

Serum microRNA Expression Profiling in Malaria Patients and Bioinformatic Analysis of Hsa-miR-106b-5p

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Research

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Abstract

Background

Malaria, caused by *Plasmodium*, is one of the three major infectious diseases that seriously endangers public health. Resistance to anti-malarial drugs and insecticides has made the prevention and control of malaria shown little improvement in the last four years. This study aimed to explore the changes in microRNA (miRNA) expression profiling of malaria patient and predict malaria-related miRNA by bioinformatics methods to provide theoretical basis for further verification of the correlation between specific miRNAs and immune regulation of malaria.

Methods

Serum of patients infected by *Plasmodium falciparum* and healthy people from Myanmar border area was collected. miRNA expression profiling was obtained by RT-qPCR. Then the differentially expressed miRNA was screened and target genes were predicted by four miRNA databases (TargetScan, DIANA-microT, miRDB, and miRTarbase) and an intersection of target genes was obtained by Venn analysis. GO and KEGG analysis were performed for the overlapping target genes via Metascape. The results were further visualized by Cytoscape. Finally, Protein-protein interaction (PPI) network of predicted overlapping target genes was built by STRING.

Results

Among the 341 tested serum miRNAs, 64 were differentially expressed in malaria patients ($P < 0.05$), 27 miRNAs were up-regulated and 37 miRNAs were down-regulated. The miRNA with the most significant difference was hsa-miR-106b-5p (FC=14.871, adjusted. P .value<0.01); GO and KEGG analysis found that its overlapping predicted target gene set was remarkably enriched in biological functions such as GO:0007264~small GTPase mediated signal transduction, GO:0051056~regulation of small GTPase mediated signal transduction, GO:0051020~GTPase binding, GO:0048514~blood vessel morphogenesis($P < 0.01$) and signal pathway such as hsa04144: Endocytosis, hsa01521:EGFR tyrosine kinase inhibitor resistance, hsa05212:Pancreatic cancer ($P < 0.01$); Besides, a PPI network containing 39 predicted target genes of hsa-miR-106b-5p was constructed, and 5 hub genes VEGFA, STAT3, RACGAP1, OCRL, and RBBP7 have been selected.

Conclusion

The bioinformatics analysis results indicated that hsa-miR-106b-5p has a great relationship with malaria, it plays a part in inhibiting the emergence of ARTs resistance in *Plasmodium* and tumor progression, which may be achieved by regulating vascular morphogenesis, endocytosis, and VEGFA. The underlying mechanism needs to be further elucidated. We believe that this finding will facilitate an in-depth research on the association between malaria and miRNA.

Background

Malaria is one of the three major infectious diseases that seriously endangers public health, is mainly transmitted by a bite of *Plasmodium*-infected female Anopheles mosquitoes [1]. According to the World malaria report 2020, in 2019, approximately 229 million cases of malaria were reported worldwide, resulting in 409,000 deaths, most of which were children under 5 years of age in sub-Saharan Africa [2]. In the past two decades, tremendous progress has been made in preventing, controlling, and eliminating malaria. However, to date, the global mortality of malaria remains high. The spread of insecticide resistance [3], emergence of antimalarial drug resistance [4], and lack of safe, effective, and easily accessible malaria vaccine all have made the prevention and control of malaria challenging.

Lots of studies have indicated that microRNAs (miRNAs) perform a crucial biological function in the immune regulation of *Plasmodium* and its host [5]. miRNAs are endogenous conservative single-stranded non-coding RNAs containing approximately 22 nucleotides. miRNAs do not possess any coding function of their own, but they can bind to the 3' end (3'-untranslated region) of specific target mRNAs in a complementary base pairing manner, thereby inhibiting translation of the transcript and regulating physiological functions [6]. miRNAs are biomarkers for various diseases, and they are widely involved in diverse physiological processes such as cell proliferation, differentiation, and apoptosis [7]. Some studies have shown that miRNA has potential to be biomarkers for malaria [8]. Additionally, our group also have confirmed that the downregulation of miR-106b in mice is positively correlated with a decrease in the immune protection of malaria vaccine. For miRNA has a complex regulatory network, it is possible to improve the problem of preventing and controlling of malaria through regulating function of malaria-related miRNA. The study of miRNA is important for understanding gene regulatory networks, gene functions and the biological processes. However, current findings of the relationship between miRNA and malaria, and the underlying mechanisms are only the tip of the iceberg. Predicting target genes and conducting systematic bioinformatic analyses of miRNA are essential for an in-depth investigation. Therefore, in this study, we analyzed the serum miRNA expression profiling of malaria patients by bioinformatics methods to obtain a comprehensive and systematic understanding of the biological processes and miRNA interactions in malaria.

Methods

Sample collection

The study was performed on two groups, five *Plasmodium falciparum* (*P.falciparum*) infected patients (experimental group) and three healthy people (control group), all participants were from the countryside in Myanmar border area. Blood samples of the participants were collected using vacuum tubes.

Subsequently, serum was separated out naturally by stored at room temperature for 30 mins – 1 h, and then stored at -20°C for follow up miRNA expression profiling experiment.

Total RNA isolation and reverse transcription

This experiment was performed at the Biomedical Analysis Center of Army Medical University. First, the Trizol method was used for RNA isolation. Following this, 200µL serum was immediately mixed with 800µL Trizol in an Eppendorf tube. The Total RNA Isolation Kit (Life Technologies) was used for subsequent experiments, according to manufacturer's instructions. The concentration and purity of RNA were determined using electrophoresis by enzyme-labeling measuring instrument (Gen5-CHS1.09). Finally, E.coli poly-A-polymerase was used to generate polyadenylated tails to the 3'-end of all mature miRNA sequences. cDNA was synthesized using reverse transcriptase with the qScript Flex cDNA synthesis kit (Quanta Biosciences).

