Technology in Cancer Research & Treatment
Volume 18: 1-11

DOI: 10.1177/1533033819875130
journals.sagepub.com/home/tct
SAGE

Possible Molecular Mechanisms for the Roles of MicroRNA-21 Played in Lung Cancer

Qiang Wang, MD[1] and Linyou Zhang, MD, PhD[2]

Abstract

Background: We aimed to find the possible molecular mechanisms for the roles of microRNA-21 underlying lung cancer development. **Methods:** MicroRNA-21-5p inhibitor was transfected into A549 cells. Total RNA was isolated from 10 samples, including 3 in control group (A549 cells), 3 in negative control group (A549 cells transferred with microRNA-21 negative control), and 4 in SH group (A549 cells transferred with microRNA-21 inhibitor), followed by RNA sequencing. Then, differentially expressed genes were screened for negative control group versus control group, SH group versus control group, and SH group versus negative control group. Functional enrichment analyses, protein–protein interaction network, and modules analyses were conducted. Target genes of hsa-miR-21-5p and transcription factors were predicted, followed by the regulatory network construction. **Results:** Minichromosome maintenance 10 replication initiation factor and cell division cycle associated 8 were important nodes in protein–protein interaction network with higher degrees. Cell division cycle associated 8 was enriched in cell division biological process. Furthermore, maintenance 10 replication initiation factor and cell division cycle associated 8 were significantly enriched in cluster 1 and micro-RNA-transcription factor-target genes regulating network. In addition, transcription factor Dp family member 3 (transcription factor of maintenance 10 replication initiation factor and cell division cycle associated 8) and RAD21 cohesin complex component (transcription factor of maintenance 10 replication initiation factor) were target genes of hsa-miR-21-5p. **Conclusions:** Micro-RNA-21 may play a key role in lung cancer partly via maintenance 10 replication initiation factor and cell division cycle associated 8. Furthermore, microRNA-21 targeted cell division cycle associated 8 and then played roles in lung cancer via the process of cell division. Transcription factor Dp family member 3 and RAD21 cohesin complex component are important transcription factors in microRNA-21-interfered lung cancer.

Keywords

lung cancer, microRNA-21, differentially expressed genes, transcription factors, biological process

Abbreviations

cDNA, complementary DNA; DEGs, differentially expressed genes; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; miRNA, microRNAs; mRNA, messenger RNA; NC, negative control; NSCLC, nonsmall cell lung carcinoma; PPI, protein–protein interaction; qPCR, quantitative PCR; RT-PCR, reverse transcription polymerase chain reaction; SCLC, small cell lung carcinoma; TF, transcription factor.

Received: January 01, 2019; Revised: June 11, 2019; Accepted: August 15, 2019.

Introduction

Lung cancer, also called lung carcinoma, is the most common cause of major cancer incidence and mortality in men, whereas in women it is the third most common cause of cancer incidence and the second most common cause of cancer mortality.[1] Lung cancer can be mainly divided into 2 subtypes: nonsmall cell lung carcinoma (NSCLC) and small cell lung carcinoma (SCLC).[2] Shortness of breath, coughing (including coughing up blood), chest pains, and weight loss are the most common symptoms of lung cancer.[3] It is reported that long-term tobacco smoking is the cause of the vast majority (85%) of cases.[4] In

[1] Department of Orthopedics, The Second Affiliated Hospital of Harbin Medical University, Harbin, China
[2] Department of Thoracic Surgery, The Second Affiliated Hospital of Harbin Medical University, Harbin, China

Corresponding Author:
Linyou Zhang, Department of Thoracic Surgery, The Second Affiliated Hospital of Harbin Medical University, Harbin 150086, China.
Email: wenqiangL8@163.com

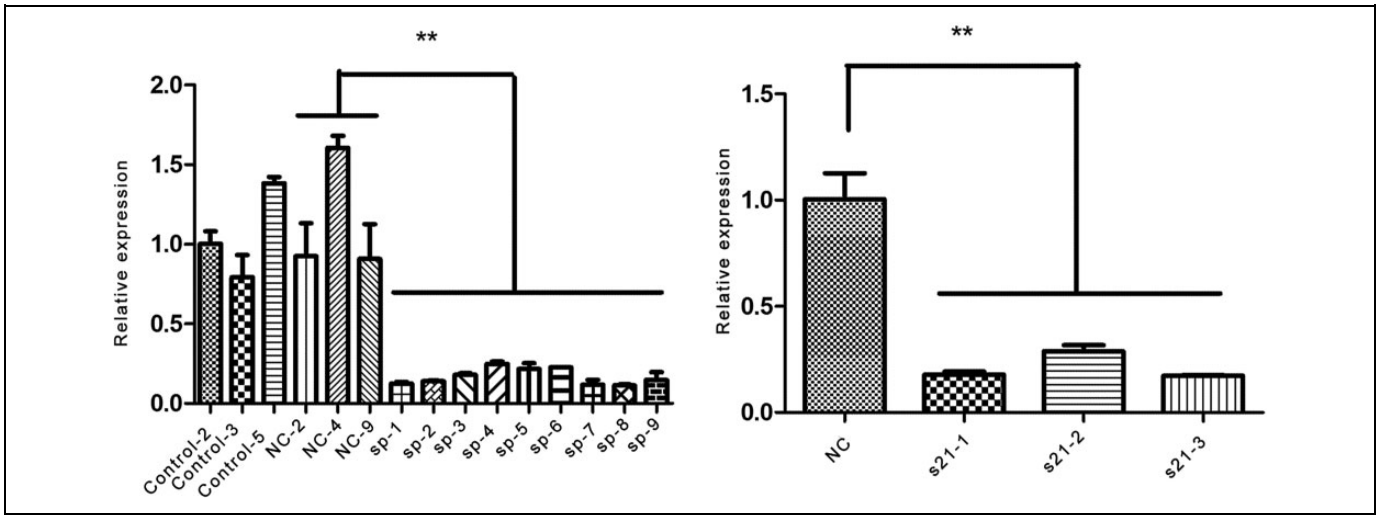

Figure 1. The results for qualitative polymerase chain reaction (qPCR) validation. Control, A549 cells (x_2, x_3, and x_5); negative control (NC), A549 cells + miRNA-21 negative control (nc_2, nc_4, and nc_9); SH, A549 cells+miRNA-21 inhibitor (s21_1, s21_2, s21_3, and sp_1- sp_9). $^{**}P < .01$, compared to NC group.

