



miRNA-218/FANCI is associated with metastasis and poor prognosis in lung adenocarcinoma: a bioinformatics analysis

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Background: In this study, tumor microarray analysis was used to screen the key messenger RNAs (mRNAs) and microRNAs related to the progression of lung adenocarcinoma (LUAD), in order to provide a theoretical basis for early diagnosis, therapeutic targets, and prognosis evaluation of patients with LUAD.

Methods: The mRNA and miRNA expression datasets came from the Gene Expression Omnibus (GEO) project database. Differentially expressed genes (DEGs) and microRNAs (DEMs) between LUAD tissues and adjacent lung tissue were obtained using GEO2R. The Search Tool for the Retrieval of Interacting Genes website was also employed to construct and visualize the interactions of overlapped DEGs. The overall survival of DEMs was investigated using the Kaplan-Meier plotter. The TargetScan website (<http://www.targetscan.org/>) was used to verify the relationship between FA Complementation Group I (FANCI) and the expression of miRNA-218 (miR-218). The expression of FANCI was verified using the GEO and Human Protein Atlas databases, as well as Real Time Quantitative PCR using our own samples. Next, we analyzed the relationship between the expression of FANCI and the clinicopathological characteristics as well as the prognosis of patients with LUAD. We also explored whether the FANCI was related to immune cell infiltration in LUAD.

Results: FANCI was identified as a hub gene and associated with poor OS. We found that miR-218 negatively regulates FANCI mRNA expression. At the mRNA expression and protein level, FANCI was more highly expressed in LUAD tissues. The expression of FANCI in LUAD was related to tumor size ($\chi^2=13.96$, $P<0.001$), lymphatic metastasis ($\chi^2=3.88$, $P<0.05$), distant metastasis ($\chi^2=45.39$, $P<0.001$), and stage ($\chi^2=11.03$, $P<0.05$). In addition, the Cox regression model found that FANCI mRNA expression was an independent predictive factor of patient survival ($P<0.05$). FANCI expression was both weakly related to B cells and neutrophil infiltration in LUAD.

Conclusions: miR-218 may negatively regulate FANCI, and FANCI could promote metastasis via extracellular matrix (ECM) receptor interaction, leading to poor prognosis of LUAD. FANCI may be a key gene to determine metastasis and poor prognosis in patients with LUAD. Changes in the immune microenvironment may be the mechanism through which FANCI leads to poor prognosis of LUAD.

Keywords: Lung adenocarcinoma (LUAD); miR-218; FANCI; metastasis; poor prognosis

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Introduction

At present, lung cancer has the highest mortality rate worldwide, and lung adenocarcinoma has become the most common form of lung cancer (1). LUAD is typically diagnosed in advanced stages, when the cancer has metastasized to nearby tissues (2). Late diagnosis leads to delayed treatment, and failure of prognosis evaluation leads to a low survival rate among LUAD patients (3). Furthermore, drug resistance has also limited the clinical use of targeted drugs. Therefore, exploring the mechanisms of LUAD progression is urgently needed, which could aid in the diagnosis and discovery of prognostic biomarkers and potential therapeutic targets (4). Numerous biomarkers and therapeutic targets have been shown to play an effective role in the mechanisms of treatment for LUAD patients. However, there remains a pressing need to identify more genetic information of the key genes in LUAD progression.

Currently, comprehensive bioinformatics analysis, which is mainly focused on screening and further analyzing differentially expressed molecules, has been widely used in the diagnosis and treatment of LUAD, as well as the identification of potential biomarkers associated with prognosis. Accumulating data on malignancy is available from the authoritative GEO genomics data repository, which includes microarray and high-throughput sequencing data. In the current study, we used one microRNA (miRNA) microarray and three messenger RNA (mRNA) microarrays to obtain DEMs and DEGs in order to reduce false positive results. Functional enrichment of DEGs and DEMs was carried out. The protein-protein interaction (PPI) network analysis was used to screen out the hub genes related to LUAD through the Molecular Complex Detection (MCODE) module. Key miRNAs and mRNAs in LUAD were identified by miRNA-mRNA interaction analysis and OS analysis. Mir-218 was been shown to be downregulated in a variety of malignant tumors, thereby playing an important function similar to that of tumor suppressor genes. In the current study, we confirmed that miR-218 is downregulated and related to poor prognosis in LUAD patients. In the hub DEGs, FANCI was observed to be an important potential prognostic biomarker for LUAD. We identified that miR-218 is negatively correlated with the FANCI expression in LUAD for the first time.

Additionally, we verified FANCI expression in LUAD using The Cancer Genome Atlas (TCGA) and GEO databases, and explored the correlation between FANCI expression and the clinicopathological characteristics as well as the prognosis of LUAD patients. Our results provide more evidence to confirm that FANCI is a LUAD biomarker that may be involved in the mechanisms of metastasis as well as the poor prognosis of LUAD patients. This may help to provide targeted therapy for LUAD patients with novel targets.

We present the following article in accordance with the REMARK reporting checklist (available at <https://dx.doi.org/10.21037/atm-21-3823>).

Methods

Microarray data

The GEO Series (GSE)63805 miRNA microarray and the GSE40791, GSE27262, and GSE75307 mRNA microarrays were downloaded from the GEO database. The GSE40791 dataset included 100 normal tissues and 94 LUAD tissues. GSE27262 comprised 50 samples, including 25 LUAD tissues and 25 normal tissues. GSE75037 contained 166 samples, including 83 LUAD samples and 83 normal samples. GSE63805 included 30 LUAD samples and 30 normal samples.

Clinical specimen collection

Thirty surgical specimens from LUAD patients, including lung cancer tissues and paracancerous tissues, were collected at the Department of Thoracic Surgery in the First Affiliated Hospital of Zhengzhou University from September 2019 to January 2020. These patients had primary tumors and did not receive preoperative chemoradiotherapy, biotherapy, or antitumor therapy. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University (NO.2019-KY-255), and the patients and their families agreed to the tissue samples collection and their use in research.

DEGs and DEMs analysis

In this study, GEO2R (<https://www.ncbi.nlm.nih.gov/geo/geo2r/>), a comprehensive tool that can analyze different expression data groups, was employed to identify DEGs and DEMs. An adjusted P value <0.01 and logFC >1.5 was applied for DEGs, and an adjusted P value <0.05 and log FC >1 was used for DEMs. The overlapping DEGs were derived from the GSE40791, GSE27262, and GSE75037 datasets.

Pathway analysis for DEMs and DEGs

Database for Annotation, Visualization, and Integrated Discovery (DAVID, <https://david.ncifcrf.gov/>), an online bioinformatics database, was used for pathway analysis of DEGs and DEMs. We then visualized the results using RStudio software (Boston, RStudio, Inc.). Similarly, enrichment analysis was performed for DEMs using FunRich (<http://www.funrich.org>).

Construction of the PPI network

Search Tool for the Retrieval of Interacting Genes (STRING, <https://string-db.org/>) is an online website that was designed to predict PPI network. In this study, we set an interaction score >0.4, and analyzed the PPI network for DEGs. We subsequently visualized the results using Cytoscape software. MCODE, a Cytoscape plugin, was used to locate the hub genes of the PPI network.

Kaplan-Meier (KM) analysis of DEMs

We used the KM plotter to perform an overall survival (OS) analysis of the DEMs in 513 and 866 LUAD patients, respectively. We separated patients into two groups according to the gene transcriptional expression level, and drew KM plots.