Mirna Qrt-pcr

An Echo550 instrument was used to inject samples (mix, template, primers). The ViiA7 Real-Time PCR Thermocycles and SYBR green-based real-time quantitative PCR method were used to quantify the relative expression of mature miRNAs, as described previously []. Two duplicate holes were prepared for each sample. All primers and probes used for miRNA quantification were purchased from Quanta Biosciences, and a total of 341 miRNAs were detected. The $2^{-\Delta\Delta Ct}$ method was applied to analyze the relative expression levels of miRNAs.

Analysis Of Serum Mirna Expression Profiling

The fold change (FC) approach was used to calculate serum miRNA expression levels between *P.falciparum*-infected and healthy control groups. The miRNA expression level in the infected group changed twice as compared to that in the healthy group was defined as differential expression, and differentially expressed miRNAs were obtained. By controlling the false discovery rate to correct the false positive rate, and using the SAM library, we screened the differentially expressed miRNAs ($P < 0.05$, $|\log FC| > 1$) and selected the most significant one (adjusted P -value < 0.05 , $|\log FC| > 1$) for bioinformatic analysis.

Prediction of the target gene of miRNA and bioinformatic analysis

To predict differentially expressed miRNA target genes, we used the following classic and authoritative miRNA target gene databases: TargetScan [], DIANA-microT [], miRDB [], and miRTarbase []. Subsequently, we used Venny2.1.0 [] to obtain an intersection of analysis results, and Draw Venn Diagrams [] to obtain overlapping target gene sets.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway analyses were performed for the predicted overlapping target genes using Metascape online tools [] to reveal the biological functions and potential pathways for genes involved in systemic regulation. The results were visualized using Cytoscape (ver 3.8.0) [] and RStudio (ver 1.2.1335).

Finally, the predicted overlapping target gene set was imported into the STRING online database (ver 11.0) [] for Protein-protein interaction (PPI) analysis. The minimum required interaction score was set at a medium confidence (0.400), and disconnected nodes in the network were excluded to construct a PPI network. The analysis results of STRING were imported into Cytoscape for further visual analysis and the hub genes, which played a pivotal role in the whole network, were screened.

Statistical analysis

miRNA expression profiling data were analyzed using SPSS (ver18.0) computer software (SPSS for Windows, SPSS Inc., 2009). Statistically significant difference was set at $P < 0.05$.

Results

Analysis of serum miRNA expression profiling in malaria patients

To identify a diagnostic serum miRNA signature for malaria, Vii7 and a real-time based high-throughput PCR array were used to compare the serum miRNA expression profiling of five *P.falciparum* infected patients and three healthy controls. Of the 341 miRNAs detected, 64 were differentially expressed in malaria patients ($P < 0.05$). Among these, 27 were up-regulated and 37 were down-regulated (Additional file 1). Additionally, the miRNA with the most significant difference was hsa-miR-106b-5p (adjusted P -value < 0.01 , $|\log FC| > 1$). Its expression level of *Plasmodium* infected group was up-regulated by 14.871 times compared with the control group, and it was selected for subsequent bioinformatic analysis.

Prediction of the target gene of hsa-miR-106b-5p

Four target gene sets of hsa-miR-106b-5p, 415–2037–1384 and 1091 target genes, were predicated by TargetScan, DIANA-microT, miRDB, and miRTarbase respectively (Fig. 1). A total of 78 overlapping genes were obtained using Venn analysis, accounting for 2.6% of the target genes predicted by all databases. These genes may be potential targets of hsa-miR-106b-5p (Fig. 2, Table 1).

Bioinformatic analysis of the predicted target genes of hsa-miR-106b-5p

The 78 overlapping target genes of hsa-miR-106b-5p were imported into Metascape for GO analysis (Additional file 2), and they were further visualized as bubble plot using RStudio (Fig. 3). The first three functions that significantly enriched of overlapping target genes were GO:0007264 ~ small GTPase-mediated signal transduction ($P < 0.01$), GO:0051056 ~ regulation of small GTPase-mediated signal transduction ($P < 0.01$), and GO:0051020 ~ GTPase binding ($P < 0.01$) (Fig. 4). Overall, among the 197 significantly enriched pathways in Biological processes (BPs, $P < 0.05$), the first three were GO:0007264 ~ small GTPase-mediated signal transduction, GO:0051056 ~ regulation of small GTPase-mediated signal transduction and GO:0048514 ~ blood vessel morphogenesis (Fig. 3A). Among the 29 pathways enriched in Cellular components (CCs, $P < 0.05$), the first three were GO:0010008 ~ endosome membrane,

GO:0000323 ~ lytic vacuole and GO:0005764 ~ lysosome (Fig. 3B). Among the 39 pathways enriched in Molecular functions (MFs, $P < 0.05$), the first three were GO:0051020 ~ GTPase binding, GO:0017016 ~ Ras GTPase binding and GO:0005547 ~ phosphatidylinositol-3,4,5-trisphosphate binding (Fig. 3C).

KEGG analysis of the 78 predicted overlapping target genes of hsa-miR-106b-5p revealed that the target genes were significantly enriched in nine pathways ($P < 0.05$) (Fig. 3D, Table 2). The top three included hsa04144: Endocytosis ($P < 0.01$), hsa01521: EGFR tyrosine kinase inhibitor resistance ($P < 0.01$) and hsa05212: Pancreatic cancer ($P < 0.01$), which may be the potential pathways involved in target genes.

A PPI network of the predicted target genes of hsa-miR-106b-5p was constructed using STRING, consisting of 39 nodes and 43 edges (Fig. 5). Following analysis, 78 predicted overlapping target genes were used as input, while 39 genes, which were disconnected from any other node after analysis, were removed. It has been demonstrated that complex interactions such as co-expression, neighborhood, and gene fusion, exist between target genes. Furthermore, the five hub genes (genes that interact with at least four other target genes) VEGFA, STAT3, RACGAP1, OCRL, and RBBP7, in the PPI network were speculated to play essential roles in the biological effects regulated by hsa-miR-106b-5p. Further analyses of the five hub genes revealed that all of them played vital roles in tumorigenesis.

