

Identification of The Key miRNAs And Target Genes In Basal Cell Carcinoma By Bioinformatics Analysis

Hao Liu

Chongqing Medical University <https://orcid.org/0000-0001-8926-6320>

Ling Chen (✉ chenling@cqmu.edu.cn)

Chongqing Medical University

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Identification of the key miRNAs and target genes in basal cell carcinoma by bioinformatics analysis

Hao Liu¹, Ling Chen^{2,*}

¹ School of Stomatology, Chongqing Medical University, Chongqing 400016, China;

² The Center of Experimental Teaching Management, Chongqing Medical University, Chongqing 400016, China.

*Correspondence: chenling@cqmu.edu.cn

Abstract

Background

The highly tissue-destructive and localized accumulation of basal cell carcinoma (BCC) makes it one of the most important cancers affecting people's lives. Existing therapeutic approaches, including surgical treatment, chemotherapy, and Hedgehog pathway inhibitors, have failed to achieve broad therapeutic effects for various reasons. This study aims to explore additional potential therapeutic targets and possible diagnostic and prognostic biomarkers using bioinformatics analysis.

Material/Methods

The Gene Expression Omnibus (GEO) database identified the microarray dataset GSE34535. The GEO2R tool was used to screen out differentially expressed genes (DEGs) between BCC and non-lesional skin. Potential target genes of DE-miRNA were screened using the miRWalk, mirDIP and miRTarBase databases. Gene Ontology function and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis for target genes were established using the DAVID database. Protein-protein interaction network and miRNA-hub gene network were analyzed based on the STRING database and visualized by Cytoscape software.

Results

51 up-regulated DE-miRNAs and 38 down-regulated DE-miRNAs were identified from the BCC samples. miR-455-5p was mainly up-regulated and miR-139-5p was mainly down-regulated. Two key hub genes MAPK1 and EGFR were identified in the PPI network. Four out of the ten hub genes were regulated by up-regulated miR-18a and four by down-regulated miR-133b. Viral infections were also identified in the study.

Conclusions

Bioinformatics identified four miRNAs and two important hub genes that may be associated with BCC, and it was suggested that viruses may play a role in BCC.

Keywords

bioinformatics analysis; basal cell carcinoma; miRNAs.

1 | Background

Basal cell carcinoma (BCC), previously known as basal cell epithelioma, is the most common cancer in humans that originates in the basal cell layer of the epidermis and skin appendages. Clinically, BCC mostly occurs at the site of exposure, with the head and neck being the most common. The majority of BCCs grow slowly and rarely metastasize, but when cancerous tissue is not adequately removed or treated in a timely manner, BCCs may develop local metastasis, which is highly destructive and causes a structural destruction-like appearance of surrounding tissues[1], and may even lead to a small number of deaths. Therefore, improving the early detection rate and carrying out effective treatment are effective ways to avoid the progression of BCC in an undesirable direction. BCC is mainly prevalent in older men[2], but in recent years the trend of younger and more feminine people is also becoming more apparent[3]. The interaction between environmental, phenotypic and genetic factors is a risk factor for the development of BCC[4], but the molecular mechanisms underlying its occurrence and development have not yet been fully elucidated. Surgical approach is the first choice for the treatment of BCC, especially Mohs micrographic surgery, which has a high cure rate. If surgery is contraindicated, radiotherapy is the main alternative treatment for BCC. However, these methods are not always applicable, especially for institutions that lack experienced specialists and relevant pathology testing equipment[5]. And for patients with Local advanced BCC (LaBCC), the cancer has caused extensive tissue destruction in the surrounding anatomical region, which makes it impossible to treat the tumor through surgery or radiation therapy[4]. Hedgehog (Hh) pathway inhibitors have been developed for use in patients with locally advanced or rare metastatic BCC[6], but the prevalence of drug resistance[7] and side effects[8] has limited their widespread use. Therefore, it is urgent to explore the underlying molecular mechanisms of BCC and find more useful early diagnostic techniques and more reliable molecular markers to monitor recurrence, assess prognosis and provide new ideas for targeted therapy.

MicroRNAs (miRNAs) are small RNAs between 21–25 nucleotides in length, they do not have the potential to encode proteins, but can influence genes. They are present in a variety of biological processes and play an important regulatory role in the translation and degradation of messenger RNAs (mRNAs) through binding to the 3'-untranslated region of mRNAs[9]. Many studies have shown that miRNAs are closely associated with the development and progression of a variety of diseases. Especially in tumors, there have been a large number of discoveries targeting different types of tumors, which provide effective ways to diagnose and treat the diseases. For BCC, similar studies have been conducted to investigate the differences in miRNAs expression in lesioned versus non-lesioned regions in patients with general BCC[10], in patients with different subtypes of BCC[11] or in an extensive cranial BCC patient receiving oral vismodegib therapy[12]. Their study identified a series of up- and down-regulated miRNAs, including miR-203, miR-183, miR-17, miR-29c, and others. These initial studies demonstrated that miRNAs are involved in BCC and revealed the importance of miRNAs in the development and prognosis of BCC. However, more studies, such as gene array studies to investigate the role of miRNAs and target genes, are needed to better reveal the role of miRNAs in the pathogenesis of BCC.