MiRNA target gene prediction

MiRecords (<http://c1.accurascience.com/miRecords/>) was used to predict the target mRNA of survivable DEMs, which consisted of 11 miRNA target gene prediction algorithms. We accepted target mRNA of DEMs that were predicted in at least three databases. Next, we visualized the regulatory network using Cytoscape (Institute for Systems Biology).

miRNA-mRNA network construction

Previous studies have demonstrated that miRNA regulated the expression of mRNA by translation inhibition or degradation, which indicated that miRNA was negatively related to mRNA expression. The reverse trend of DEM-DEG (upregulated DEM-downregulated DEG or downregulated DEM-upregulated DEG) was screened, and Cytoscape was used to visualize DEM-DEG network was used.

Relationship between FANCI and miR-218 expression

The online prediction website TargetScan (http://www.targetscan.org/vert_72/) was used to predict the miRNA target genes and verify the relationship between FANCI and miR-218 expression.

Verification of FANCI expression by real time quantitative PCR (RT-qPCR)

We isolated total RNAs from LUAD tissues and reverse-transcribed them into complementary DNA (cDNA), and then used RT-qPCR to validate the expression of FANCI. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was amplified as an internal control. The primer sequences were as follows:

- ❖ FANCI-forward: CCACCTTTGGTCTATCAGCTTC.
- ❖ FANCI-reverse: CCACCTTTGGTCTATCAGCTTC.
- ❖ GAPDH-forward: GGAGCGAGATCCCTCCAAAAT.
- ❖ GAPDH-reverse: GGCTGTTGTCATACTTCTCATGG.

Human Protein Atlas (HPA) analysis

Immunohistochemistry (IHC) staining data were used from the HPA database to analyze the protein level of FANCI in LUAD samples. FANCI protein expression included four levels: high, medium, low, and undetected. The scoring system involved the percentage of positively-stained cells as well as the intensity of staining.

Exploring the relationship between the expression of FANCI and clinical features in TCGA database

We downloaded the clinical information and mRNA sequencing data of LUAD patients from the TCGA database, and the clinicopathological features and FANCI mRNA expression values of 475 patients with LUAD

were obtained via screening. Patients were divided into a high and low group according to the median expression of FANCI. We analyzed the correlation between the expression of FANCI and tumor size, gender, lymph node metastasis, age, clinical stage, distant metastasis, and survival. The effects of clinicopathological features and FANCI expression on survival were analyzed by univariate and multivariate analyses.

Tumor Immune Estimation Resource (TIMER) analysis

The TIMER online tool (<https://cistrome.shinyapps.io/timer/>), which is a website that analyzes immune infiltration across multiple cancer types, was used to assess these infiltrates on the LUAD sample data. We explored the connection between FANCI and immune cells as well as their marker genes. The parameters were selected as follows: “survival”, cancer type: “LUAD”, gene type: “FANCI”, clinical: “All”, and immune infiltration: “Default”, and survival analysis was conducted to establish a multivariate Cox proportional risk model, which adopted a Cox proportional hazard model and used R package (<https://www.rstudio.com/products/rpackages/>) “survival” to fit.

Statistical analysis

We used GraphPad Prism 8.0 (American, GraphPad Software, LLC) software to test the differential expression. Pearson correlation analysis was used to determine the relationship between the expression of FANCI and miR-218. The χ^2 test was applied to analyze the relationship between FANCI expression and clinicopathological characteristics. We performed univariate and multivariate survival analysis using the Cox proportional hazards regression models. We used SPSS (American, IBM) statistical software and RStudio (<https://www.rstudio.com/>) to analyze the clinical characteristics. A P value <0.05 was considered statistically significant.

Results

DEGs and microRNAs analysis

We obtained 2,708, 1,159, and 1,756 DEGs from the GSE40791, GSE27262, and GSE75037 datasets, respectively (Figure 1A–1C). Among them, 608 DEGs overlapped; 177 were upregulated, and 431 were downregulated (Figure 1). From the GSE63805 dataset, a total of 27 DEMs (16

upregulated and 11 downregulated) miRNAs were extracted (Figure 1D and Table S1). These miRNAs and genes were recognized as differentially expressed in LUAD samples compared with normal samples.

Functional enrichment analysis

The biological process that analyzed the overlapping DEGs included positive regulation of angiogenesis, positive regulation of cell-substrate adhesion, and extracellular matrix organization (Figure 2A). Regarding molecular functions (MF), it included heparin binding, calcium ion binding, and adenosine-triphosphate (ATP)-dependent microtubule motor activity (Figure 2B). Regarding cellular components (CC), it included extracellular space, proteinaceous extracellular matrix, and extracellular exosome (Figure 2C). Additionally, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analyzed included malaria, extracellular matrix (ECM)-receptor interaction, and protein digestion and absorption (Figure 2D).

Regarding biological processes (BP) analysis of DEMs, the pathways were concentrated in the regulation of nucleobase, nucleoside, nucleotide, and nucleic acid metabolism (Figure 3A). In addition, the most enriched Gene Ontology (GO) terms in MF were transcription factor activity (Figure 3B). Regarding CC, the DEMs were concentrated in the actin cytoskeleton, cytoplasm, and nucleus (Figure 3C). As shown in Figure 3D, the biological pathway analysis included proteoglycan syndecan-mediated signaling events, the erythroblastic leukemia viral oncogene homolog (ERBB) receptor signaling network, the nectin adhesion pathway, plasma membrane estrogen receptor signaling, and signaling events mediated by the hepatocyte growth factor receptor.

PPI network analysis

We used the STRING website to analyze the protein interactions of the overlapping genes. We constructed the network of DEGs, comprising 552 protein (Figure 4A), and used the MCODE Cytoscape plug-in to assess the functional modules. Module 1 was identified as consisting of 48 nodes and 1,075 edges, including FANCI, ANLN, GINS2, TPO2A, etc. (Figure 4B).