Discussion

Analysis of serum miRNAs expression profiling between malaria patients and healthy controls revealed that 64 of the 341 detected miRNAs were differentially expressed. Among them, expression of the miRNA, hsa-miR-106b-5p, was significantly higher in malaria patients than in the healthy controls (the expression level is 14.871 times that of the control group), which had the smallest P value and adjusted. P .value, and largest logFC value. Thus, we confirmed that the downregulation of miR-106b is positively correlated with a decrease in the immune protection of malaria vaccines. It has been reported that increasing the expression of miR-106b in vitro can enhance the expression of transmembrane activator and CAML interactor (TACI) on the surface of memory B cells (MBCs) [1]. This indicated that hsa-miR-106b-5p is closely related to malaria, therefore we focused on its analysis.

We used bioinformatic analysis such as target gene prediction, GO and KEGG analyses to understand the biological functions of hsa-miR-106b-5p and its association with malaria comprehensively and systematically. First, in order to make up for the inevitable false positives and false negatives of bioinformatic predictions, four authoritative miRNA databases, TargetScan, DIANA-microT, miRDB, and miRTarbase, were used to predict the target genes of hsa-miR-106b-5p. The intersection of these results was analyzed using Venn, and a more reliable target gene set, containing 78 overlapping predicted target genes was obtained (Fig. 1). Subsequently, Metascape was used to perform GO and KEGG analyses for overlapping target genes.

GO results revealed that the target genes were significantly enriched in the related functions of small GTPases (Additional file 2). Small GTPases are key participants in a series of pathophysiological processes, most of which are associated with the regulation of protein secretion, endocytosis and

vesicular transport [,]. Previous studies have shown that the dysfunction and negative regulation of certain small GTPases, such as members of the Ras and Arf subfamilies, are related to the promotion and progression of tumors [,], and recent studies have confirmed that GO:0007264 ~ small GTPase mediated signal transduction is related to the occurrence of breast cancer []. Studies have shown that miR-106b-5p is the most regulated miRNA in cancer tissues. It is involved in the emergence and development of pancreatic cancer, lung cancer, and breast cancer among others [,,,], which not only verifies the accuracy of our approaches and directions, but also suggests a link between malaria and tumors.

In 2011, antitumor effects were observed in a Lewis lung cancer model mouse after infection with *Plasmodium yoelii*. The proliferation of tumor cells was significantly inhibited and the survival of tumor-bearing mice was remarkably prolonged []. An epidemiological survey also showed a significantly negative correlation between the incidence of malaria and tumor mortality []. In general, there are three reasons: (1) Periodic high fevers bouts occur in the host in the acute phase of malaria infection, which can kill certain non-heat-resistant cancer cells. Hyperthermia has been clinically used for the treatment of some cancers [,]. (2) *Plasmodium* infection can stimulate the innate immunity of host, activating and enhancing antitumor immunity. It mainly occurs through activation of NK and DC cells to promote the production of a large number of cytokines such as IFN- γ and TNF- α , which antagonize the tumor immunosuppressive microenvironment, leading to an antitumor effect. In fact, malaria infection can enhance the host immune response and it has been used as an adjuvant to treat cancer []. GO analysis showed that GO:0002376 ~ immune system process ($P < 0.01$), GO:0048518 ~ positive regulation of biological process ($P < 0.01$) and GO:0008283 ~ cell proliferation ($P < 0.01$) are all related to immune system. (3) Excluding immune responses, malaria infection also acts on VEGFR2 through miRNAs in exosomes and lncRNA F63 in tumor tissues, thereby inhibiting tumor angiogenesis. Angiogenesis is the formation of new blood vessels from pre-existing blood vessels. It plays an important role in tumor development, and it is responsible for the transportation of nutrients needed for tumor growth. Cutting off the nutrient supply from tumor blood vessels will cause the cancer cells to "starved to death" [25,]. This is strongly associated with GO:0048514 ~ blood vessel morphogenesis ($P < 0.01$), suggesting that malaria infection inhibits tumor progression. Besides, GO:0051781 ~ positive regulation of cell division and GO:003052 ~ intracellular receptor signaling pathway are also important for further investigation.

KEGG analysis results revealed that the overlapping target gene set was enriched in hsa04144: Endocytosis ($P < 0.01$), hsa01521: EGFR tyrosine kinase inhibitor resistance ($P < 0.01$), hsa05212: Pancreatic cancer ($P < 0.01$) and other pathways (Table 3). The most significant enrichment was observed in endocytosis, which plays an essential role in malaria infection. For *Plasmodium*, the importance of endocytosis is self-evident []. In the pre-erythrocytic stage of malaria, endocytosis mediates the uptake and degradation of up to 80% of host cell cytoplasm which is mainly composed of hemoglobin by the malaria parasite. However, the molecular mechanism of this process remains unclear []. In general, endocytosis requires core transport factors, including Rab-GTPase [,], which is similar to the results of GO analysis, and thus, it further provides evidence for the link between hsa-miR-106b-5p and malaria. Interestingly, a recent study found that mutations of Kelch13 (K13) protein in *Plasmodium* are