Therefore, this study aimed to identify miRNAs and target genes associated with prognosis or treatment in BCC using bioinformatics analysis. The study included screening for DE-miRNAs, analysis of functional and pathway enrichment, and construction of PPI networks, to identify the

novel targets.

2 | MATERIALS AND METHODS

2.1 | Data source

The gene expression data of BCC were obtained from the GEO database(<https://www.ncbi.nlm.nih.gov/geo/>). The microarray dataset GSE34535 based on GPL15019, which included 14 samples from the center of the tumors and from sites of adjacent non-lesional skin, was selected and used in this study.

2.2 | Screening for DE-miRNA

The online GEO2R analysis tool (<https://www.ncbi.nlm.nih.gov/geo/geo2r/>) from GEO database was used to compare the differences between BCC tissue and normal tissue, and calculate the adjusted P-value and $|\log_{2}FC|$. The screening threshold for DE-miRNA was set to adjust P-value < 0.05 and $|\log_{2}FC|$ was defined as > 1.

2.3 | Analyses of miRNA-mRNA targets

In this section, we identified the top ten up-regulated and down-regulated DE-miRNAs and then predicted the targets of the DEGs by employing three miRNA-target tools: miRWalk V3.0 database, mirDIP, and miRTarBase. Statistical analysis of prediction results for each tool was carried and intersecting parts were identified using the Venn diagram webtool (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) to screen the miRNA targets. Then, use the Cytoscape software (<http://cytoscape.org/>) to visualize the miRNA-mRNA networks.

2.4 | GO and KEGG pathway analysis of DEGs

GO and KEGG pathway analysis are widely used in bioinformatics research, which revealed the biological processes (BPs), cellular components (CCs), molecular functions (MFs) and pathways associated with the object of study. In this study, GO annotation analysis and KEGG pathway enrichment analysis of DEGs were performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) (<https://david.ncifcrf.gov>). $P < 0.05$ was considered statistically significant.

2.5 | PPI network construction and hub gene identification

The Search Tool for the Retrieval of Interacting Genes (STRING) database (<http://string-db.org/>) was used to analyze PPI network. The results were visualized by Cytoscape software. To determine the key genes, we use Cytoscape's plug-in cytoHubba to calculate the degree of each protein node.

3 | RESULTS

3.1 | Identification of DE-miRNAs

The microarray dataset GSE34535 was downloaded from the GEO database included 7 cases of BCC(GSM850677-GSM850683) and 7 intraindividual controls(GSM850684-GSM850690). Adjust P-value<0.05 and $|\log_{2}FC| > 1$ were considered as criteria for screening DE-miRNAs. 89 DE-miRNAs were identified from dataset (Figure 1), including 51 up-regulated miRNAs and 38 down-regulated miRNAs. Noteworthy, the expression of HCMV, HSV1 and HSV2 virus-associated miRNAs was lower in BCC tissues than in control tissues, especially hcmv-miR-UL70-3p was in the 9th place among the down-regulated miRNAs and hsv1-miR-H17 was in the 11th place. Table 1 lists the 10 most significantly up-regulated miRNAs and the 10 most significantly down-regulated miRNAs(exclusion of virus-associated miRNAs). Table 2 lists the down-regulated viral-associated miRNAs.

3.2 | miRNAs-target gene interactions

After processing and analyzing the data predicted from the three databases, a total of 874 overlapping genes regarding twenty miRNAs were obtained. These overlapping genes are possible target genes that interact with the miRNAs. The possible targets are validated by more than four algorithms, including TargetScan, miRDB, RNA22 and RNAhybrid, with high confidence. The miRNA-mRNA network was visualized by Cytoscape and is displayed in Figure 2. The count of target genes for the corresponding miRNAs is listed in Table 1.

3.3 | Enrichment analyses of the target genes

To explore the functions of the target genes, we analyzed 874 genes screened using the DAVID online tool. In the GO annotation, three items closely related to function, BP, CC and MF, were chosen for annotation. The significantly enriched entries for BP were transcription, DNA-templated, positive regulation of transcription from RNA polymerase II promoter and negative regulation of transcription from RNA polymerase II promoter (Fig.3A). Furthermore, the nucleus, cytoplasm and nucleoplasm were the most concentrated entries in CC term (Fig.3B). The most enriched MF were protein binding, DNA binding and ATP binding (Fig.3C). KEGG pathway enrichment analysis showed that the regulation of DE-miRNAs were significantly enrichment in pathways in cancer, PI3K-Akt signaling pathway and MicroRNAs in cancer. Intriguingly, the enrichment in HTLA-I infection was found to have a possible role in BCC (Fig.3D).