Kaplan-Meier analysis of DEMs

We used the KM plotter to predict the prognostic values

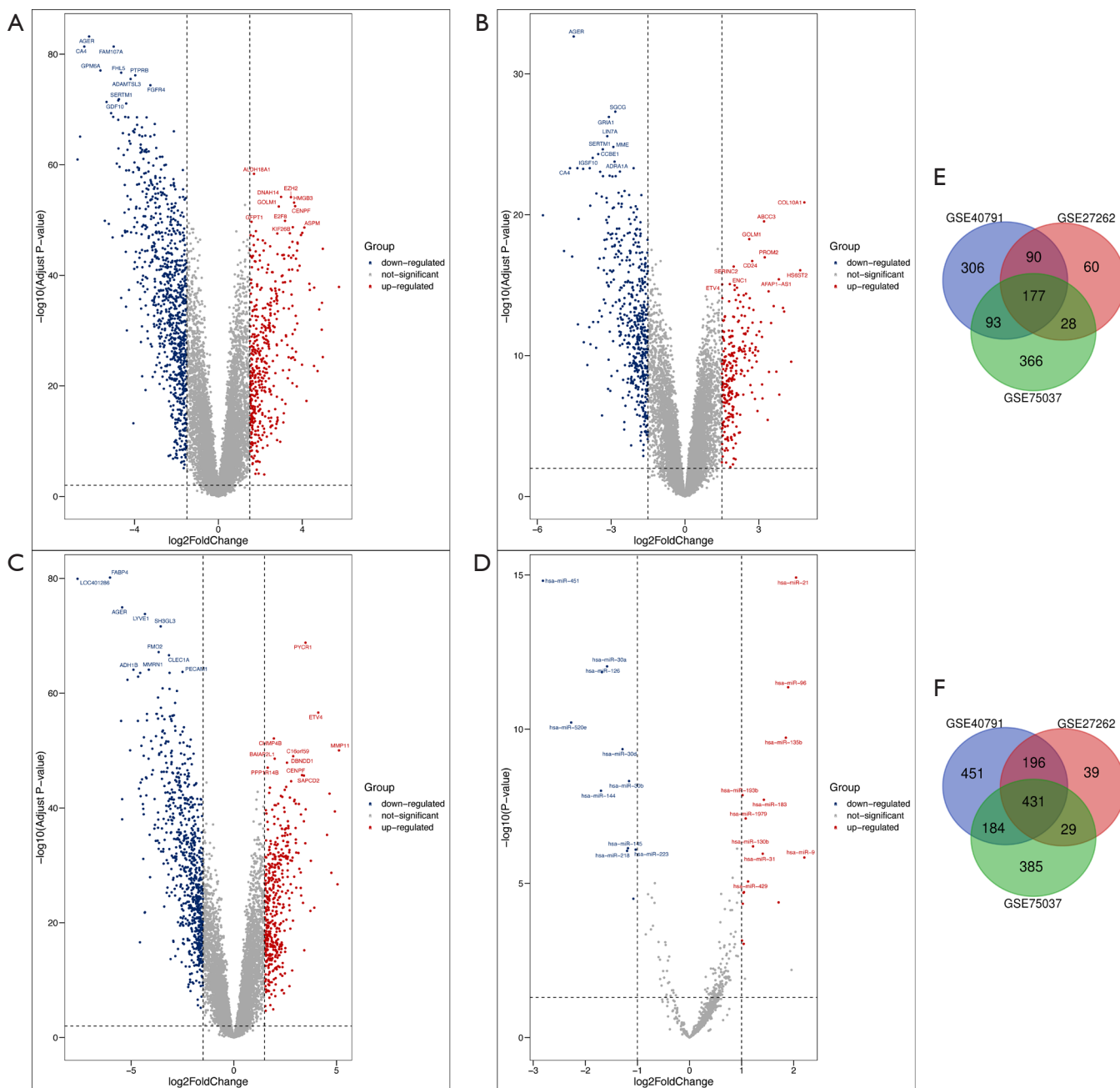


Figure 1 Screening of differentially expressed mRNA and miRNAs. (A) Differentially expressed mRNA in GSE75037; (B) differentially expressed mRNA in GSE40791; (C) differentially expressed mRNA in GSE27262; (D) differentially expressed miRNA in GSE63805; (E) the upregulated differentially expressed mRNA in three datasets; and (F) the downregulated differentially expressed mRNA in three datasets.

of the 27 identified DEMs. The results showed that the high expression of miR-21, miR-193b, miR-31, and miR-9 ($P < 0.05$) were associated with poor OS. Additionally, the low expression of miR-30a, miR-126, miR-30d, miR-218, and miR-30b ($P < 0.05$) was related with worse OS (Figure 5).

DEM target mRNA prediction and miRNA-mRNA pairs

A total of 2,805 DEM target genes were predicted using the miRecord database, including 798 target genes predicted by downregulated DEMs and 2,007 target genes predicted by

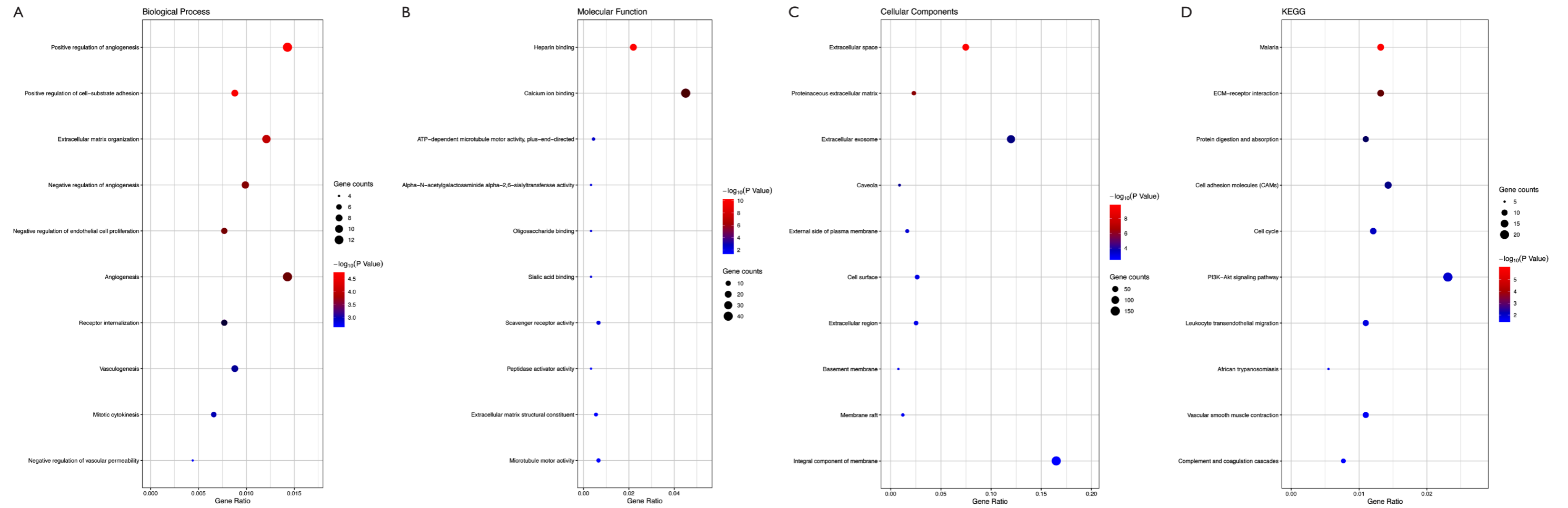


Figure 2 Related pathways of the differentially expressed genes in lung adenocarcinoma. (A) BP, (B) MF, (C) CC, and (D) KEGG pathway analyses.

