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Original Article

Identification of key miRNAs and targeted genes involved in the progression of oral squamous cell carcinoma

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KEYWORDS
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Abstract *Background/purpose:* Oral squamous cell carcinoma (OSCC) is one of the most common types of head and neck squamous cell carcinoma. Accurate biomarkers are needed for early diagnosis and prognosis of OSCC. MicroRNAs (miRNAs) have shown great values in different types of cancers including OSCC. However, most of the miRNAs involved in the development of OSCC remain uncovered. This study aimed to identify hub miRNAs and mRNAs in OSCC.

Materials and methods: We explored the roles of key miRNAs, target genes and their relationships in OSCC using an integrated bioinformatics approach. Initially, Two OSCC microarray datasets from the Gene Expression Omnibus database were obtained to analyze miRNA expression. MiRNA-targeted mRNAs were acquired, and gene ontology/kyoto encyclopedia of genes and genomes analyses were performed. Thereafter, we constructed a protein–protein interaction (PPI) network to identify hub genes and a miRNA-mRNA interaction network was used to identify key miRNAs. Furthermore, differential gene expression and Kaplan–Meier Plotter survival analysis was performed to evaluate their potential clinical application values.

Results: Four upregulated, two downregulated miRNAs and 608 target genes of the differentially expressed miRNAs were identified. The PPI and miRNA-mRNA interaction networks highlighted 10 hub genes and two key miRNAs, and pathway analyses showed their correlative involvement in tumorigenesis-related processes. Of these miRNAs and genes, miR-125b, β-actin, vinculin and histone deacetylase 1 were correlated with overall survival ($P < 0.05$).

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Conclusion: These findings indicate that miR-21 and miR-125b, associated with the 10 hub genes, jointly participate in OSCC tumorigenesis, offering insight into the molecular mechanisms underlying OSCC as potential targets for early diagnosis, treatment and prognosis.

Introduction

Oral squamous cell carcinoma (OSCC) of the head and neck squamous cell carcinoma ranked as the sixth most common cancer worldwide.[1] Over 30,0000 new cases occur worldwide every year, with annual deaths exceeding 140,000. Lacking of early signs contribute to its high prevalence and morbidity.[2,3] In spite of the various clinical therapeutic methods, the prognosis of OSCC remains to be improved. In fact, only 50% of patient's survival is up to five years.[4] Thus, identifying novel biomarkers and therapeutic targets is crucial to detect OSCC at early stages, and also for the diagnosis, treatment and prognosis of OSCC patients.

MicroRNAs (miRNAs) are short non-coding RNAs (ncRNAs) with an average length of 20–25 nucleotides. Presented the growing needs for suitable biomarkers to diagnose and treat OSCC at early stage, miRNAs, which have contributed to a large number of biological processes, involving cell proliferation, cellular differentiation and cell migration, finally influencing tumor development in different types of cancer,[5–7] are drawing considerable attention. Several miRNAs are shown to be dysregulated in OSCC and may impact tumor development. For example, MiR-155–5p plays an oncogenic role in oral cancer.[8] MiR-770 is identified as an oncomiR regulating the tumor metastasis and invasion.[9] However, given the severity and complication of OSCC, other related transcripts among the large number of differentially expressed miRNAs regulating OSCC pathogenesis remain to be explored.

Today, bioinformatics plays a new emerging role in biological studies and is providing full-scale methods for the sketch map construction of hub genes and crucial non-coding RNAs in OSCC development.[10,11] Whereas, relationships between miRNAs and target genes and their prognostic values in OSCC remain uninvestigated. In the current work, we investigated two gene expression omnibus datasets to identify differentially expressed miRNAs in OSCC utilizing bioinformatic approaches. We used the encyclopedia of RNA interactomes to locate the target genes of the differentially expressed miRNAs. Gene ontology and kyoto encyclopedia of genes and genomes pathway analyses are retrievable on database for annotation visualization and integrated discovery. The miRNA-gene interaction network was visualized utilizing Cytoscape. Six differentially expressed miRNAs and several of their targeted hub genes dysregulated in OSCC were unmasked, and two key differentially expressed miRNAs, with their hub-gene targets, were further analyzed for their expression and prognosis in OSCC patients. Our study aimed to illuminate the miRNA-mRNA interaction network in OSCC development and provided potential miRNAs and target genes which may be profitable biomarkers for diagnosis, prognosis and therapy of OSCC.

Materials and methods

Data selection

Two OSCC miRNA expression microarray datasets (GSE103931, GSE137865) were retrieved from the gene expression omnibus database (https://www.ncbi.nlm.nih.gov/geo). GSE103931 included 30 cancer (OSCC group) and 19 normal specimens (control group),[12] and GSE137865 contained 6 cancer (OSCC group) and corresponding contralateral normal tissues (control group).[13] The patients were from 30 to 77 years old, male or female, with primary site of buccal mucosa, gum, hard palate, mouth floor, tongue, uvula or lip. All patients were under primary treatment and did not receive any preoperative radiotherapy or chemotherapy.

Differentially expressed miRNA screening

NetworkAnalyst (https://www.networkanalyst.ca/was used to standardize the data and identify differentially expressed miRNAs in each dataset, the thresholds were set as: adjusted $P < 0.05$ and log2 fold change ≥ 1.[14] Overlapping miRNAs were screened out utilizing Draw Venn Diagram (http://bioinformatics.psb.ugent.be/Webtools/Venn/).

MiRNA target prediction

The encyclopedia of RNA interactomes (http://starbase.sysu.edu.cn/index.php), an opensource platform of RNA interactomes , was utilized to locate target genes of the differentially expressed miRNAs.[15]

Gene ontology and kyoto encyclopedia of genes and genomes enrichment analyses

Gene ontology/kyoto encyclopedia of genes and genomes enrichment analyses of the predicted target genes were performed on the database for annotation visualization and integrated discovery (http://david.abcc.ncifcrf.gov/).[16-18] P value < 0.05 was considered significant.