3.4 | PPI network construction and hub genes identification

To explore the interactions between target genes, we used the STRING database to predict the PPI network. A total of 366 nodes and 1,559 edges were involved in the PPI network, as shown in Figure 4. The top ten hub genes of up- and down-regulated miRNAs were identified and evaluated separately by degree, as shown in Table 3. For up-regulated miRNAs, MAPK1, EP300, KRAS, PTEN, CCND1, ESR1, CCNB1, SMAD4, ATM and IGF1R were the top ten hub genes, and MAPK1 showed the highest node degree (node degree=91). For down-regulated miRNAs, the leading ten hub genes were EGFR, KRAS, NOTCH1, EP300, MAPK1, CDC42, SIRT1, SMAD4, ERBB2 and BCL2L1 and EGFR showed the highest node degree (node degree=39). Figure 5 shows the networks of the top 10 hub genes.

3.5 | miRNA-hub gene network

To further clarify the interaction between DE-miRNAs and hub genes, the miRNA-hub gene network was mapped, as shown in figure 6. For up-regulated miRNAs, four out of ten genes (ESR1, PTEN, ATM and CCND1) could be potentially modulated by has-miR-18a. Has-miR-182, has-miR-96 and has-miR-183 each could target three genes. Has-miR-196b, has-miR-181c and has-miR-181d each could potentially modulate one gene. Also, for down-regulated miRNAs, four out of ten hub genes (SIRT1, BCL2L1, CDC42 and EGFR) could be potentially modulated by has-miR-133b. Two hub genes could be potentially modulated by has-miR-30a*. Has-miR-193a-5p, has-miR-452, has-miR-378a-3p, has-miR-29c* and has-miR-139-5p each could potentially regulate one gene. Therefore, has-miR-18a and has-miR-133b were thought to be the most associated with the the development and progression of BCC.

4 | DISCUSSION

BBC is a less malignant tumor. Although it rarely metastasizes, its high degree of tissue destruction and local accumulation make it one of the most important cancers affecting people's lives. Existing

therapeutic approaches, including surgical treatment, chemotherapy, and Hedgehog pathway inhibitors, have failed to achieve broad therapeutic effects for various reasons. Therefore, more potential therapeutic targets and possible diagnostic and prognostic biomarkers need to be explored.

In this study, 89 DE-miRNAs in BCC tissues compared to normal skin tissue samples were identified by differential expression analysis of miRNA arrays downloaded from the GEO database. In BCC, has-miR-455-5p was mainly up-regulated and has-139-5p was mainly down-regulated. Meanwhile, the miRNA-hub gene interaction network showed that has-miR-18a and has-miR-133b were the most associated with the hub genes among the up- or down-regulated miRNAs. Thus, the four miRNAs with the most significant expression differences or the most extensive interactions are the most likely key DE-miRNAs affecting the development of BCC.

For up-regulated miRNAs, miRNA-455-5p and miRNA-18a were confirmed to have the same expression difference in another but more targeted single-case BCC study[12]. miR-455-5p is characterized as a tumor-associated miRNA in cancer research. In colon cancer, miR-455-5p functions as a potential oncogene, which promoted HT29 cell proliferation and inhibited HT29 cell apoptosis by suppressing galectin-9 expression[13]. Also, it has been suggested that miRNA-455-5p is associated with bladder cancer progression and its overexpression may suggest a poor prognosis of bladder cancer[14]. In non-small cell lung cancer, the expression of miR-455-5p was up-regulated in tumor tissues compared to corresponding noncancerous tissues. Further studies showed that miR-455-5p could promote the growth and metastasis of NSCLC by inhibiting SOCS3[15]. The pro-carcinogenic effect of miR-455-5p has also been demonstrated in other tumors[16, 17]. In this study, miRNA-455-5p was considered to be the most significantly up-regulated expression in BCC, but at the same time, it was not involved in the regulation of the top ten hub genes of up-regulated miRNAs. Further studies on the molecular mechanism of miRNA-455-5p involved in the development of BCC are still needed. Considering its significant high expression in cancer tissues, we suggest that miRNA-455-5p has a suggestive role. Expression of miRNA-18a is associated with several human malignancies and appears to exhibit opposing effects. In a study on clear cell renal cell carcinoma, investigators suggested that the miRNA18a / HIF1A / PVT1 pathway may play a key role in ccRCC progression and the upregulation of miRNA-18a has a positive effect on ccRCC cell migration and invasion[18]. In contrast, another study on breast cancer suggested that miRNA-18a plays an oncogenic role in the early development of breast cancer, and its high expression is beneficial for survival[19]. In addition, miRNA-18a was also shown to be highly overexpressed in patients with esophageal squamous cell carcinoma, but did not correlate with tumor invasion, node positivity, metastatic disease and advanced stage of tumor, and its level of expression was significantly reduced after completion of chemoradiotherapy[20]. In this study, miRNA-18a was closely associated with four of the ten hub genes and may play a key role in the development of BCC. However, its up-regulated expression plays a positive or negative role in BCC still needs further investigation.