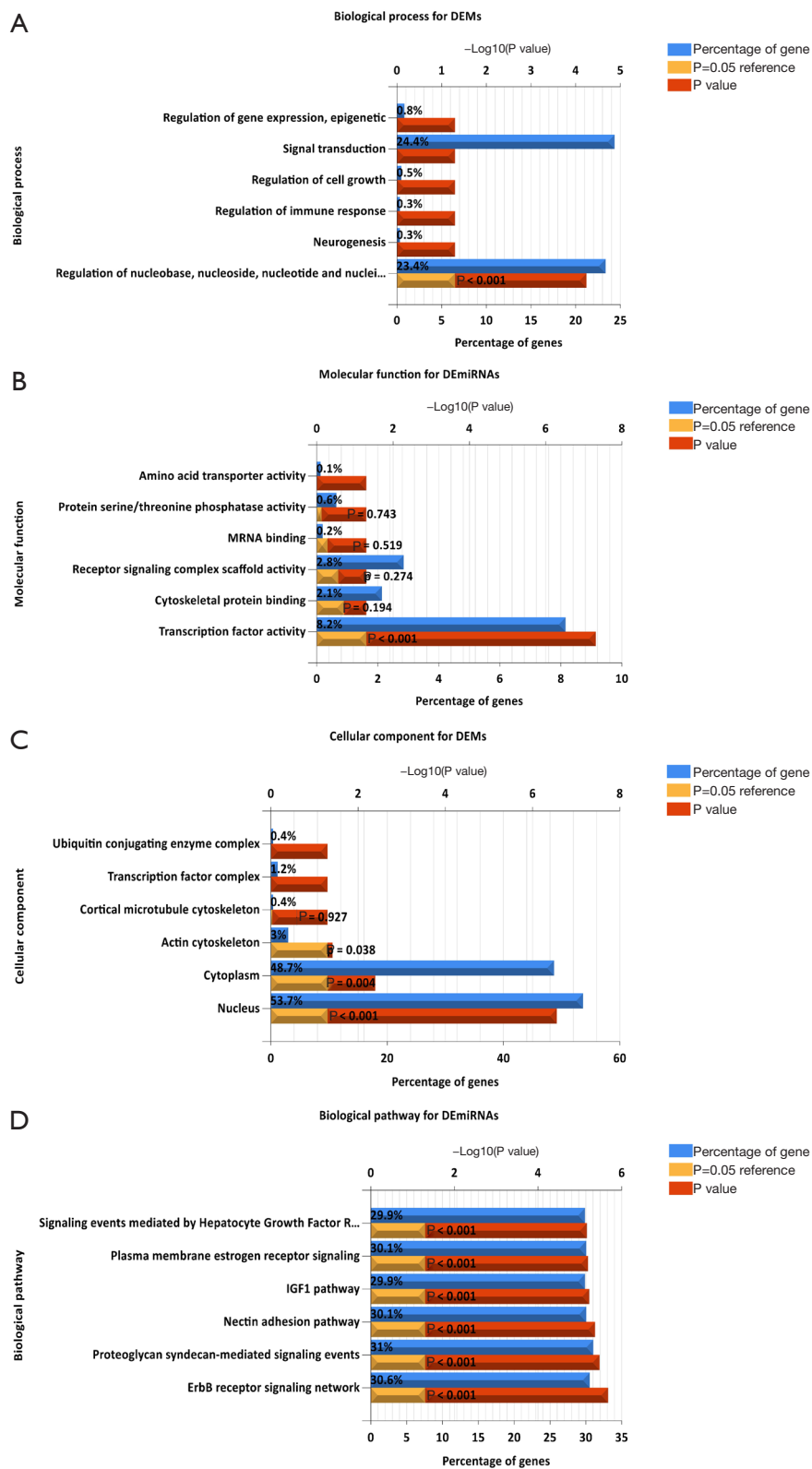


Figure 3 Related pathways of the overlapping differentially expressed miRNA in lung adenocarcinoma. (A) BP, (B) MF, (C) CC, and (D) biological pathway analysis.

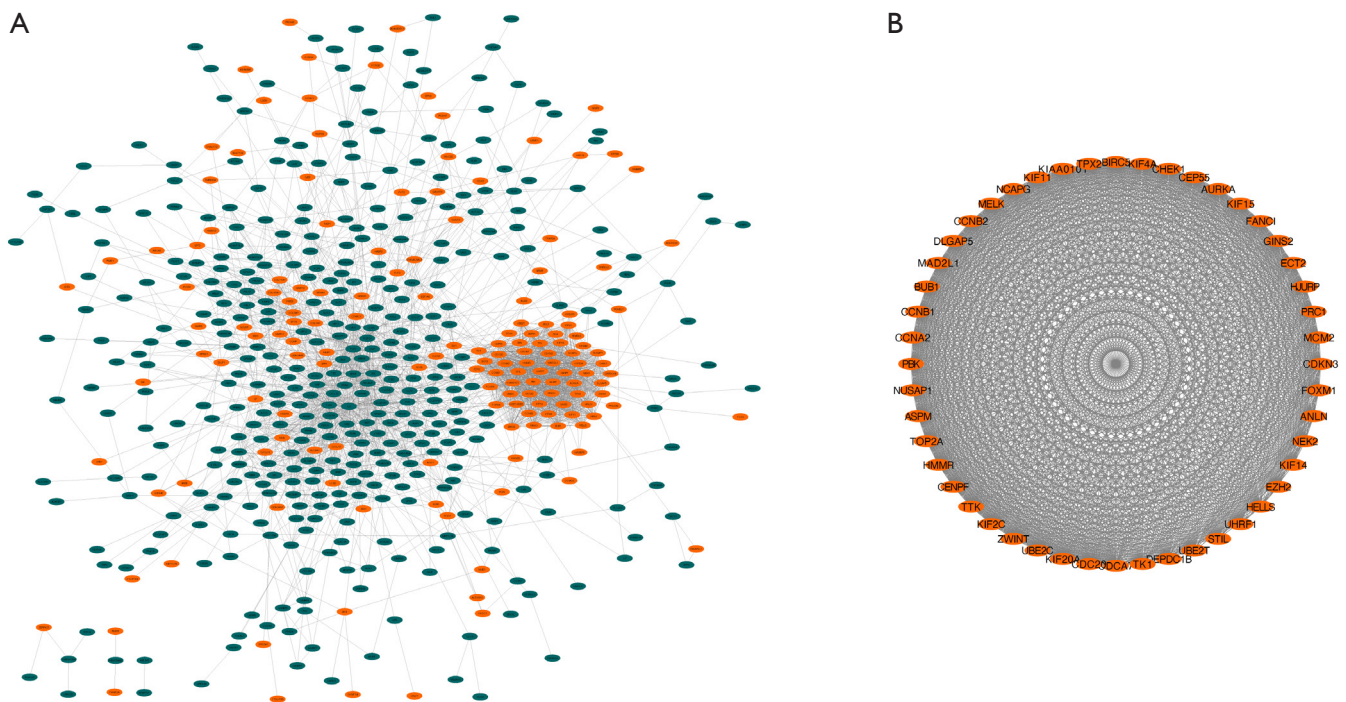


Figure 4 Protein interactions of: (A) the overlapping DEGs; (B) DEGs in Module 1. Green nodes: downregulated genes. Red nodes: upregulated genes. DEG, differentially expressed gene.

upregulated DEMs (Figure 6). These targets predicted by downregulated DEMs and upregulated DEMs intersected with upregulated DEGs and downregulated DEGs, respectively. Six upregulated DEGs were predicted by downregulated DEMs, and 20 downregulated DEGs were predicted by upregulated DEMs. A DEM-DEG regulatory network was constructed using Cytoscape (Figure 7).

MiR-218 negatively regulates FANCI mRNA expression

TargetScan was used to predict that miR-218 could target FANCI, and the binding site of FANCI was identified in the sequence of miR-218 (Figure 8A). In addition, FANCI expression and miR-218 in LUAD samples was detected using GEO data, and the potential connection between FANCI and miR-218 was explored by the TargetScan database. Compared with normal lung samples, FANCI mRNA expression in LUAD tissues was significantly upregulated (Figure 8B), and miR-218 expression in LUAD samples was markedly downregulated (Figure 8C), which indicated that FANCI was negatively correlated with miR-218 (Figure 8D).

FANCI mRNA expression detection by qRT-PCR and protein expression by immunohistochemistry (IHC)

The IHC staining for FANCI in the HPA database was compared between LUAD tumor samples and normal lung samples using the HPA039972 antibody. FANCI was “undetected” in normal lung tissue (Figure 9A), and was detected as “moderate” in tumor samples (Figure 9B). By analyzing the correlation between FANCI expression and prognosis in the HPA database, a total of 696 high expression samples and 298 low expression samples were obtained. The OS was significantly lower in the FANCI high expression group (log-rank $P=0.0068$, Figure 9C). We also verified the FANCI expression using our own samples by RT-qPCR, and FANCI was notably high in LUAD samples ($t=12.21$, $P<0.0001$, Figure 9D).