Construction of protein–protein interaction network

To better learn the cross-actions among the target genes, a PPI network was performed and analyzed using STRING.[19] The combined score >0.4 were considered significant and crucial genes in the PPI network were screened out based on the cutoff criteria of degree from cytoHubba in Cytoscape (http://cytoscape.org/).[20] Gene ontology and kyoto

encyclopedia of genes and genomes analyses of key genes were conducted on the database for annotation visualization and integrated discovery.

Construction of miRNA-gene interaction network and miRNA-target coexpression validation

The resulting miRNA-gene network was visualized utilizing Cytoscape (http://cytoscape.org/). Validation of miRNA-target coexpression was undertaken on the encyclopedia of RNA interactomes.

MiRNA pathway analysis and construction of miRNA regulatory network

Pathway analysis of the key miRNAs was performed using MiRPathDB (miRPathDB (uni-sb.de).[21] Key miRNA regulatory network with hub genes were constructed based on kyoto encyclopedia of genes and genomes pathway analyses (https://www.kegg.jp/).[17]

Expression validation and survival analyses

Expression levels of hub genes and key miRNAs was analyzed using the university of alabama cancer database (http://ualcan.path.uab.edu/).[22] Kaplan–Meier Plotter (http://kmplot.com/analysis/) was used to analyze the correlation of key miRNAs and the prognosis of head and neck squamous cell cancers.[23] The correlation of hub genes and the prognosis of head and neck squamous cell cancers were analyzed on the university of alabama cancer database.

Results

The overview of this study is presented in Fig. 1.

Identification of differentially expressed miRNAs and target genes in oral squamous cell carcinoma

Data of the two gene expression omnibus datasets (GSE103931 and GSE137865) [12,13] was retrieved from the gene expression omnibus database and standardized utilizing NetworkAnalyst 3.0. According to the thresholds ($|Log2FC|>1$ and p value < 0.05), 42 and 12 differentially expressed miRNAs were screened out in GSE103931 and GSE137865, respectively (Fig. 2a and b). The differentially expressed miRNAs from the two datasets were overlapped with a Venn diagram, giving out four upregulated and two downregulated differentially expressed miRNAs (Fig. 2c and d). Six overlapped differentially expressed miRNAs were demonstrated (Table 1). And the differentially expressed miRNAs in each Dataset were demonstrated in Supplementary Table S1 and Supplementary Table S2. Subsequently, we used the encyclopedia of RNA interactomes database to predict target genes of the six differentially expressed miRNAs. 462 potential target genes were found for the four upregulated hub miRNAs, whereas 146 target genes were identified for the two downregulated hub miRNAs.

Enrichment analysis of target genes

Gene ontology and kyoto encyclopedia of genes and genomes enrichment analyses of the 462 and 146 target genes were performed using database for annotation visualization and integrated discovery website. The top 10 gene ontology terms (biological processes, molecular functions and cellular components) and top 5 kyoto encyclopedia of genes and genomes pathways were observably gathered (Fig. 3a–h). For the 462 targets of the four upregulated miRNAs, results of gene ontology analysis showed that the most enriched terms were 'transcription' and 'regulation of transcription' in the biological process category, 'protein binding' and 'DNA binding' in the molecular function category, and 'nucleus' and 'cytoplasm' in the cellular component category (Fig. 3a–c). For the 146 targets of the two downregulated miRNAs, the most enriched terms were 'apoptotic process' and 'viral process' in the biological process category, 'protein binding' and 'ATP binding' in the molecular function category, and 'nucleus' and 'acytoplasm' in the cellular component category (Fig. 3e–g). For target genes of the upregulated miRNAs, kyoto encyclopedia of genes and genomes pathway analysis demonstrated that pathways were most enriched in 'Regulation of actin cytoskeleton', 'PI3K-Akt signaling pathway', and 'Proteoglycans in cancer' (Fig. 3d). For the downregulated miRNAs, the target genes were most enriched in 'Proteoglycans in cancer', 'Hippo signaling pathway', and 'Insulin signaling pathway' (Fig. 3h).

Construction of protein–protein interaction and miRNA-gene interaction networks

To distinguish the connections and identify key genes among the targets genes, we constructed the PPI networks on STRING and Cytoscape. The top 20 key genes were screened out with degree as the criterion (Fig. 4a and b). For the four upregulated miRNAs, the key targets were β-actin (ACTB), heterogeneous nuclear ribonucleoprotein A1 (HNRNPA1), mitogen-activated protein kinase 1 (MAPK1), tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein β (YWHAB), vinculin (VCL), integrin alpha V (ITGAV), retinoblastoma 1 (RB1), tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta (YWHAZ), phosphoinositide-3-kinase, regulatory subunit 1 (PIK3R1), Raf-1 proto-oncogene, serine/threonine kinase (RAF1). For the two downregulated miRNAs, the key targets were actin gamma 1 (ACTG1), cyclin-dependent kinase 2 (CDK2), protein phosphatase 2, catalytic subunit, alpha isozyme (PPP2CA), histone deacetylase 1 (HDAC1), exportin 1 (XPO1), eukaryotic translation initiation factor 4E binding protein 1 (EIF4EBP1), heterogeneous nuclear ribonucleoprotein U (HNRNPU), lysine (K)-specific methyltransferase 2C (KMT2C), ELAV like RNA binding protein 1 (ELAVL1), DEAD box helicase 5 (DDX5). To explore potential molecular mechanisms underlying OSCC, we conducted gene ontology/kyoto encyclopedia of genes and genomes analyses on these key genes. Results 1were significantly enriched in a wide range of gene ontology functions, such as 'viral process' and 'positive regulation of transcription from RNA polymerase II promoter' in the