resistant to artemisinin (ART) and its derivatives due to a reduction of hemoglobin endocytosis [1]. Artemisinin is a frontline antimalarial drug. However, drug resistance has gradually emerged in Cambodia and the Greater Mekong Subregion due to long-term use and abuse. The main clinical manifestation of artemisinin resistance is that the clearance of malaria parasite is delayed or reduced after treatment with ART [2]. The mutated K13 gene in *Plasmodium* is related to a delayed parasite clearance after treatment with Artemisinin-based combination therapy (ACT), as recommended by WHO [3]. Molecular characteristics of the highly conserved K13 C-terminal region in *P.falciparum*, kelch13 propeller region (*Pfk-13*), are closely linked to the *Plasmodium* phenotype from Cambodia having a delayed ART clearance [4]. Moreover, K13 is related to hemoglobin internalization and catabolism. Thus, reducing K13 impairs hemoglobin catabolism and reduces artemisinin activation [5]. Studies on compartment proteins of K13 and their functions revealed the entire pathway of ART resistance, that is, K13 and its compartment proteins play important role in the endocytosis of hemoglobin uptake by malaria parasite. Hemoglobin digestion products are necessary for the activation of ARTs. Therefore, an inactivation of all kinds of K13 proteins will reduce hemoglobin uptake by malaria parasites, thereby reducing the activation and concentration of ART decreasing the susceptibility of malaria parasites to ART, and eventually resulting in the resistance of malaria parasite to ART. Moreover, the main manifestation observed in wild-type anti-ART *P.falciparum* explains that changing the stability of K13 protein can affect endocytosis and lead to resistance to ARTs from both negative and positive sides. An opportunistic inactivation of K13 gene will cause the malaria parasite to remain in the ring stages and no longer grow and divide. However, the inactivation of K13 only affects the ring stage and does not work for trophozoite stages. This may be the reason for the frequent mutations found in drug-resistant *Plasmodium* isolates due to K13 gene, out of all proteins that affect endocytosis [36, 37]. Since the main reason for the emergence of ART resistance among *Plasmodium* is the reduction of endocytosis and the predicted target genes of hsa-miR-106b-5p are significantly concentrated in the signaling pathway of endocytosis, hsa-miR-106b-5p is the key miRNA in malaria. Is it possible to enhance endocytosis by targeting hsa-miR-106b-5p and improve ART resistance?

Finally, we constructed a PPI network using STRING, containing 39 predicted target genes of hsa-miR-106b-5p. Additionally, we identified five hub genes, VEGFA, STAT3, RACGAP1, OCRL, and RBBP7, which may play vital roles in the regulation of biological effects in hsa-miR-106b-5p (Fig. 5). The most critical hub gene was VEGFA, a key factor for vascular endothelial activation, which induces the survival, migration, and proliferation of endothelial cells during angiogenesis, and promotes vascular penetration and endothelial activation during inflammation [38]. It has been proposed as a biomarker for malaria severity, and it is expressed in the plasma and brain tissues of malaria patients [39]. Endothelial activation is an early feature of malaria. It is probably beneficial for the isolation of infected RBCs and it also leads to thrombocytopenia. The number of platelets was negatively correlated with parasitemia and malaria severity. These results reveal a correlation between hsa-miR-106b-5p and malaria. Furthermore, the expression of VEGFA increased in many cancers. When its production is inhibited, tumor angiogenesis reduces, thus, decreasing the source of nutrition for cancer cells and finally achieving an anti-tumor effect. Monoclonal antibodies against VEGFA have been used to treat cancer. However, they cause some

side effects and redundancy of other antigenic factors []. Therefore, using miRNA to target genes is highly specific and safe. An equally important gene, STAT3, regulates cell growth and apoptosis, whereas RACGAP1, a GTPase-activating protein belonging to the Rho GTPase family, is highly expressed in malignant tumor cells. These hub genes are of great significance in malaria and tumors.

In brief, the GO results of our bioinformatic analysis suggest that hsa-miR-106b-5p may mediate malaria to inhibit tumor progression by controlling vascular morphogenesis and other complex functions. The KEGG results indicate that hsa-miR-106b-5p can improve ART resistance and affect the growth and division in *Plasmodium* through endocytosis. Likewise, the PPI of predicted target genes revealed the relationship between VEGFA and malaria severity, and the link between malaria parasite and tumor growth and metastasis.

However, our study had some limitations. First, the number of volunteers was small, and it may not be representative due to large individual differences in humans. However, the study detected 341 miRNAs, obtained serum miRNA expression profiling of malaria patients, and identified certain differentially expressed miRNAs. To reduce errors, we will expand the sample size in future experiments. Second, disease factors of volunteers, which may affect the results were not considered. Confounding factors can be increased in information statistics. Third, false positives or false negatives inevitably exist in bioinformatics analysis approaches. Therefore, a further experimental verification for the predicted target genes and their biological functions is needed. The dual-luciferase reporter assay can be used to identify binding sites of miRNA target genes and choose miRNA mimics or inhibitors for in vitro induction and in vivo experiments for functional verification.

In summary, this study analyzed the changes in serum miRNA expression profiling of malaria patients using bioinformatic analysis, identified differentially expressed miRNAs, and conducted a comprehensive and systematic analysis of hsa-miR-106b-5p, which shows the maximum statistical difference and has attracted tremendous research interest. Additionally, we constructed a PPI network for the predicted target genes of hsa-miR-106b-5p. The results demonstrate that it plays a role in inhibiting tumor progression and the emergence of ART resistance in *Plasmodium*, which may be achieved by regulating vascular morphogenesis, endocytosis, and VEGFA. The study findings will provide a systematic understanding of the biological processes associated with hsa-miR-106b-5p, and they will provide data support and theoretical basis for further studies to verify the link between malaria and tumors, design malaria vaccines, and regulate miRNA functions to achieve disease treatment.

Conclusion

In this study, the analysis of serum miRNA expression profiling between malaria-infected patients and healthy people revealed that there is indeed miRNA closely linked to malaria. Among the 341 serum miRNAs detected in patients infected by *Plasmodium falciparum*, 64 were differentially expressed ($P < 0.05$), hsa-miR-106b-5p showed the most significant differences. Preliminary experimental verification and follow-up bioinformatics analysis confirmed that it is closely related to malaria. Comprehensive

analysis results showed that hsa-miR-106b-5p does play a certain role in inhibiting the emergence of ARTs resistance in *Plasmodium* and tumor progression, and it may be achieved by regulating vascular morphogenesis and endocytosis and VEGFA.