Relationship between clinicopathological features and FANCI expression

Based on TCGA database, we combined the clinicopathological data and found that FANCI was

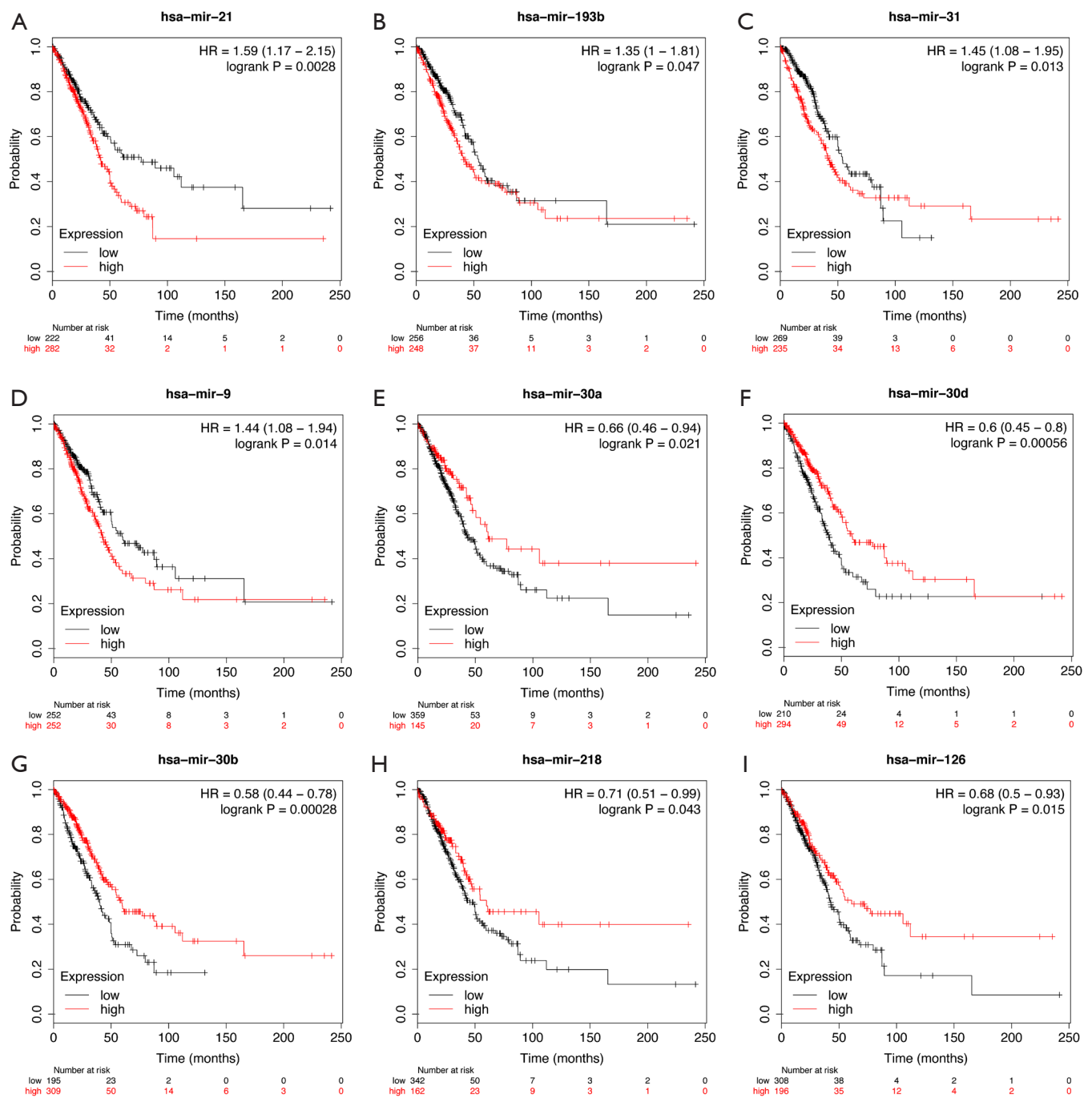


Figure 5 Overall survival analysis of DEMs. KM plotter of OS in LUAD patients. Survival analysis of: (A) miR-21; (B) miR-193b; (C) miR-31; (D) miR-9; (E) miR-30a; (F) miR-30d; (G) miR-30b; (H) miR-218; and (I) miR-126. DEM, differentially expressed microRNA; K-M, Kaplan-Meier; OS, overall survival; LUAD, lung adenocarcinoma.

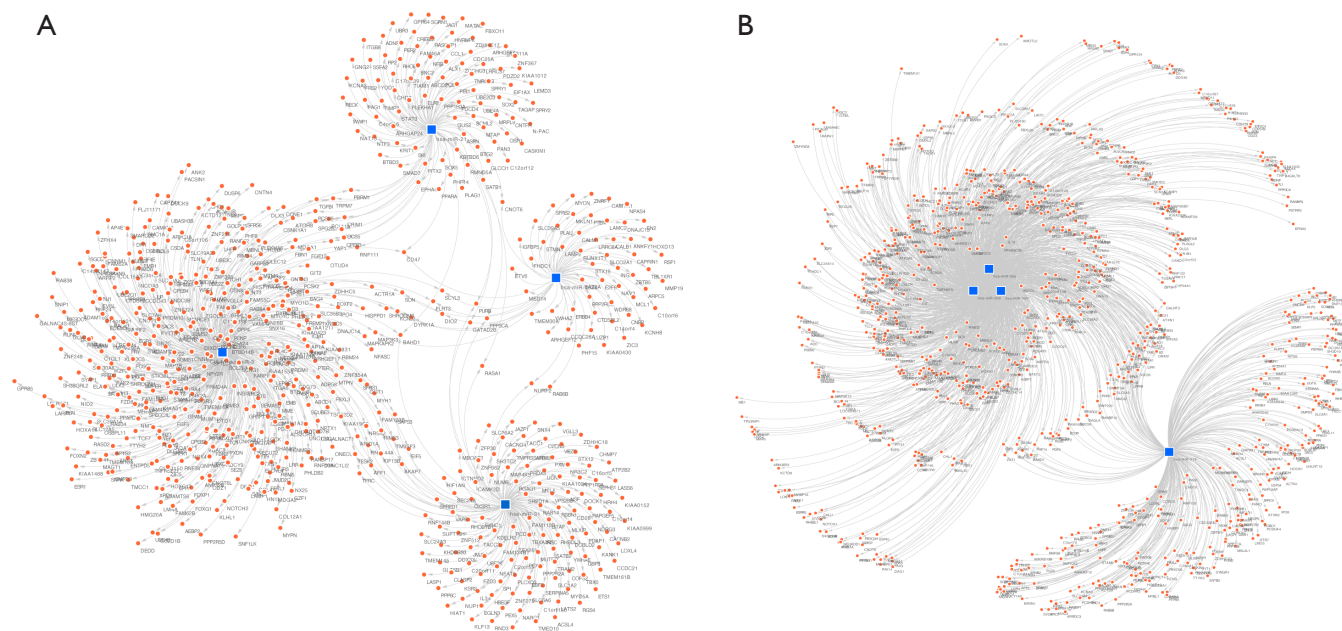


Figure 6 Potential target genes predicted by DEMs using miRecords. (A) Potential downregulated target genes predicted by upregulated DEMs were constructed by Cytoscape. (B) Potential upregulated target genes predicted by downregulated DEMs were constructed by Cytoscape. DEM, differentially expressed microRNA.