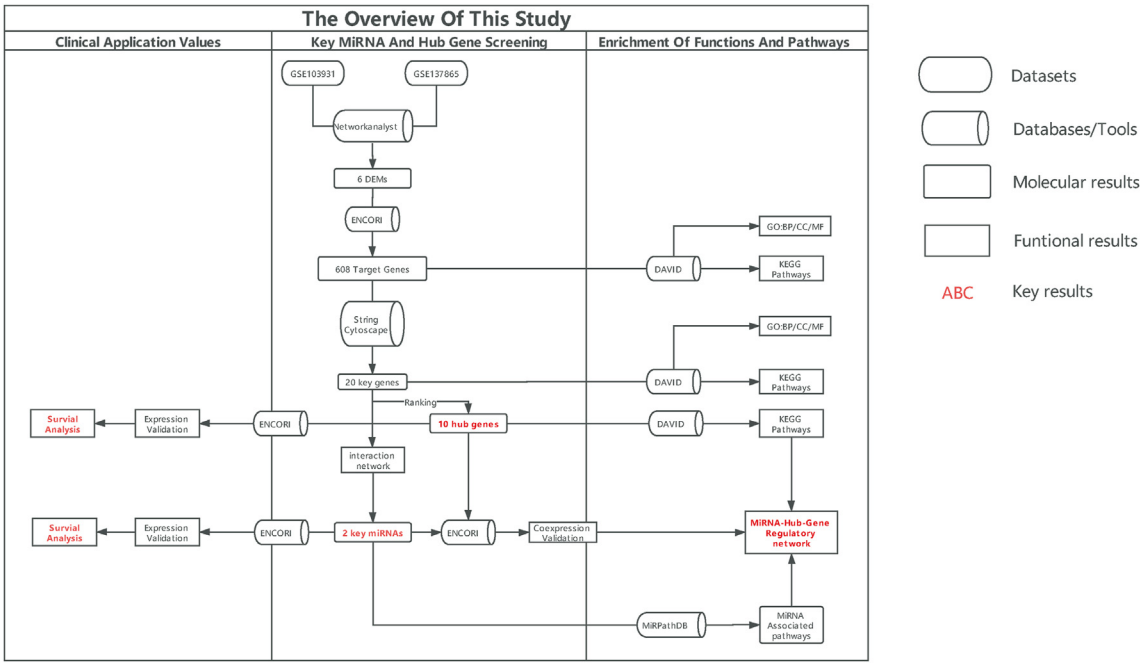
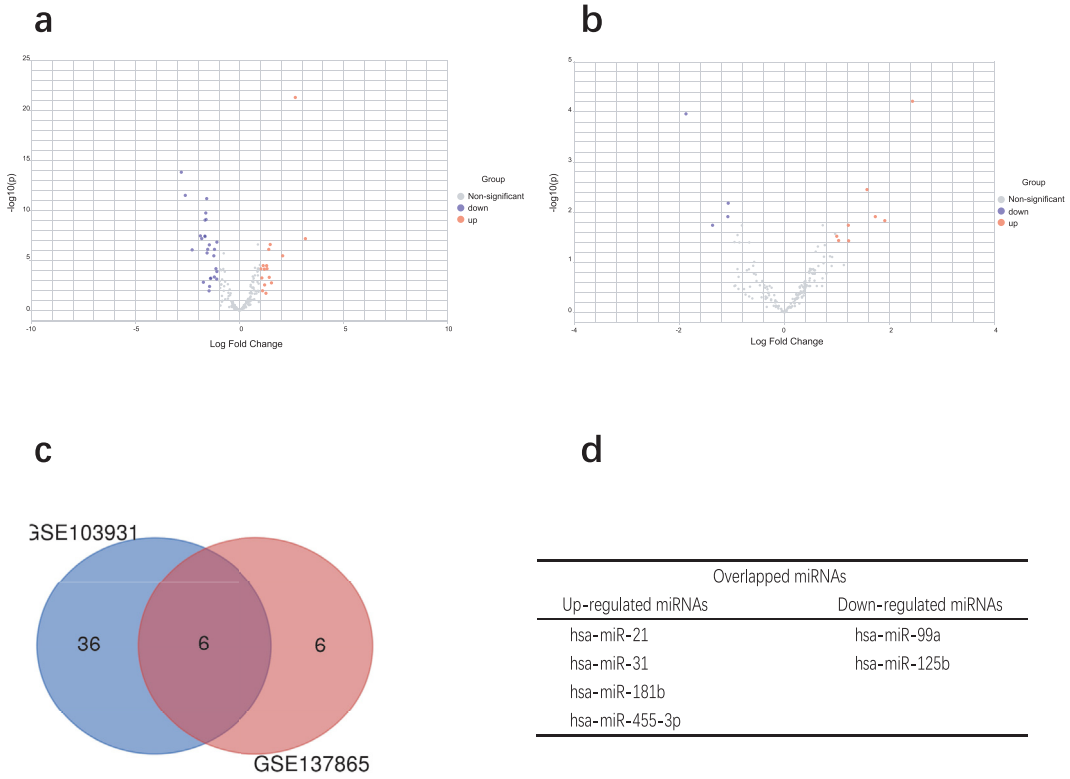


Figure 1 **Overview of this study**. Flow of this study, derivated from GSE103931 and GSE 137865 and validated in TCGA-head and neck squamous cell carcinoma cohort. Red fonts represent the key results.



d

Overlapped miRNAs	
Up-regulated miRNAs	Down-regulated miRNAs
hsa-miR-21	hsa-miR-99a
hsa-miR-31	hsa-miR-125b
hsa-miR-181b	
hsa-miR-455-3p	

Figure 2 **Identification of differently expressed miRNAs in oral squamous cell carcinoma (OSCC)** (**a**, **b**) Volcano plots of differentially expressed miRNAs inGSE103931 and GSE137865. (c) Venn plot of the differentially expressed miRNAs in the two gene expression omnibus datasets. (**d**)The overlapped four upregulated and two downregulated miRNAs.