For down-regulated miRNAs, miRNA-139-5p was also confirmed to be down-regulated in a similar study[12]. In several studies, miRNA-139-5p has been suggested to be a negative regulator of cancer development, exhibiting potent anti-oncogenic and antimetastatic activity. In esophageal cancer, the expression level of miR-139-5p was significantly lower compared with that in paracancerous tissues,

and the expression level in serum also closely correlated with tumor stage. Further studies revealed that high expression of miR-139-5p inhibited the proliferation of esophageal cancer cells by regulating VEGFR and the signaling pathways of its downstream primers, which played a regulatory role in inhibiting the development of esophageal cancer[21]. In contrast, low expression of miR-139-5p in tumor patients suggested poor prognosis. Similar effects have been demonstrated in other studies, where miR-139-5p was significantly under-expressed in cancer tissues, while multiple pathways such as CXCR4, PKM2, RPRD1B and Notch1 could be negatively regulated by regulating miR-139-5p levels to inhibit the development of various cancers such as oral squamous cell carcinoma[22], gallbladder cancer[23], and breast cancer[24]. In this study, miRNA-139-5p downregulation in BCC tissues may suggest poor prognosis. And the high expression of miR-139-5p may be involved in inhibiting the proliferation, migration and invasion process of cancerous basal cells through similar pathways, exerting an oncogenic effect. The differential expression of miRNA-133b in BCC was not mentioned in other studies. In this study, BCC tissues expressed lower miRNA-133b compared to normal skin tissues ($\log_{2}FC = -4.25411$), which was located in the 5th position of the top 10 down-regulated DE-miRNAs. miRNA-hub gene network showed that miRNA-133b may be involved in regulating four of the ten hub genes (EGFR, SIRT1, BCL2L1 and CDC42). In other tumor studies, miRNA-133b exhibited similar oncogenic effects to miR-139-5p which was also lowly expressed in cancerous tissues. When overexpressed, it inhibits the development of osteosarcoma[25], FAP-derived desmoid tumor[26], clear cell renal cell carcinoma[27] and hepatocellular carcinoma[28] through the FGFR1, SIRT1, JAK2/STAT3, and SF3B4 pathways. The low expression of miRNA-133b in BCC in this study may have the same suggestive effect as miR-139-5p. Therefore, we speculate that miR-139-5p and miRNA-133b might be a negative modulator for the development of BCC, but more experimental validation is needed.

In the present study, we performed GO terms and KEGG pathway enrichment for 20 miRNAs which were significantly up-regulated and significantly down-regulated with the help of DAVID database. GO analysis revealed a significant enrichment of DEGs in cell replication and apoptosis, with these pathways showing a great role in BCC progression. KEGG pathway analysis showed that DEGs were significantly enriched in various tumor pathways and PI3K-Akt pathway. The role of PI3K-Akt signaling pathway in BCC remains unclear. In previous studies, PI3K-Akt was considered to be the framework of malignant behavior, deeply involved in the genesis, proliferation and apoptosis of many malignancies, and even in the whole process of tumorigenesis[29]. PI3K-Akt is also gaining attention as a potential therapeutic target against a variety of cancers.

In this study, MAPK1 and EGFR were positioned as the most concluded hub genes. MAPK1 belongs to the MAP kinase family, which is extensively involved in cell differentiation, proliferation, transcriptional development and regulatory processes[30]. As a potential target gene for miRNAs, MAPK1 has been reported to be extensively involved in the development of tumor in several studies. Various miRNAs are involved in the progression of several cancers by targeting MAPK1, including pancreatic cancer[31], bladder cancer[32] and endometrial cancer[33]. In the present study, it was suggested that MAPK1 may play a role in the process of BCC by PPI network analysis, but this finding needs to be verified in further experiments. EGFR is considered as an oncogenic driver and there is substantial evidence that EGFR is involved in the pathogenesis and progression of various cancers[34]. Previous studies have shown that significant EGFR expression in BCC is associated

with BCC aggressiveness and tumor differentiation to different histological subtypes[35], but the mechanism of its action in BCC is unclear.