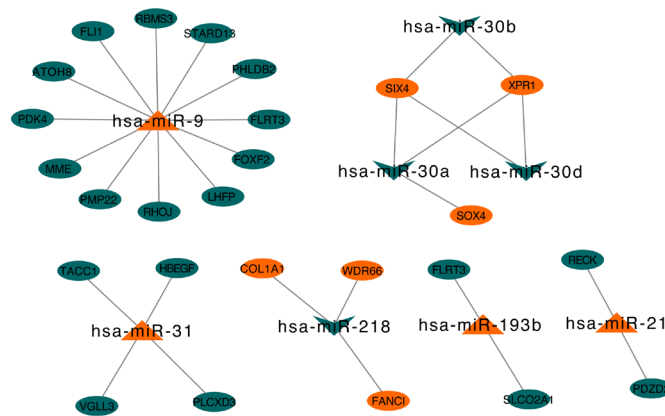


Figure 7 DEMs-DEGs target prediction network. DEM, differentially expressed microRNA; DEG, differentially expressed gene.

correlated with tumor size ($\chi^2=13.96$, $P<0.001$), distant metastasis ($\chi^2=45.39$, $P<0.001$), lymphatic metastasis ($\chi^2=3.88$, $P<0.05$), and stage ($\chi^2=11.03$, $P<0.05$, *Table 1*). Meanwhile, FANCI expression was significantly correlated with tumor size ($P<0.01$), distant metastasis ($P<0.0001$), survival status ($P<0.0001$), and tumor (T), regional lymph node (N), metastasis (M) and clinical stage ($P<0.01$, *Figure 10*). Furthermore, multivariate Cox analysis showed that FANCI was a significant independent factor for poor OS ($P<0.05$,

Table 2). Based on TCGA database, the overall 5-year survival rate of the FANCI high expression group was poorer than the low expression group ($P<0.01$; *Figure S1*).

Relationship between FANCI and immune infiltrates

The TIMER website analysis showed that the expression of FANCI was associated with B cell and neutrophil infiltration in LUAD ($P<0.05$, *Figure 11A*). Combined with survival

Table 1 FANCI expression with clinicopathological characteristics (N=475)

Clinicopathological feature	Total number of patients (%)	FANCI expression (%)		χ^2	P
		High	Low		
Gender				13.85	0.000**
Male	222 (46.7)	131 (55.3)	91 (38.2)		
Female	253 (53.3)	106 (44.7)	147 (61.8)		
Age (years)				1.35	0.245
<60	129 (27.2)	70 (29.5)	59 (24.8)		
≥60	346 (72.8)	167 (70.5)	179 (75.2)		
Tumor size (cm)				13.96	0.000**
<3	163 (34.3)	62 (26.2)	101 (42.4)		
≥3	312 (65.7)	175 (73.8)	137 (57.6)		
Lymphatic metastasis				3.88	0.049*
N0	313 (65.9)	146 (61.6)	167 (70.2)		
N1–N3	162 (34.1)	91 (38.4)	71 (29.8)		
Distant metastasis				45.39	0.000**
M0	411 (86.5)	180 (75.9)	231 (97.1)		
M1	64 (13.5)	57 (24.1)	7 (2.9)		
Stage				11.03	0.012*
I	256 (53.9)	112 (47.3)	144 (60.5)		
II	118 (24.8)	67 (28.3)	51 (21.4)		
III	79 (16.6)	42 (17.7)	37 (15.5)		
IV	22 (4.6)	16 (6.8)	6 (2.5)		

*P<0.05; **P<0.01.

genes in tumor-infiltrating immune cells was evaluated. The results showed that FANCI expression was related to marker genes Cluster of Differentiation (CD) 19 and CD79A in B cells (Figure 11C). The marker genes of neutrophils were CECAM8, ITGAM, and CCR7 (Figure 11D).

Discussion

Cancer is a major global public health problem, and lung cancer has been the most common cause of cancer-related mortality in recent years (5). Standard treatment for lung cancer involves surgical resection, chemotherapy, radiotherapy, and immunotherapy. Despite these treatments, there has been no significant change in the OS rate of LUAD patients (6), and thus, finding new therapeutic targets for treatment is crucial. Through

accurate microarray analysis of tumors, researchers can screen out biomarkers related to tumor occurrence and development for early diagnosis, therapeutic targets, and prognosis evaluation of cancer patients (7).

In the present study, the GO analysis results indicated that DEGs were primarily concentrated in the positive regulation of angiogenesis, positive regulation of cell-substrate adhesion, heparin binding, calcium ion binding, extracellular space, and proteinaceous extracellular matrix. These results are consistent with previously published findings that angiogenesis and cell substrate adherence are closely related to tumor invasion and metastasis (8,9). The KEGG analysis of the DEGs was mainly concentrated in ECM-receptor interaction, malaria, as well as protein digestion and absorption. In addition, previous studies have shown that ECM receptor interactions play a critical

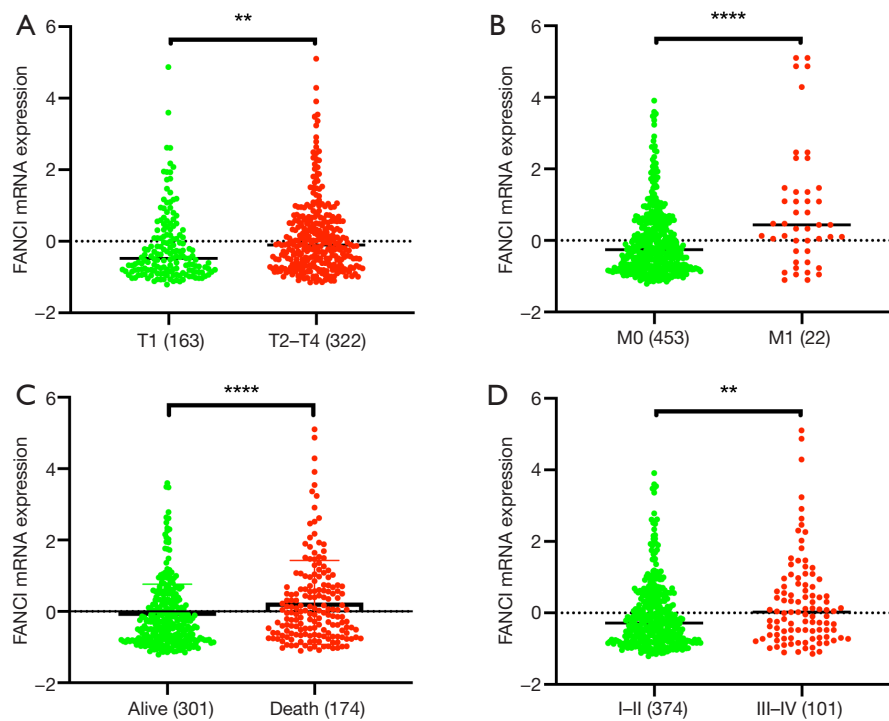


Figure 10 Relationship between the expression of FANCI mRNA and clinicopathological parameters in LUAD in TCGA database. (A) The relationship between FANCI expression and T stage. (B) The relationship between FANCI expression and M stage. (C) The relationship between FANCI expression and survival state. (D) The relationship between FANCI expression and TNM clinical stage. ** $P < 0.01$; **** $P < 0.0001$. LUAD, lung adenocarcinoma.