Abbreviations

ART

Artemisinin

ACT

Artemisinin-based combination therapy

BP

Biological process

CC

Cellular component

FC

Fold change

GO

Gene Ontology

KEGG

Kyoto Encyclopedia of Genes and Genomes

MBC

memory B cell

MF

Molecular function

PPI

Protein-Protein Interaction

RT-qPCR

Real-Time quantitative PCR

TACI

Transmembrane activator and CAML interactor

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and with the approval of the Ethics Committee of the Guilin Medical University Institute of Medical Research, and a written informed consent was obtained from all participants.

Consent for publication

Not applicable.

Availability of data and materials

The main data generated during this study are included in this published article and its supplementary information files. And the other datasets analyzed during the current study are available from the corresponding author on reasonable request.

Competing interest

The authors declare that they have no competing interests.

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Authors' contributions

XP designed this study, supervised the whole work, and revised the manuscript. JP conducted the bioinformatic analysis and drafted the manuscript. YW collected the samples of experiment and performed the miRNA expression profiling experiment. LJ, DL and PT contributed to quality control of the experiment and provided some constructive suggestions for the revision of the manuscript. All the authors listed have approved the final version for published and agreed to be accountable for all aspects of the study.

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Tables

Table 1. Predicted overlapping target genes of hsa-miR-106b-5p

miRNA databases	Total	Overlapping target genes
TargetScan, DIANA-microT, miRDB, miRTarBase	78	CHD9, PPP1R15B, CEP170, RACGAP1, OCRL, FAM126B, PRRX1, LDLR, MAP7, GNS, UBE2Q2, ARHGAP12, HMBOX1, VEGFA, C2orf69, YES1, ZBTB4, PANK3, ELK4, WEE1, FAM102A, TNFSF11, ARHGAP1, SH3BP5, RBBP7, UXS1, NABP1, ZNF532, TMEM123, CHIC1, SUCO, ITCH, LIMK1, MYO1D, HECA, NUFIP2, SOX4, LAPTM4A, MECP2, YOD1, DCBLD2, LPGAT1, REEP3, ACBD5, CADM2, DYNC1LI2, UBR5, NRBP1, AKTIP, ZRANB1, STAT3, EIF5A2, EREG, TXLNA, POLQ, RAPGEF4, ZBTB6, SNTB2, TNFAIP1, AGO1, SESN3, DAB2, NFIB, FMNL3, ANKFY1, ARAP2, ANKRD50, IQSEC1, SMOC1, RAB12, RAB30, GBF1, CIT, JAK1, BTBD7, ARID4B, SMAD5, USP3

Table 2. KEGG pathway analysis of overlapping target genes of hsa-miR-106b-5p

KEGG ID	Term	<i>P</i> value	Genes
hsa04144	Endocytosis	1.12E-04	DAB2, LDLR, GBF1, IQSEC1, ITCH, ARAP2
hsa01521	EGFR tyrosine kinase inhibitor resistance	1.41E-03	JAK1, STAT3, VEGFA
hsa05212	Pancreatic cancer	1.57E-03	JAK1, STAT3, VEGFA
hsa05145	Toxoplasmosis	4.21E-03	JAK1, LDLR, STAT3
hsa04120	Ubiquitin mediated proteolysis	6.81E-03	UBR5, ITCH, UBE2Q2
hsa04550	Signaling pathways regulating pluripotency of stem cells	9.03E-03	JAK1, SMAD5, STAT3
hsa05160	Hepatitis C	1.18E-02	JAK1, LDLR, STAT3
hsa05167	Kaposi sarcoma-associated herpes infection	1.70E-02	JAK1, STAT3, VEGFA
hsa05163	Human cytomegalovirus infection	2.96E-02	JAK1, STAT3, VEGFA