The relationship between the virus and BCC is another finding of this study. Virus-associated miRNAs were significantly down-regulated in the lesioned area compared to the adjacent non-lesioned skin tissue of BCC, including miRNAs of HCMV, HSV1, HSV2 and KSHV. Meanwhile, KEGG pathway analysis revealed a high enrichment of DEGs in the HTLV-I infection pathway. Previous studies have found HPV to be detected in BCC and suggest that it may play a role in BCC. However, the association of HCMV, HSV1, HSV2, KSHV and HTLV-I with BCC has not been reported in studies. Some studies have suggested that viral infections may be responsible for specific human cancers worldwide[36]. HCMV is carried by the majority of the world's population. It has been found to be present in gliomas and may play a role in gliomas through specific local tumor microenvironment or other pathways[37, 38]. HSV1 and HSV2 belong to the two most common types of Herpes simplex virus. HSV infections are common and widespread worldwide[39]. HSV1 infections mostly occur in the oropharynx and have clinical symptoms similar to those of conventional infections. HSV2 infections mostly occur in the genitalia with symptoms of a herpes-like appearance. Although HSV infection has shown a trend of progressive increase and difficulty in control in recent years, there is no direct evidence of a direct relationship with the development of tumors. Although HSV infection has shown a trend of progressive increase and difficulty in control in recent years, there is no direct evidence of a direct relationship with the development of tumors. It is believed that KSHV infection can directly induce tumorigenesis through a complex interaction of multiple viruses, cellular angiogenesis and inflammatory markers[40]. It is involved in the development and progression of a variety of malignancies including Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castleman's disease[41]. HTLV-I is the first pathogenic human retrovirus to be identified. In the past 30 years of research, HTLV-I is thought to be associated with a variety of malignancies, particularly non-Hodgkin lymphomas, Kaposi sarcoma, and cervical cancer[42]. HTLV-I indirectly plays a role in cancer through microenvironmental or immune surveillance attenuation. In conclusion, these viruses may play a role in different types of tumors, but the exact mechanism or magnitude of the role is not well understood. In the present study, the mixed infection of multiple viruses in BCC tissues could be either an incidental opportunistic infection or a co-infection of multiple viruses playing a role in the development of BCC, which may be particularly associated with BCC expansion. Further studies are needed.

Our current study has some limitations. The small sample size we obtained from GSE34535 may produce some bias in the analysis of DE-miRNA. In addition, the predictions obtained based on bioinformatics analysis need to be validated in more cellular and animal experiments.

5 | CONCLUSION

Based on the GEO database and bioinformatics analysis, we not only identified four miRNAs and two important hub genes that may be associated with BCC, but also suggested that viruses may play a role in BCC. These preliminary findings may serve as promising novel therapeutic targets and prognostic biomarkers for BCC. However, experiments are still needed to support our results.

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Authors 'contributions

All authors were involved in the entire process of the study. t. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

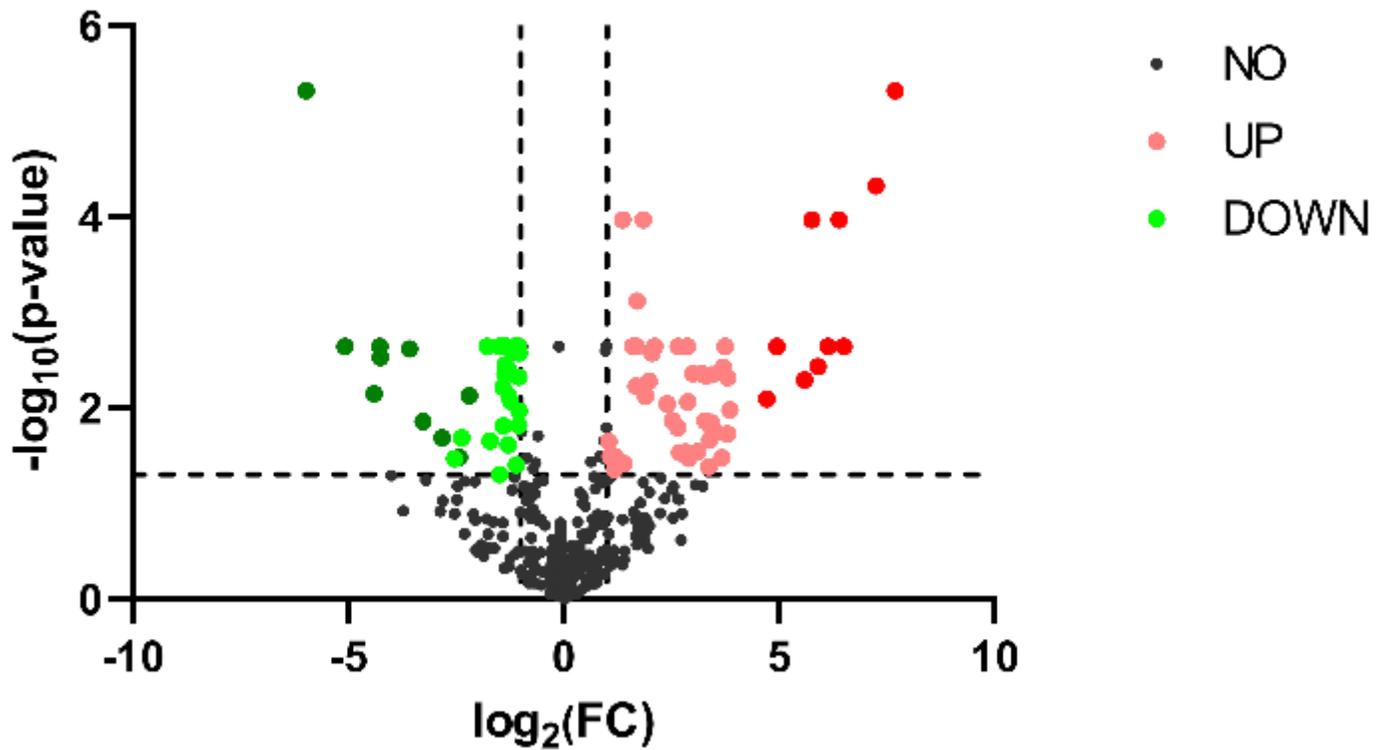


Figure 1

Volcano plot of differentially expressed miRNAs in GSE34535 The red dot represents up-regulated miRNAs and the green dot represents down-regulated miRNAs