Table 2 Predicted prognostic value of FANCI in LUAD

Clinicopathological features	Subgroup	Univariate analyses			Multivariate analyses		
		HR	95% CI	P value	HR	95% CI	P value
Gender	Male	1.00	0.67–1.21	0.49			
	Female	0.90					
Age (years)	<60	1.00	0.76–1.48	0.75			
	≥60	1.06					
Tumor size (cm)	<3	1.00	1.20–2.40	0.003**	1.00	0.93–1.91	0.115
	≥3	1.70			1.33		
Lymphatic metastasis	N0	1.00	1.83–3.32	0.000**	1.00	1.63–3.01	0.000**
	N1–N3	2.64			2.22		
Distant metastasis	M0	1.00	1.10–3.42	0.022*	1.00	0.94–2.94	0.081
	M1	1.94			1.66		
FANCI expression	Low	1.00	1.19–2.18	0.002**	1.00	1.02–1.89	0.039*
	High	1.61			1.39		

* $P < 0.05$; ** $P < 0.01$. LUAD, lung adenocarcinoma.

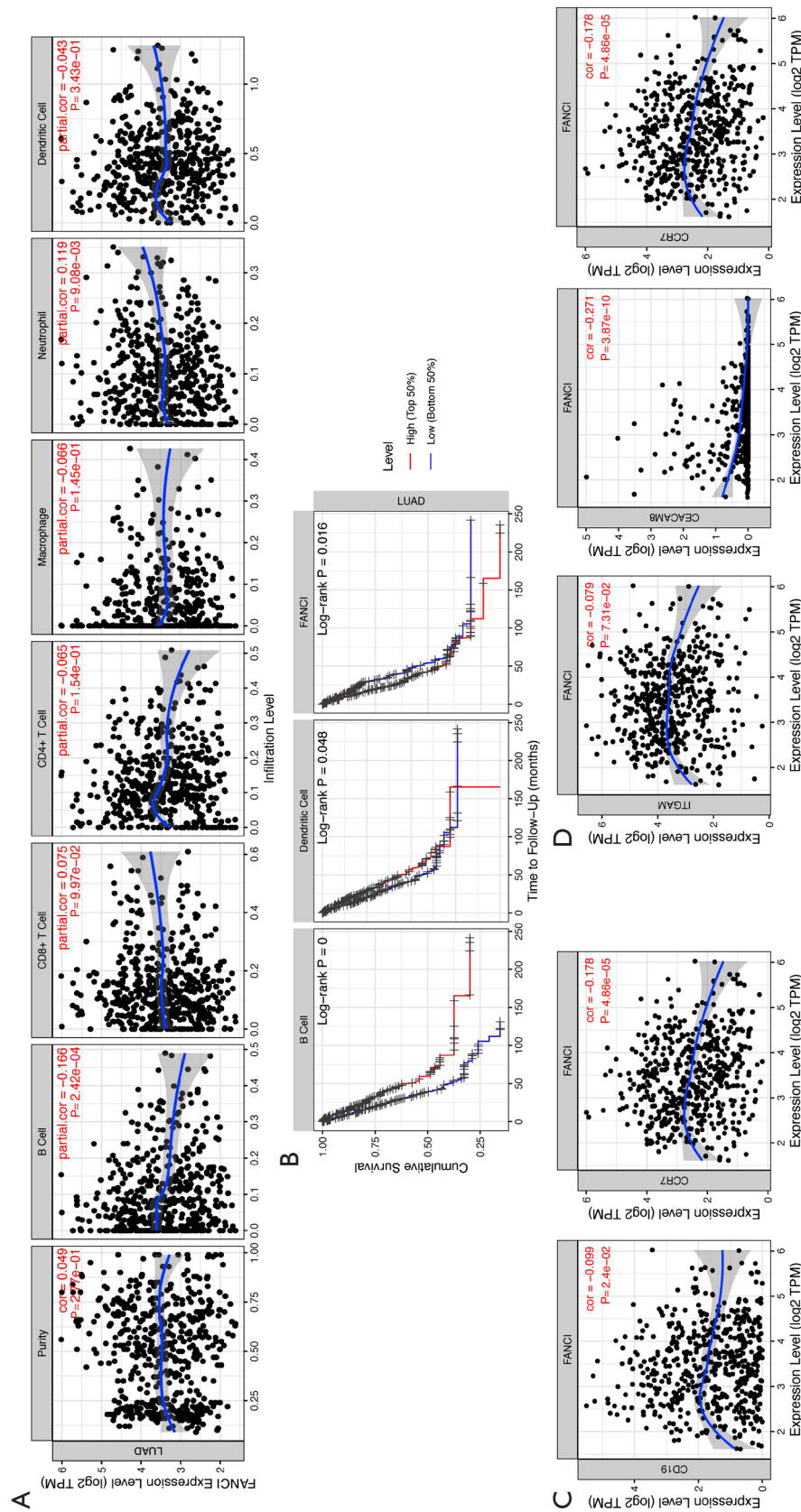


Figure 11 Correlation between FANCI and immune cell infiltration level in LUAD. (A) FANCI expression with six types of immune cell infiltration. (B) The survival of immune cells. (C) FANCI expression with B cell marker genes. (D) FANCI expression with neutrophil marker genes. LUAD, lung adenocarcinoma.

role in tumor progression (10). ECM is upregulated in prostate cancer tissues (11), and is involved in the metastasis of gastric cancer (12). ECM may also promote epithelial-mesenchymal transition (EMT) in colorectal cancer (13). In addition, ECM in the glioblastoma microenvironment is very important for the pathological characteristics of abnormal neovascularization and diffuse tumor cell infiltration (14). These previous studies indicate that ECM is involved in tumor progression and metastasis.

MicroRNAs (miRNAs) are small, noncoding RNA molecules (20–22 nucleotides in length) that regulate gene expression through translation inhibition or mRNA degradation (15). MiRNAs can act as both oncogenes and tumor suppressors by inhibiting the expression of target genes (16). Considerable research has shown that changes in the expression of miRNAs are associated with a variety of cancers, including lung cancer (17).

In total, 2,805 genes were targeted by DEMs that were associated with poor OS for LUAD patients, and 26 DEGs were obtained through the intersection of DEM-targeted genes with DEGs. These 26 DEGs and eight DEMs constituted 30 pairs of miRNA-mRNA regulatory relationships. Among the DEGs in these regulatory relationships, only FANCI was identified as the hub gene. Our bioinformatics prediction showed that miRNA-218 is closely related with FANCI, and may be an upstream miRNA that negatively regulates the mRNA expression of FANCI. Numerous studies have demonstrated that miR-218 is involved in the development of various types of cancer by targeting a single gene, and FANCI also plays an important role in many types of cancer. However, the fact that miR-218 negatively regulates FANCI mRNA expression in LUAD has not been reported previously.

MiR-218 has been shown to be downregulated in various malignancies, playing vital functions similar to tumor suppressor genes. Zhang *et al.* reported that miR-218 inhibits tumor angiogenesis in gastric cancer by targeting Roundabout Guidance Receptor 1 (ROBO1) (18). Furthermore, Ahmadinejad *et al.* demonstrated that the miR-218 is associated with poor prognosis in breast cancer (19). Xu *et al.* found that miR-218 inhibits LUAD progression by directly targeting BMI1 Proto-Oncogene, Polycomb Ring Finger (BMI-1) to regulate the EMT and phosphatidylinositol 3 kinase (PI3K)/protein kinase B (AKT) signaling pathways (20). Our finding that lower expression of miR-218 suppresses LUAD progression is consistent with these reports.

