.37E-07 |
| hsa05140 | Leishmaniasis | 17 | 1.19E-06 |
| hsa05167 | Kaposi sarcoma-associated herpesvirus infection | 29 | 1.68E-06 |
| hsa05146 | Amoebiasis | 19 | 3.14E-06 |
| hsa04512 | ECM-receptor interaction | 17 | 5.38E-06 |
| hsa04510 | Focal adhesion | 29 | 6.69E-06 |
| hsa04933 | AGE-RAGE signaling pathway in diabetic complications | 18 | 2.26E-05 |
| hsa04940 | Type I diabetes mellitus | 11 | 3.20E-05 |
| hsa05165 | Human papillomavirus infection | 38 | 6.93E-05 |
| hsa05163 | Human cytomegalovirus infection | 29 | 7.05E-05 |
| hsa04974 | Protein digestion and absorption | 16 | 7.51E-05 |





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| ID | Description | Count | *P*-value |
|---|---|---|---|
| hsa05134 | Legionellosis | 12 | 7.74E-05 |
| hsa05164 | Influenza A | 24 | 7.76E-05 |
| hsa05132 | Salmonella infection | 15 | 0.000157 |
| hsa05166 | Human T-cell leukemia virus 1 infection | 27 | 0.000263 |
| hsa04514 | Cell adhesion molecules (CAMs) | 20 | 0.00035 |
| hsa05144 | Malaria | 10 | 0.00054 |
| hsa04151 | PI3K-Akt signaling pathway | 37 | 0.000605 |
| hsa05170 | Human immunodeficiency virus 1 infection | 25 | 0.000858 |
| hsa04210 | Apoptosis | 18 | 0.001211 |
| hsa04380 | Osteoclast differentiation | 17 | 0.00158 |
| hsa04672 | Intestinal immune network for IgA production | 9 | 0.002223 |
| hsa04670 | Leukocyte transendothelial migration | 15 | 0.002691 |
| hsa05014 | Amyotrophic lateral sclerosis (ALS) | 9 | 0.00296 |
| hsa04010 | MAPK signaling pathway | 30 | 0.002984 |
| hsa04218 | Cellular senescence | 19 | 0.003215 |
| hsa05130 | Pathogenic Escherichia coli infection | 9 | 0.005004 |
| hsa04650 | Natural killer cell mediated cytotoxicity | 16 | 0.005015 |
| hsa05160 | Hepatitis C | 18 | 0.005171 |
| hsa00532 | Glycosaminoglycan biosynthesis – chondroitin sulfate/dermatan sulfate | 5 | 0.005557 |
| hsa04142 | Lysosome | 15 | 0.006575 |

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