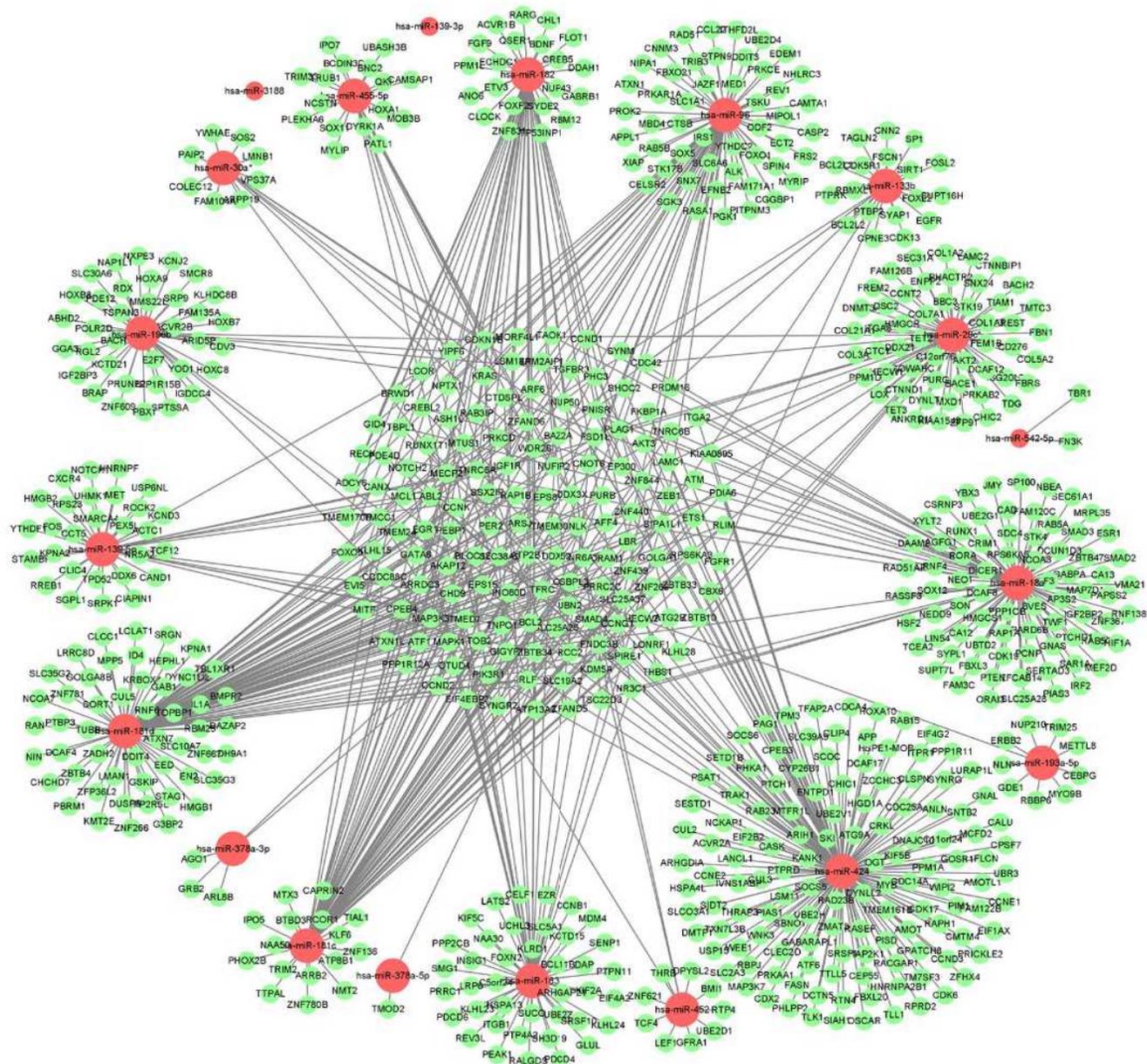


Figure 2

miRNA-target gene network The red dot represents miRNAs and the green dot represents target mRNAs.