Table 1 Differentially expressed miRNAs overlapped in both gene expression omnibus datasets.

Symbol	logFC		P-value		Up /Down
	GSE 103931	GSE 137865	GSE 103931	GSE 137865	
hsa-miR-31	3.1685	1.9153	4.23E-09	6.10E-04	Up
hsa-miR-21	2.6832	2.4429	2.79E-24	3.61E-07	Up
hsa-miR-455 −3p	1.1953	1.7349	1.38E-05	4.35E-04	Up
hsa-miR-181b	1.1388	1.0001	4.52E-06	2.50E-03	Up
hsa-miR-99a	−2.7971	−1.8731	1.63E-16	1.29E-06	Down
hsa-miR-125b	−1.6629	−1.0678	1.76E-09	1.57E-04	Down

LogFC: log2 foldchange; hsa: homosapiens.

biological process category (Fig. 4c), 'protein binding' and 'ATP binding' in the molecular function category (Fig. 4d), and 'nucleus' and 'cytosol' in the cellular component category (Fig. 4e). These genes were also enriched in typical signaling pathways in cancers (Fig. 4f). Whereafter, a miRNA-key gene interaction network was built utilizing Cytoscape, which suggested that among the 10 targets of the upregulated miRNAs, six (VCL, PIK3R1, YWHAZ, RB1, HNRNPA1, MAPK1) are latently regulated by miR-21 (Fig. 4g), while all of the key genes targeted by the downregulated miRNAs are potentially regulated by miR-125b (Fig. 4h). Later, top 5 target genes of upregulated differentially expressed miRNAs and downregulated differentially expressed miRNAs were considered as hub genes (*ACTB, HNRNPA1, MAPK1, YWHAB, VCL, ACTG1, CDK2, PPP2CA, HDAC1, XPO1*) and were demonstrated respectively (Table 2). Among the hub genes, *VCL, PPP2CA, HNRNPA1* and *MAPK1* are targets of miR-21, *PPP2CA, ACTG1, HDAC1, MAPK1, VCL, XPO1, CDK2* and *YWHAB* are targets of miR-125b. To verify the coexpression of the two miRNAs and their target hub genes, we undertook the miRNA-target coexpression utilizing the encyclopedia of RNA interactomes. Coexpression of key differentially expressed miRNAs and hub gene targets was presented in Table S3. *MAPK1* was found inversely correlated with miR-21(Fig. S1a-d). *PPP2CA, ACTG1* and *YWHAB* were found negatively while *XPO1* and *CDK2* were positively correlated with miR-125b (Fig. S1e-l).

Pathway analysis and regulatory network for key differentially expressed miRNAs

Using the miRpathDB, we explored the pathways which involved miR-21 and miR-125b. These pathways were found closely associated with tumor development, involving miRNAs in cancer, apoptosis, pathways in cancer, and PI3K-Akt signaling pathway (Fig. 5a). Subsequently, regulatory network for the two differentially expressed miRNAs and their target hub genes with significant coexpression level was constructed based on kyoto encyclopedia of genes and genomes pathway analyses (Fig. 5b). Results demonstrated that under miRNA regulation, 6 hub genes participated 7 different pathways and controlled all kinds of biological processes in tumorigenesis. Regulated by miR-21, MAPK1 participated in Influenza A and PI3K-Akt signaling pathway, regulating cell apoptosis, proliferation and DNA repair. *ACTG1, YWHAB, PPP2CA, CDK2* and *XPO1*, under regulation of miR-125b, were involved in pathways like Hippo signaling pathway, viral carcinogenesis and focal adhesion controlling degradation, apoptosis, proliferation, cell contact, viral protein expression, cell circle progression and actin polymerization.

Prognostic value of miR-21, miR-125b and hub genes in oral squamous cell carcinoma

To further advance our study, we validated the expression of miR-21, miR-125b and hub genes in TCGA using the university of alabama cancer database. The expression of the ten hub genes (*ACTB, HNRNPA1, MAPK1, YWHAB, VCL, ACTG1, CDK2, PPP2CA, HDAC1, XPO1*) were observed all considerably upregulated in head and neck squamous cell carcinoma (Fig. 6a—j). And survival analysis of the hub gene targets on the university of alabama cancer database was presented in Table S4. The results showed that high ACTB and VCL expression were related to worse prognosis in OSCC patients, and high HDAC1 expression was related to better prognosis in OSCC patients, whereas no prognostic significance was found for *HNRNPA1, MAPK1, YWHAB, ACTG1, CDK2, PPP2CA* and *XPO1*(Fig. S2). MiR-21 was higher expressed, while miR-125b was lower expressed, in head and neck cell carcinoma tissues compared with normal samples (Fig. 6k—m). And survival analysis on the Kaplan—Meier Plotter presented that high miR-125b expression was related to better prognosis in OSCC patients, while no prognostic correlation was observed for miR-21 (Fig. 6n and o).

Discussion

Emerging evidence reveals the roles of miRNA in multiple cancers including OSCC. MiRNAs are crucial functional molecules in tumor progression and a number of studies have shown that miRNAs function as tumor suppressors or oncogenes in cancer cells.[24,25] MiRNAs dysregulation plays a major role in tumorigenesis and metastasis and have attracted great interest as potential prognostic markers.[26–28] However, in consideration of numerous miRNAs that participate in tumor cell regulation, selecting significant targets for cancer diagnosis and treatment is still challengeable. What's more, the intriguing field of interactions between miRNAs and target genes in OSCC are still unmined, so are their clinical application values.