Figures

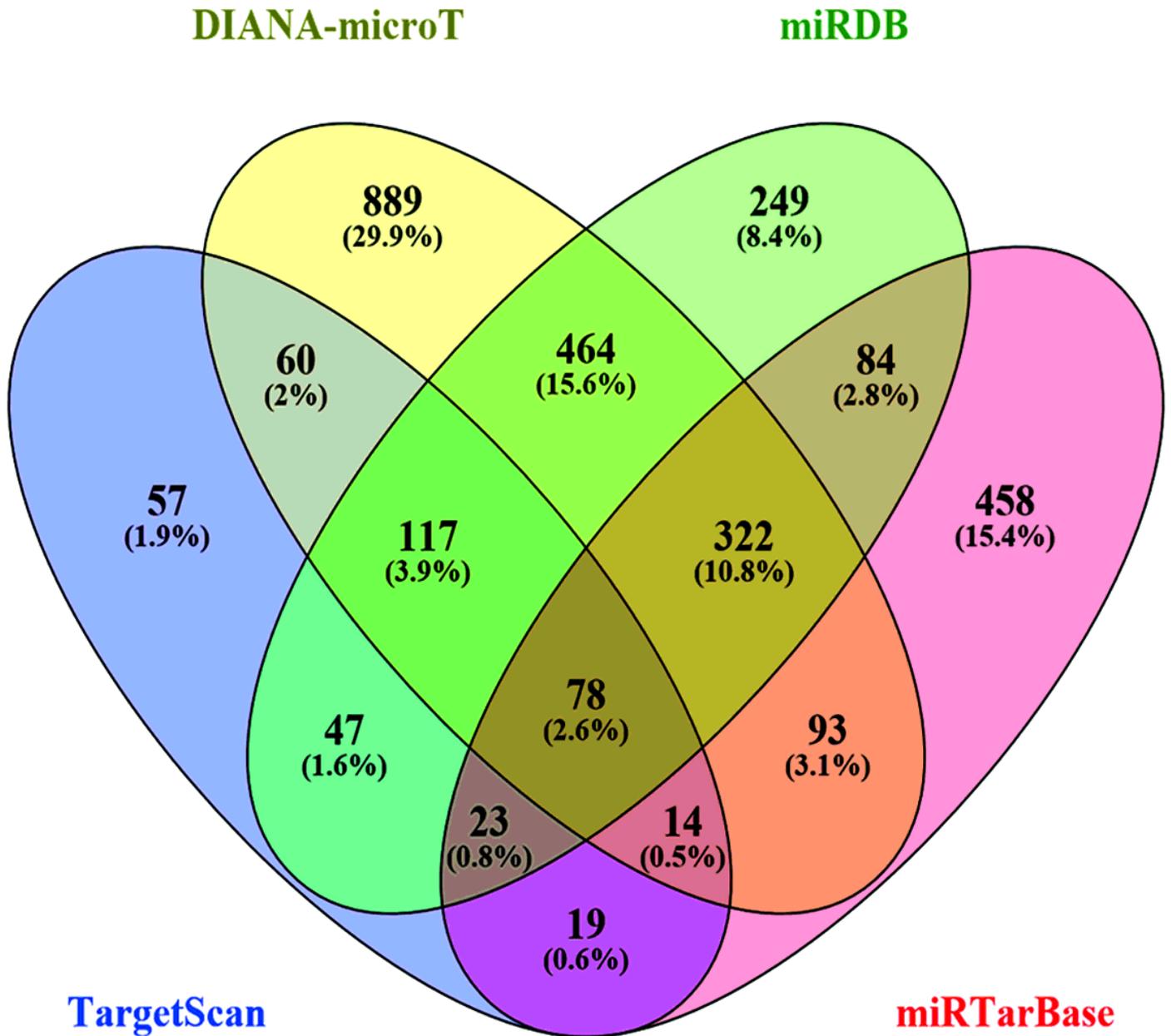


Figure 1

The prediction of hsa-miR-106b-5p target genes by four online tools. Seventy-eight overlapping genes in the intersection of four algorithms' results, created by Venn analysis.

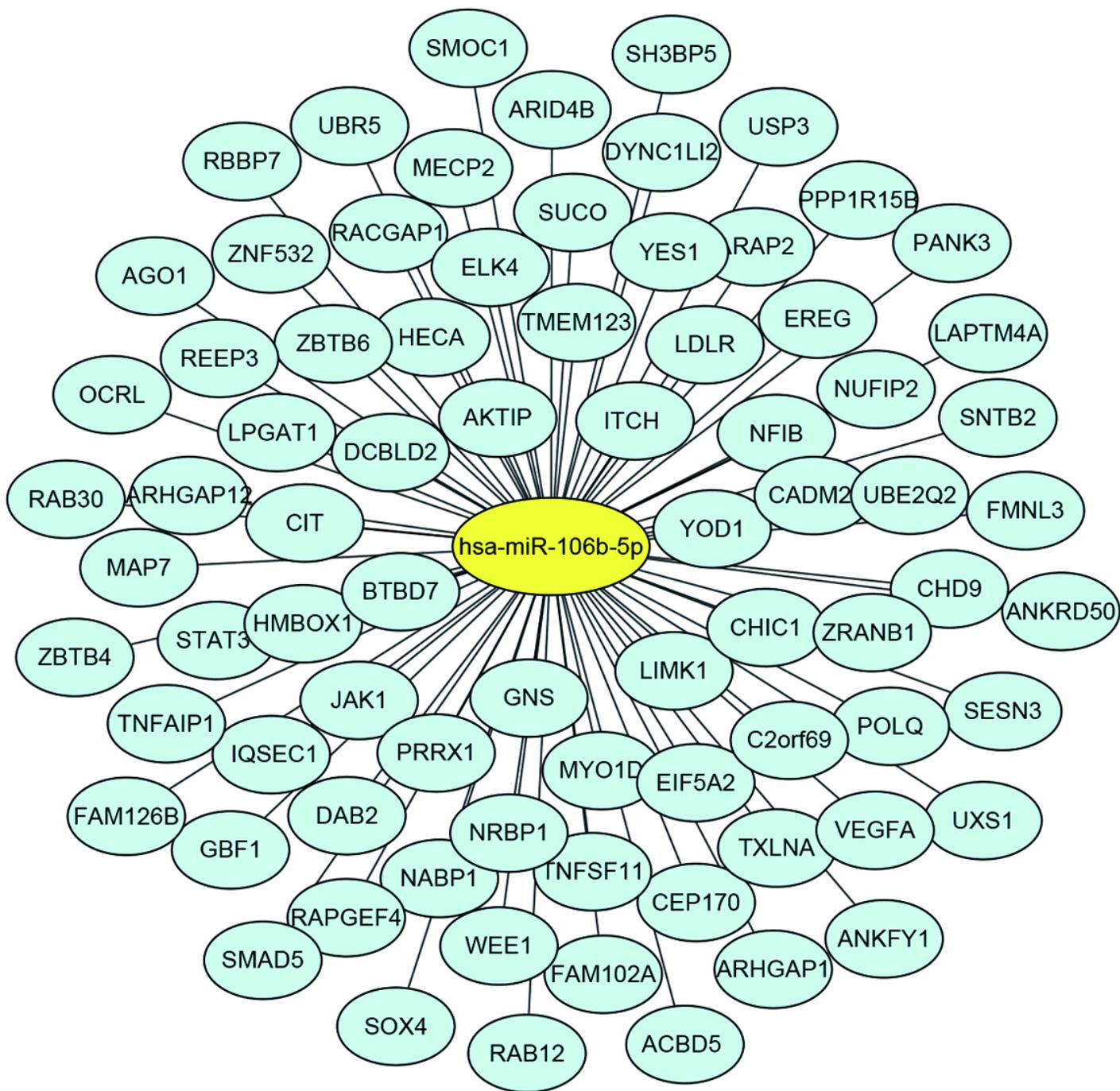


Figure 2

Seventy-eight overlapping predicted target genes of hsa-miR-106b-5p. hsa-miR-106b-5p—overlapping target genes interaction network visualized by Cytoscape (ver 3.8.0).