Fanconi anemia (FA) is caused by the mutation of the FA

family protein, and can lead to chromosome instability and other diseases, including cancer (21,22). Abbasi *et al.* showed that FANCA was a breast cancer-susceptibility allele, which is related to familial breast cancer (23). Rockfield *et al.* reported that mutation of both FA Complementation Group D2 (FANCD2) was associated ovarian cancer (24). Zheng *et al.* emphasized that FANCI may act as an oncogene in LUAD and promote cell proliferation via the mitogen-activated protein kinase kinase (MEK)/extracellular regulated protein kinases (ERK)/matrix metalloproteinases (MMPs) pathway in collaboration with Inosine Monophosphate Dehydrogenase 2 (IMPDH2) (25).

In this study, bioinformatics analysis revealed that FANCI is the hub gene of LUAD, and was highly expressed in LUAD samples and associated with metastasis and poor prognosis. According to GEO and HPA databases, FANCI expression in LUAD tissues was higher than in the normal controls at both the mRNA and protein levels. Based on TCGA database, FANCI was correlated with tumor size, distant metastasis, lymphatic metastasis, and stage. In addition, FANCI has also been demonstrated to be an independent factor of LUAD patient prognosis, with higher expression indicating poorer prognosis.

Tumor-infiltrating immune cells in the lung cancer microenvironment can identify and also promote tumor cell apoptosis to inhibit tumor progression. Meanwhile, immune cells can screen tumor cells more suitable for survival in the microenvironment to promote tumor progression (26). A previous study has shown that FANCI is negatively correlated with dendritic cells, and the high expression of FANCI has fewer dendritic cells, leading to fewer T cells. T cell infiltration usually predicts better clinical outcomes in patients (27). In addition, the role of tumor-infiltrating B cells in the tumor microenvironment has also attracted increasing attention. Most studies have shown that B cell infiltration in lung cancer is associated with a good prognosis. This is consistent with the results of this study; the prognosis of high B cell infiltration in LUAD was better than that of low B cell infiltration (28). Our results also showed that the expression of FANCI was negatively correlated with B cells, indicating that the infiltration of B cells was less when FANCI was highly expressed, which may be one of the reasons for the poor prognosis of patients with high FANCI expression. In summary, changes in the immune microenvironment may be the mechanism through which FANCI leads to a poor prognosis in LUAD.

Our study obtained 608 DEGs and 26 DEMs, nine DEM-targeted DEGs, and 30 DEM-DEG pairs. The

ECM-receptor interaction process may be related to LUAD progression. FANCI and its paired miRNA-218 may be critical genes for effective biomarkers to evaluate the poor prognosis of LUAD patients in a timely manner, and to provide potential therapeutic targets. Further analysis confirmed that FANCI was highly expressed in LUAD; we observed that higher expression of FANCI in LUAD is associated with larger tumor size, lymph node metastases, distant metastasis, higher grades, and poorer prognosis. We also identified that FANCI is an independent prognostic factor in LUAD. Therefore, miR-218 may negatively regulate FANCI, promoting metastasis, which leads to late stage and poor prognosis of LUAD by ECM-receptor interaction. The data of this study mainly comes from the published public database and belongs to the exploration of bioinformatics analysis. The deep relationship between miRNA/FANCI and lung adenocarcinoma still needs to be verified by later relevant *in vivo* and *in vitro* experimental studies.

This study showed that 608 DEGs and 26 DEMs were screened out, among which 30 pairs of DEMs composed of 26 DEGs and eight DEMs were identified. As the hub gene of LUAD, FANCI was highly expressed in LUAD. MiR-218 could target FANCI, and FANCI expression was negatively correlated with miR-218 expression, which may be the upstream miRNA negatively regulating the expression of FANCI mRNA. FANCI expression was found to be related to tumor size, lymphatic metastasis, distant metastasis, and stage, and was also an independent predictor of patient prognosis. Enrichment pathway analysis indicated that ECM receptor interaction may be closely related to the progression of LUAD. FANCI may be a key gene to determine the metastasis and poor prognosis in patients with LUAD, and is expected to be a new therapeutic target for LUAD. The existence of these targeted relationships requires further study, which may elucidate the mechanisms of LUAD metastasis and provide new methods for future diagnosis and treatment exploration.

Conclusions

As described above, we speculate that miR-218 may negatively regulate FANCI via ECM receptor interaction, leading to metastasis and poor prognosis of LUAD. FANCI may be a key gene to determine the metastasis and poor prognosis in patients with LUAD. Changes in the immune microenvironment may be the mechanism through which FANCI leads to a poor prognosis of LUAD.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://dx.doi.org/10.21037/atm-21-3823>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University (No. 2019-KY-255), and the patients and their families agreed to the tissue samples collection and their use in research.

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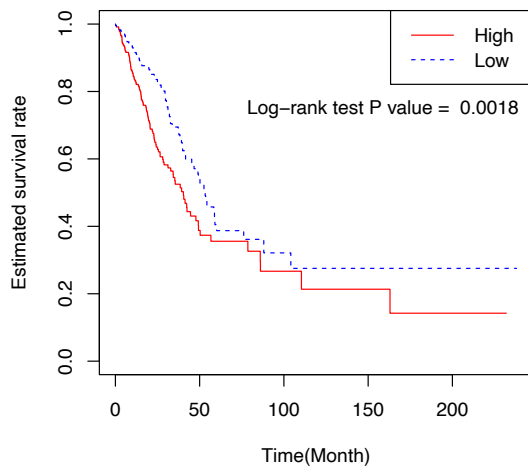


Figure S1 The overall survival of the FANCI high-expression group and the low-expression group in LUAD based on TCGA. LUAD, lung adenocarcinoma.

Table S1 The up/downregulated DEMs in GSE63805

Up/down regulated	DEMs	adj.P.Val	P value	logFC
Upregulated	hsa-miR-21	5.64E-13	1.21E-15	2.04448
	hsa-miR-96	6.41E-10	4.37E-12	1.89378
	hsa-miR-135b	1.98E-08	1.89E-10	1.84985
	hsa-miR-193b	9.22E-07	1.38E-08	1.0178
	hsa-miR-183	1.19E-06	1.95E-08	1.42641
	hsa-miR-1979	4.45E-06	7.88E-08	1.07789
	hsa-miR-130b	3.30E-05	6.29E-07	1.21872
	hsa-miR-31	4.20E-05	1.09E-06	1.4055
	hsa-miR-9	5.34E-05	1.45E-06	2.20422
	hsa-miR-429	2.77E-04	8.67E-06	1.12272
	hsa-miR-141	5.49E-04	1.92E-05	1.0451
	hsa-miR-891a	5.49E-04	2.02E-05	1.03588
	hsa-miR-1246	9.02E-04	4.14E-05	1.7101
	hsa-miR-210	9.34E-04	4.58E-05	1.02295
	hsa-miR-1290	1.12E-02	7.17E-04	1.00711
	hsa-miR-375	1.31E-02	9.16E-04	1.03906
	Downregulated	hsa-miR-451	5.64E-13	1.54E-15
hsa-miR-30a		2.25E-10	9.21E-13	-1.58344
hsa-miR-126		2.58E-10	1.41E-12	-1.68116
hsa-miR-520e		7.43E-09	6.07E-11	-2.27211
hsa-miR-30d		4.07E-08	4.44E-10	-1.28665
hsa-miR-30b		3.93E-07	4.82E-09	-1.16183
hsa-miR-144		7.28E-07	9.91E-09	-1.70085
hsa-miR-145		3.43E-05	7.25E-07	-1.18285

DEM, differentially expressed microRNA.