Bioinformatics is playing a crucial role to investigate disease mechanisms because of the complicated and multifactorial nature of diseases. For instance, miRNAs were identified to be related to esophageal squamous cell carcinoma.[29] In this study, we screened out 42 differentially expressed miRNAs and 12 differentially expressed miRNAs from gene expression omnibus datasets including OSCC and normal oral tissues. Furthermore, six collective miRNAs with differential expression in OSCC were selected. Then we detected possible targets of the six miRNAs using the encyclopedia of RNA interactomes database. Following gene ontology/kyoto encyclopedia of genes and genomes functional analyses of these targets using the database for

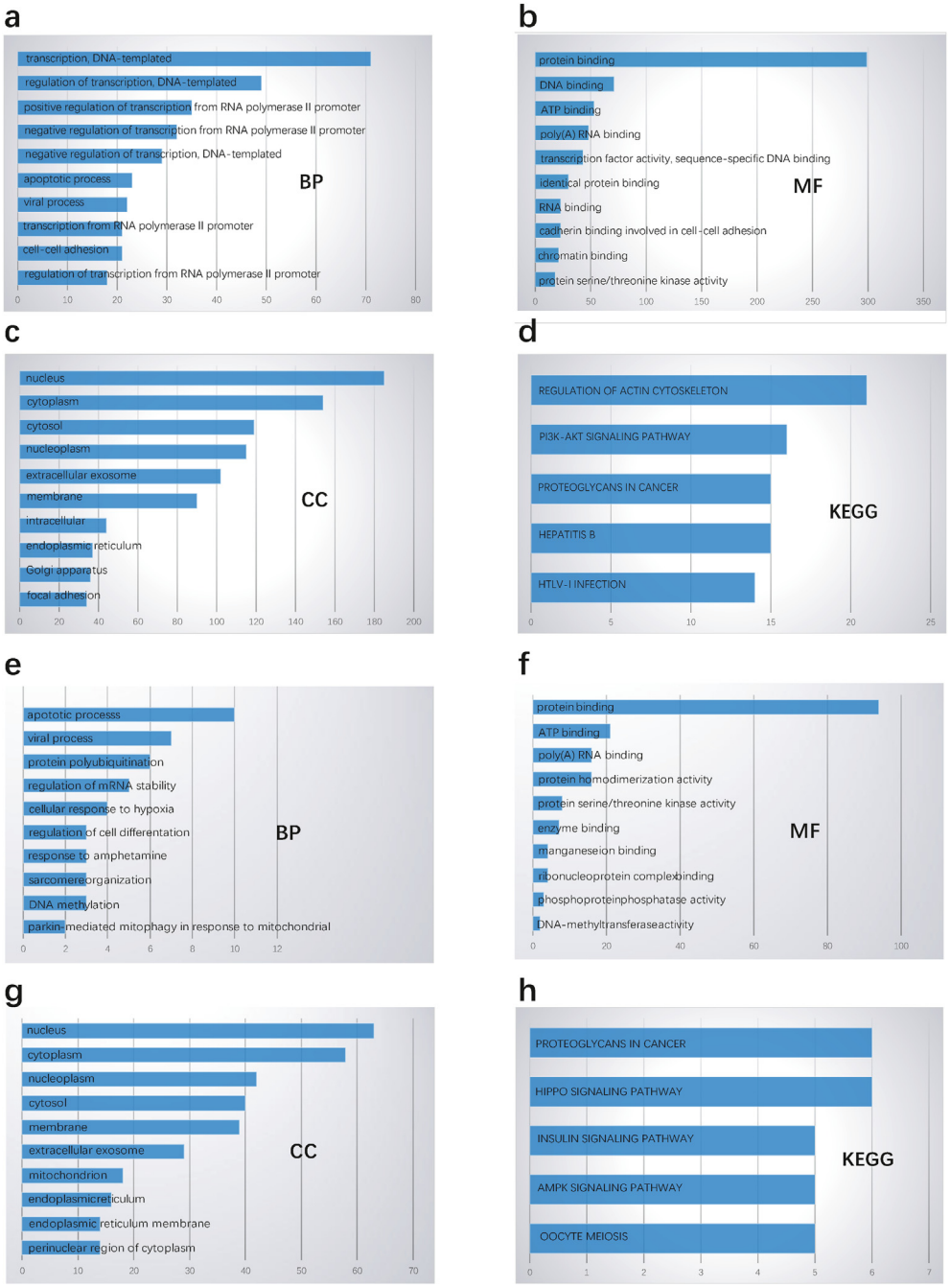


Figure 3 **Gene ontology and kyoto encyclopedia of genes and genomes analysis of target genes of the overlapped** differentially expressed miRNAs (**a**–**c**) Top 10 biological process, molecular function, and cellular component terms of the targets for the four upregulated miRNAs. (**d**) Top 5 kyoto encyclopedia of genes and genomes pathways of the targets for the four upregulated miRNAs (**e**–**g**) Top 10 biological process, molecular function, and cellular component terms of the targets for the two downregulated miRNAs. (**h**) Top 5 kyoto encyclopedia of genes and genomes pathways of the targets for the two downregulated miRNAs.

annotation visualization and integrated discovery presented their involvement in cancer cell activities (e.g., apoptotic and viral process, protein and DNA binding, and focal adhesion) and signaling pathways associated with cancer development (e.g., Regulation of actin cytoskeleton, PI3K-AKT pathways, and Proteoglycans in cancer). We next constructed a PPI network of these targets and obtained the top 10 hub genes. Subsequently, we verified their participation in critical tumor-related pathways. Later, through the miRNA-target gene interaction network, miR-21 and miR-125b were revealed as an important regulatory role, which were respectively upregulated and downregulated in OSCC, and was predicted respectively to regulate 6 and 10 of the top 10 key genes. Expression analysis of miR-21 and miR-125b targets demonstrated that *ACTB, HNRNPA1, MAPK1, YWHAB, VCL, ACTG1, CDK2,*