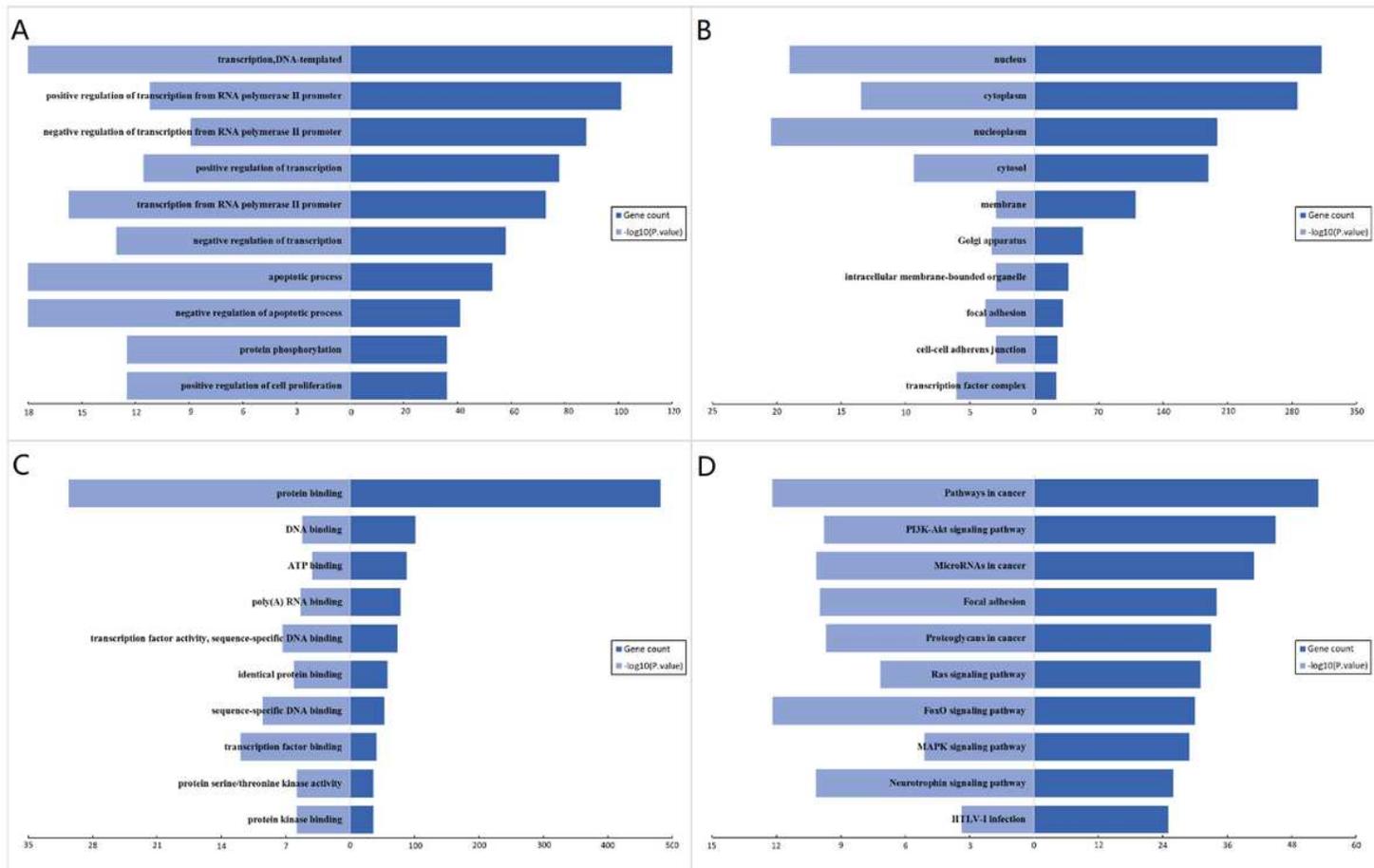


Figure 3

Top 10 significant enrichment GO and KEGG terms (A) BP: biological process;(B) CC: cellular component; (C) MF: molecular function;(D)KEGG: signaling pathway.