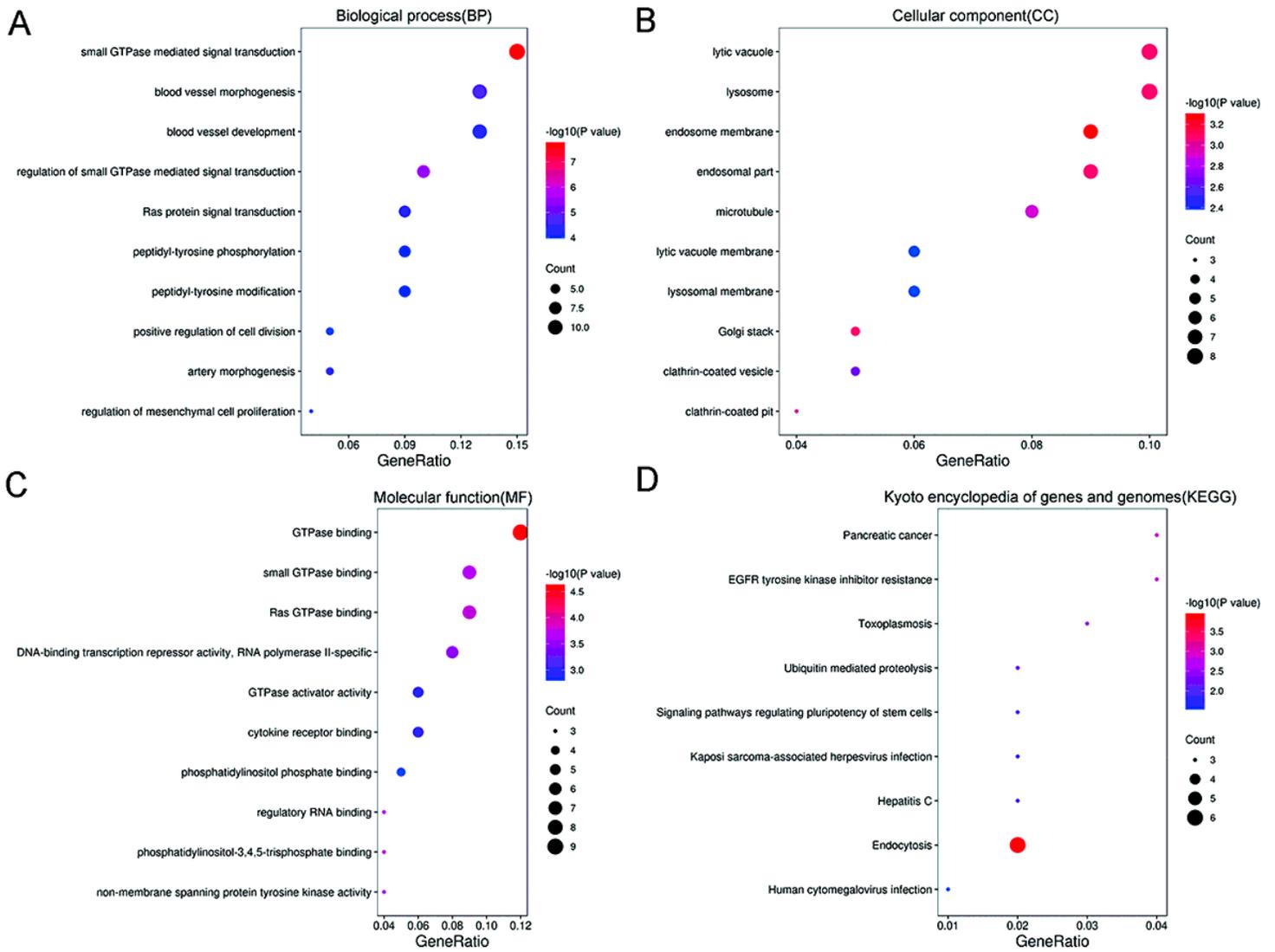


Figure 3

GO annotation and KEGG pathway analysis of target genes of hsa-miR-106b-5p (A) Enriched terms of GO Biological process (BP, $P < 0.05$); (B) Enriched terms of GO Cellular component (CC, $P < 0.05$); (C) Enriched terms of GO Molecular function (MF, $P < 0.05$); (D) Enriched terms of KEGG pathway ($P < 0.05$)