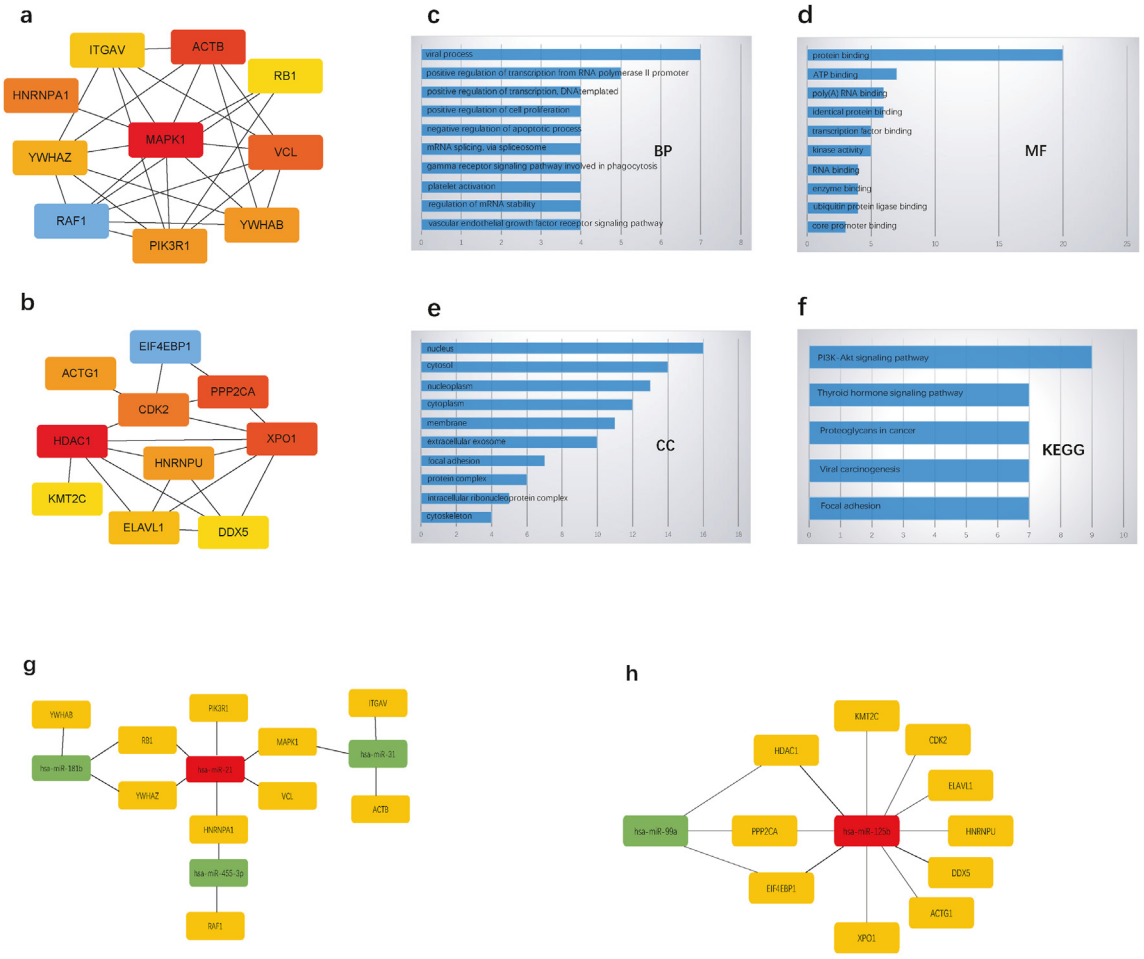


Figure 4 Gene ontology/kyoto encyclopedia of genes and genomes analysis of hub-gene targets and interaction networks of miRNAs-key-gene targets. (**a**) Top 10 key genes of the four upregulated miRNAs. (**b**) Top 10 key genes of the two downregulated miRNAs (**c**—**e**) Top 10 biological process, molecular function, and cellular component terms of the top 20 key genes. (**f**) Top 5 kyoto encyclopedia of genes and genomes pathways of the 20 key genes (**g, h**) Interaction network of the differentially expressed miRNAs and their key target genes.

Table 2 Top 5 genes in network of Upregulated/downregulated differentially expressed miRNAs by degree method.

Rank	Upregulated-DEM network		Downregulated-DEM network	
	Symbol	Score	Symbol	Score
1	MAPK1	51	HDAC1	16
2	ACTB	34	XPO1	12
3	VCL	29	PPP2CA	12
4	HNRNPA1	27	CDK2	11
5	YWHAB	26	ACTG1	9

DEM: differentially expressed miRNA.

PPP2CA, *HDAC1*, *XPO1* were overexpressed in head and neck squamous cell carcinoma. HNRNPA1, MAPK1, YWHAB, PPP2CA, HDAC1 and XPO1 have been reported as functional targets of miRNAs.[30—35] For ACTB, VCL, ACTG1 and CDK2, experimental evidence is deficient and we surmise that their expression also might be regulated by circRNAs and lncRNAs, while further study is wanted.