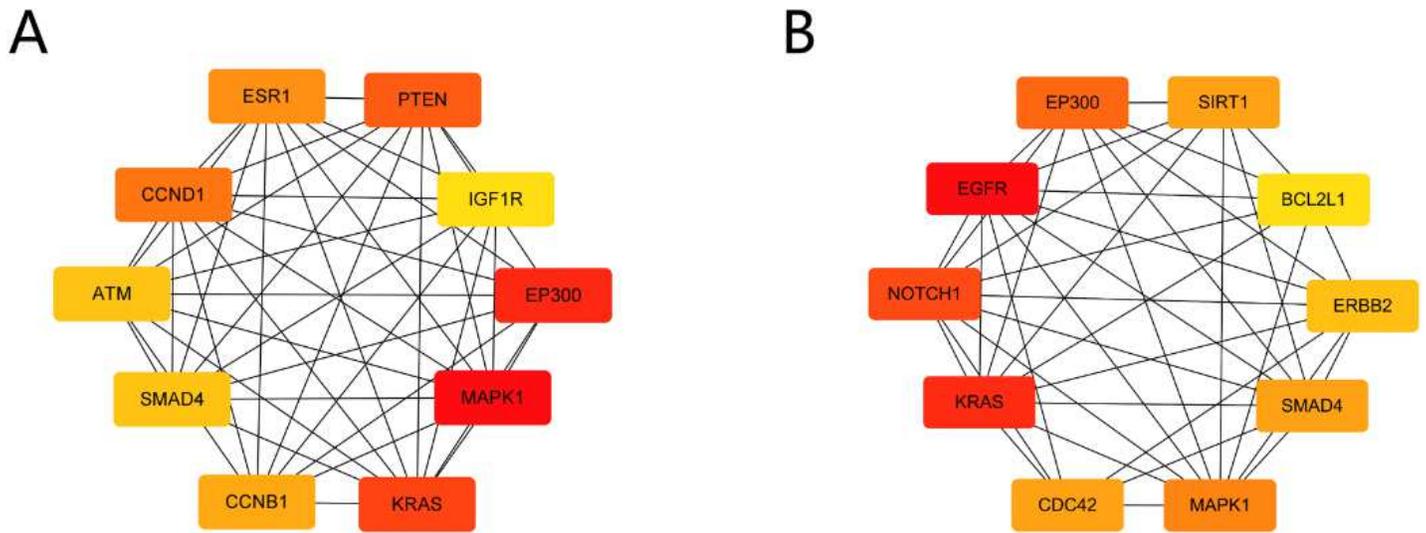


Figure 5

The networks of the top 10 hub genes (A) The mapped networks for up-regulated miRNAs;(B) The mapped networks for down-regulated miRNAs.

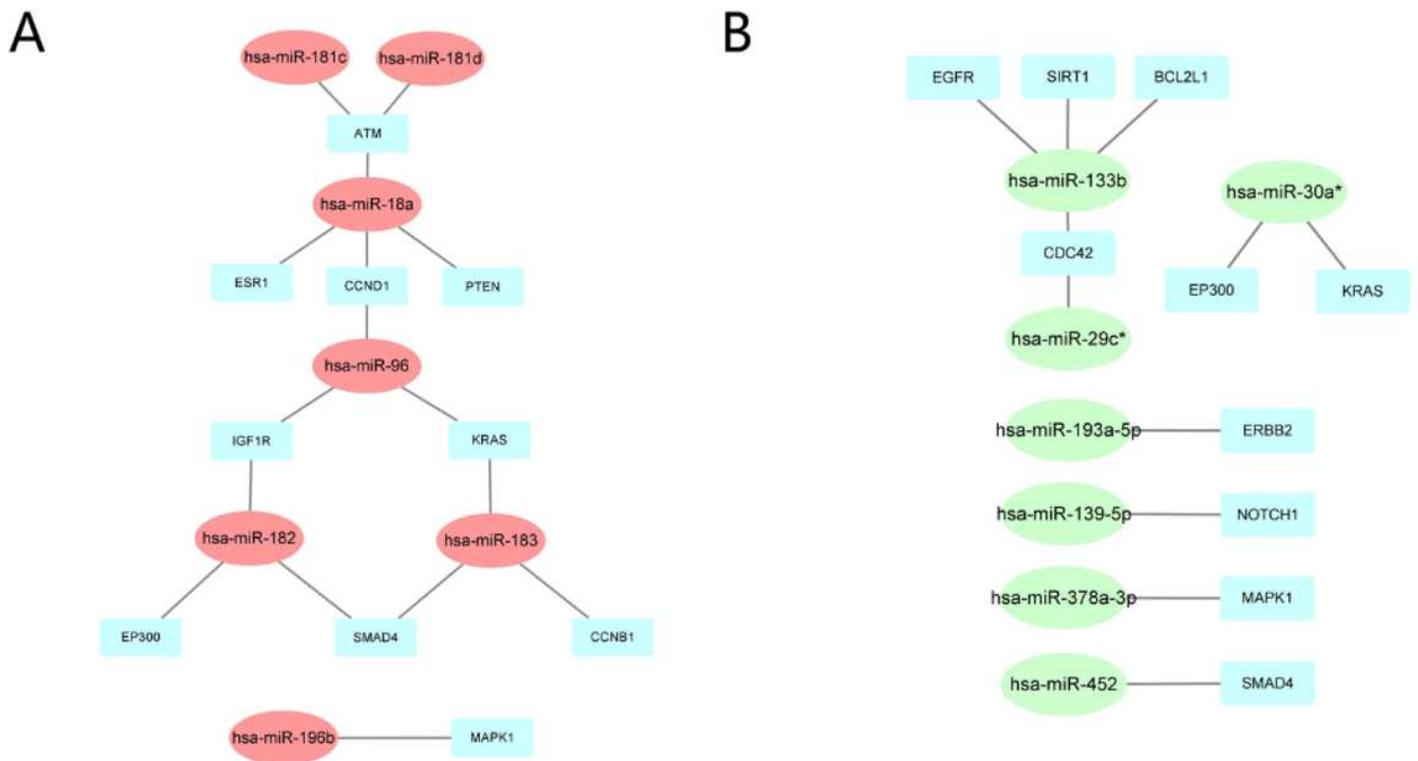


Figure 6

miRNA-hub gene networks (A)The networks for up-regulated miRNAs; (B)The networks for down-regulated miRNAs.