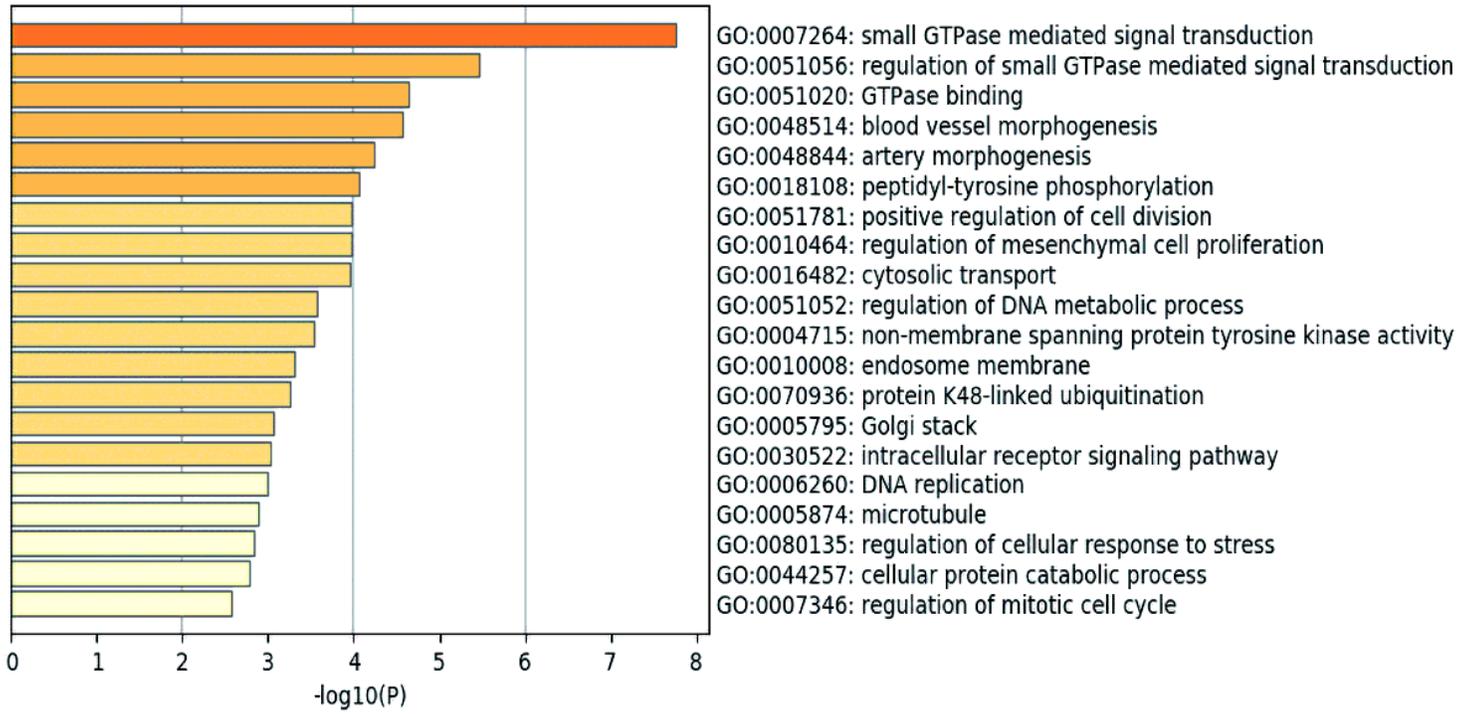


Figure 4

Heatmap of enriched terms across overlapping target gene lists of hsa-miR-106b-5p, colored by P value.

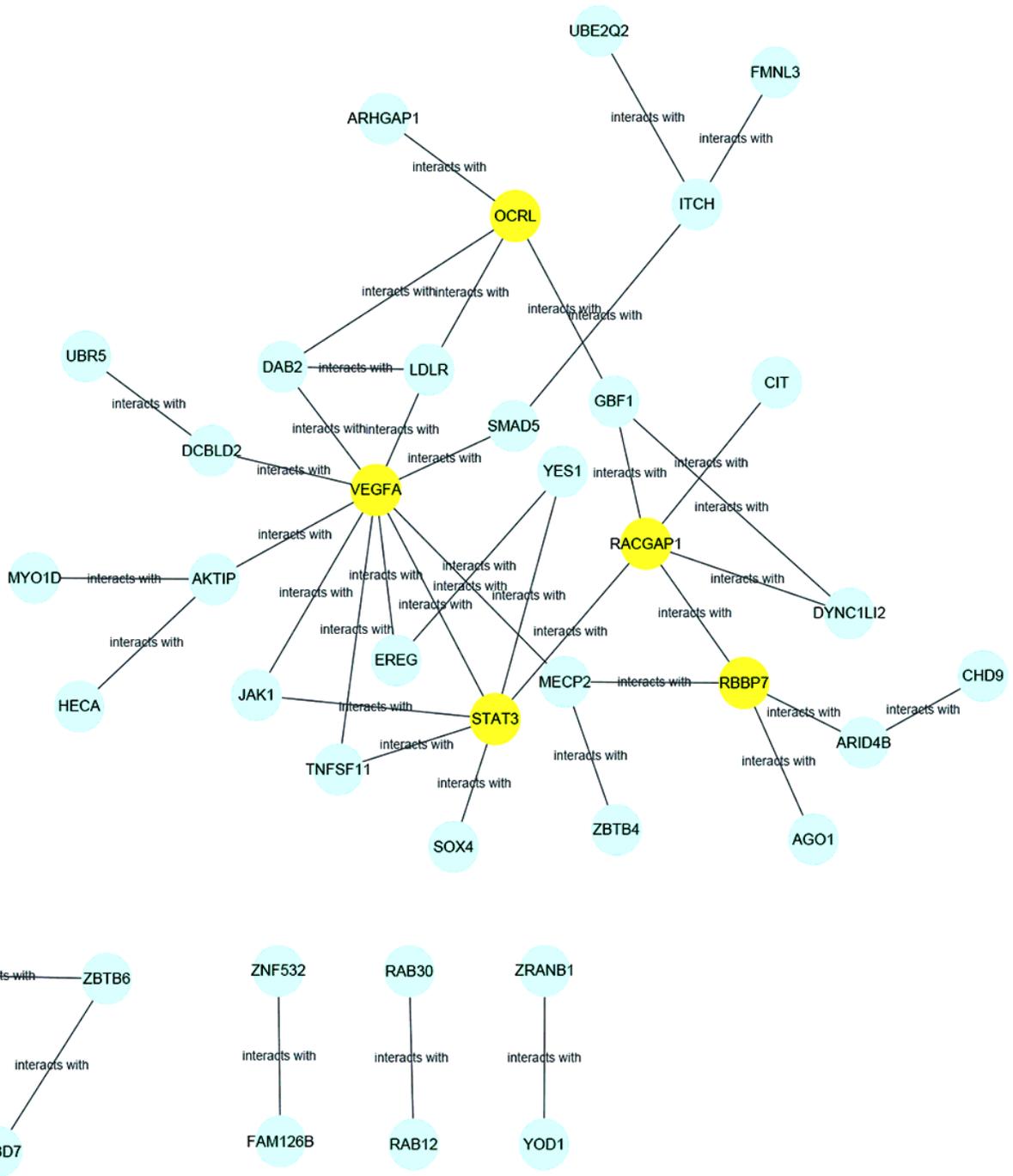


Figure 5

Protein-protein interaction (PPI) network of overlapping target genes of hsa-miR-106b-5p. The hub genes have marked in yellow, visualized by STRING (ver 11.0) and Cytoscape (ver 3.8.0).

Supplementary Files

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