Expression analysis using UALCON confirmed expression differences for miR-21 and miR-125b in OSCC, and Kaplan—Meier Plotter survival analyses demonstrated that high miR-125b expression related to better prognosis Though no correlation of miR-21 expression and survival of OSCC, former study reported that high miR-21 expression was related to poor prognosis in OSCC.[36,37] Existing studies have brought out plentiful biological processes and pathological connections of miR-21 and miR-125b in OSCC.[38—40] MiR-21 was found to affect cancer stemness by regulating yes-associated protein 1 expression.[41] MiR-125b was reported to suppress oncogenicity, increase reactive oxygen species and cisplatin sensitivity in OSCC, and lower levels of the miR-125b-PRXL2A-NRF2 axis reduced oxidative stress, hence the increased cisplatin resistance.[40] Literature also presented that miRNAs, including miR-99a, miR-105, miR-142—5p, miR-455—3p, miR-27b-3p, miR-31, and miR-181b, were related to overall survival of patients with OSCC.[42—47] MiR-31 was reported to involved in the HIF

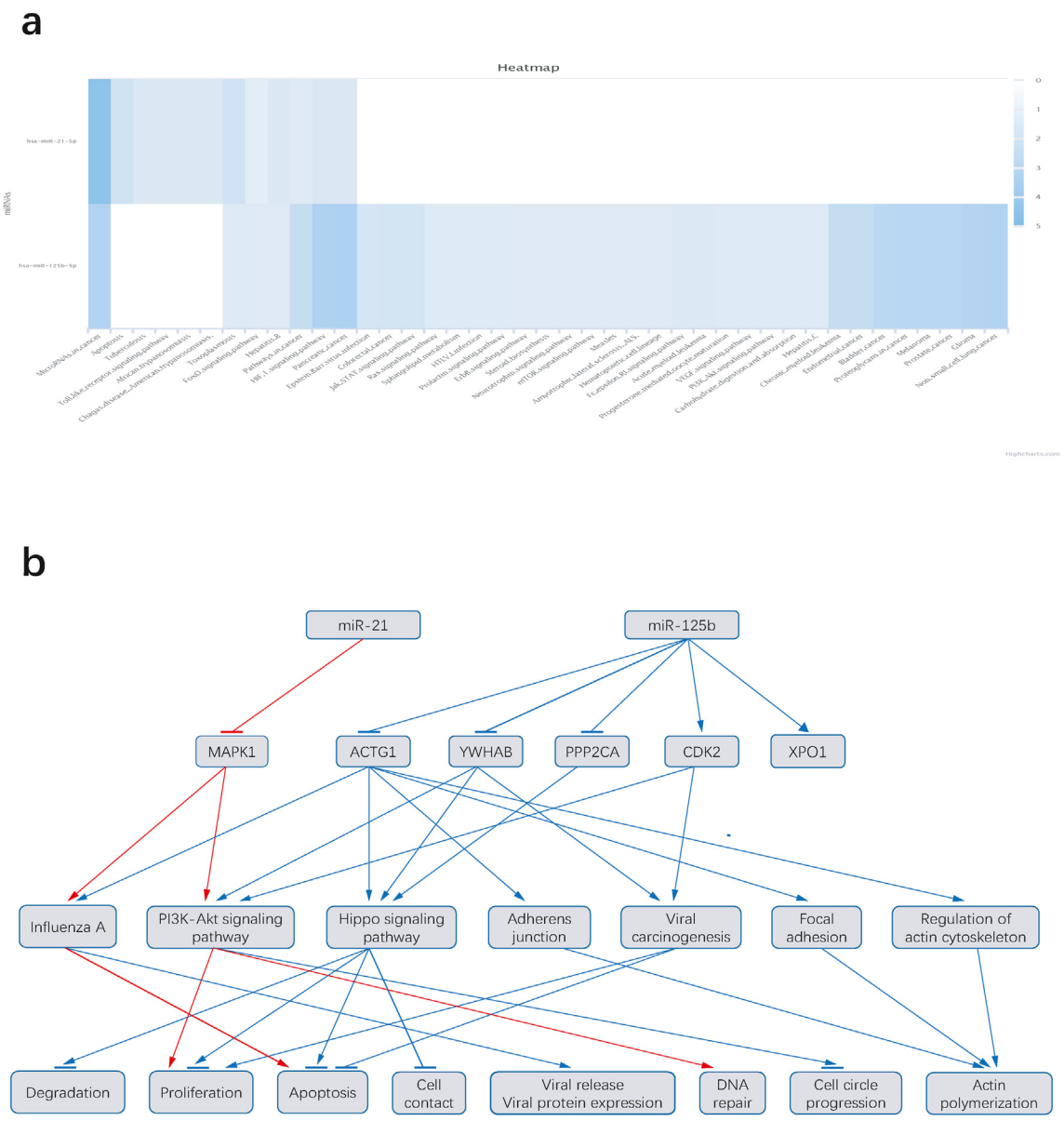


Figure 5 Regulatory network of miRNA-hub-gene targets. (**a**) Regulatory heatmap of miR-21 and miR-125b associated signaling pathways. (**b**) Regulatory network of miRNA-hub-gene targets. Blue arrows represent miR-21 associated regulatory relationships while red arrows represent miR-125b associated regulatory relationships. The interaction between miRNAs and mRNAs demonstrate miR-21 and miR-125b might regulates OSCC cellular growth, proliferation, degradation, apoptosis and migration via these genes and functions through Influenza A/PI3K-akt signaling/Hippo signaling/Adherens junction/Viral carcinogenesis/Focal adhesion/ Regulation of actin cytoskeleton pathways. → Stimulatory Modification, —| Inhibitory Modification , →| Stimulatory and Inhibitory Modification.

regulation by targeting factor-inhibiting hypoxia-inducible factor in head and neck squamous cell carcinoma development and enhance 4NQO-induced DNA damage through targeting of Ku80 in OSCC and esophageal squamous cell carcinoma.[48,49] MiR-181b was revealed to be related to lymph-node metastasis, vascular invasion, and prognosis in OSCC.[50]

Several limitations exist in our work. Considering the limited size of GSE137865, larger and more comprehensive datasets are needed. Since the selected datasets were established a few years ago, some miRNAs were not investigated and the resulted profiles were not all-sided. The expression of miR-125b, miR-21 and hub genes was not validated in OSCC cell lines and clinical OSCC cases. The roles of other ncRNAs, like circRNAs and lncRNAs, on OSCC development were not explored and worth a deep excavation. Despite of these deficiency, our study demonstrated the complicated sketch map of OSCC regulation by miRNA-gene networks and revealed several key miRNAs and undefined hub genes that latently play important roles in OSCC growth, development and metastasis.

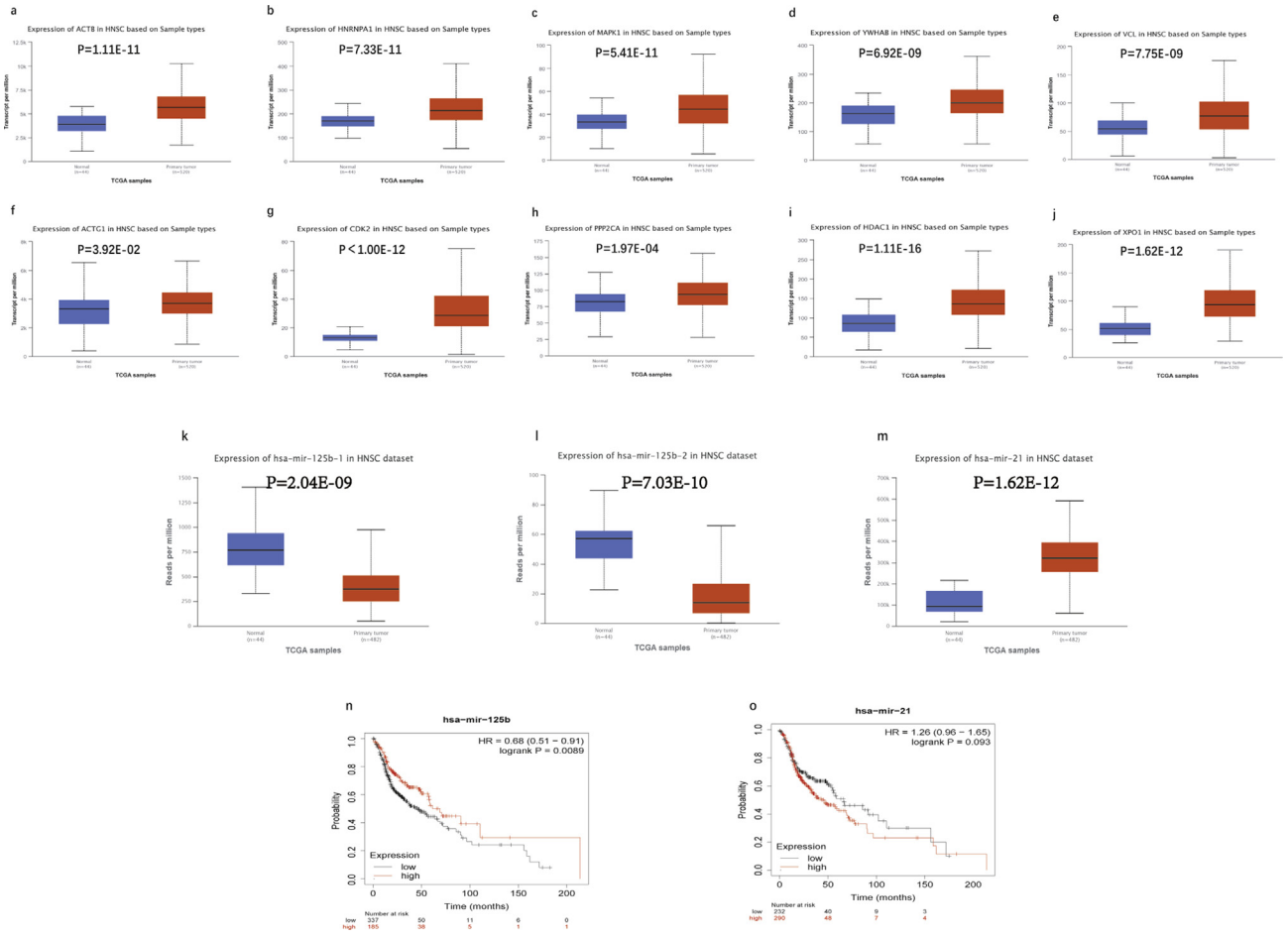


Figure 6 Expression and prognostic values of miR-21、miR-125b and their hub-gene targets in OSCC. (**a**—**j**) Relative expression of *ACTB*, *HNRNPA1*, *MAPK1*, *YWHAB*, *VCL*, *ACTG1*, *CDK2*, *PPP2CA*, *HDAC1*, *XPO1* mRNAs in head and neck squamous cell carcinoma, compared with normal tissues (**k**—**m**) Analysis of miR-21 and miR-125b expression in head and neck squamous cell carcinoma in TCGA using the university of alabama cancer database (**n**, **o**) Kaplan—Meier survival analysis of miR-21 and miR-125b in head and neck squamous cell carcinoma in TCGA performed on Kaplan—Meier Plotter. *P $<$ 0.05, **P $<$ 0.01. ***P $<$ 0.001, and ****P $<$ 0.0001.

In summary, our findings identified six differentially expressed miRNAs obviously associated with gene interaction pathways and tumorigenesis-related processes in OSCC. We also revealed several novel hub genes influenced by these miRNAs in OSCC. Most worthy of mention is that we presented a novel sketch map of regulatory network for key miRNAs with hub genes in the carcinogenesis and development of OSCC. Furthermore, miR-21 upregulation and miR-125b downregulation were discovered to be related to the prognosis of OSCC. High *ACTB* and *VCL* expression were discovered to be related to poor OS in OSCC patients, and high *HDAC1* expression was related to better prognosis in OSCC patients. Our study might aid in better understanding the molecular mechanisms of OSCC through mRNA-miRNA interactions and suggest that these miRNAs and targeted genes might be potential targets for diagnosis, therapy and prognosis of OSCC. Whereas, further study is needed to verify our findings and facilitate the clinic application of these miRNAs and genes as prognostic biomarkers or therapeutic targets in OSCC.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jds.2021.08.016.

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