addition, it was estimated in 2011 that about 1 608 800 new cases were diagnosed with lung cancers, and there will be 1 387 400 deaths worldwide.[1] Common treatments for this cancer include surgery, chemotherapy, palliative care, and radiation therapy.[5] But the efficacy of these treatments was not very satisfactory. It is noteworthy that targeted therapy for lung cancer is playing more and more important roles in recent years.

MicroRNAs (miRNAs), an abundant class of small nonprotein-coding RNAs, can regulate the expression of genes via targeting messenger RNAs (mRNAs) and triggering either mRNA cleavage or translation repression.[6] Some previous studies suggested that some specific miRNAs were associated with cancers and could be regarded as therapy targets. For example, Kim et al indicated that miR-31 functioned as a tumor suppressor and could be regarded as a novel target for the treatment of liver cancers,[7] and Lim et al suggested that miR-494 regulated G1/S transition in liver tumorigenesis and was identified as a therapeutic target for the treatment of hepatocellular carcinoma .[8] Furthermore, many studies reported the associations between miRNA-21 and lung cancers. Yang et al indicated that miRNA-21 can predict recurrence and poor survival in NSCLC.[9] MicroRNA-21 overexpression shorted survival time in human primary squamous cell lung carcinoma, suggesting miRNA-21 might be a diagnostic and prognostic marker for this disease.[6] In cancer stem-like cells, abnormal expression of miRNA-95 and miRNA-21 was related to radioresistance of lung cancer.[10] Although many studies reported the roles of miRNA-21 played in lung cancer, the exact molecular mechanism of this process was not fully be understood.

In the present study, miRNA21-5p inhibitor was transfected into A549 cells. Then, RNA was extracted, and reverse transcription polymerase chain reaction (RT-PCR) was conducted. After obtaining good results, mRNA sequencing was performed. After that, a series of bioinformatics analyses were conducted to find the possible molecular mechanism for the roles of miRNA-21 played in lung cancer.

Materials and Methods

Cell Culture and MiRNA Inhibitor Transfection

A549 cells obtained from Shanghai Cell Bank, Chinese Academy of Sciences, were maintained in Dulbecco Modified Eagle Medium at 37°C under 5% CO_2 environment. The miRNA 21 inhibitor UCAACAUCAGUCUGAUAAGCUA was synthetized by Shanghai tuoran biotechnology co, Ltd (Shanghai, China). After digestion by pancreatin, cells were seeded on 12-well plates (2.5×10^5cells/well) and cultured. When cells grew to 80%, cells were transferred with 100 nmol/L miRNA21 inhibitor and lipofectamine 2000 (Invitrogen #11668-027; Thermo Fisher Scientific, Inc, Hudson, NY) at 37°C under 5% CO_2 for 24 hours.

Quantitative RT-PCR

Total RNA of transferred A549 cells were isolated by using TRIzol (Takara, #9109). MicroRNA was extracted by miRcute miRNA isolation kit DP501 (TianGen, Beijing, China). The first strand of miRNA complementary DNA (cDNA) was reversely transcribed using miRNA cDNA synthesis kit KR211 (TianGen).

Then, the quantitative PCR reaction solution was prepared in accordance with the following reaction system: 1 μL forward primer 10 μmol/L, 10 μL SYBR Premix EX Taq (2×), 1 μL reverse primer 10 μmol/L, and 8 μL cDNA. The qPCR reaction was conducted by the following steps: 50°C for 3 minutes, 40 cycles of 95°C for 3 minutes, 95°C for 10 seconds, and 60°C for 30 seconds. Finally, melt curve analysis was performed in 60°C to 95°C using increments of 0.5°C per 10 seconds. U6 was used as the internal control. The primer sequences were listed as follows: U6 forward, 5′-CTCGCTTCGGCAGCACA-3′ and reverse, 5′-AACGCTTCACGAATTTGCGT-3′; miR-21-5p forward, 5′-TAGCTTATCAGACTGATGTTGA-3′ and reverse, 5′-GCTGTCAACGATACGCTACCTA-3′.

Table 1. Quality Control of Reads After Sequencing.

Sample	Sample_Name	Clean Read	Clean Reads Q20	Clean Read Q20(%)	Clean Reads Mean Length
nc_2	NC-2_HGVJ2ALXX_L4_1	16026528	16020668	99.96%	150
	NC-2_HGVJ2ALXX_L4_2	16026528	15781993	98.47%	150
nc_4	NC-4_HGVJ2ALXX_L4_1	15206813	15200892	99.96%	150
	NC-4_HGVJ2ALXX_L4_2	15206813	14961140	98.38%	150
nc_9	NC-9_HGVJ2ALXX_L4_1	15208792	15203236	99.96%	150
	NC-9_HGVJ2ALXX_L4_2	15208792	14984526	98.53%	150
x_2	X-2_HCT2KALXX_L7_1	15803281	15796996	99.96%	150
	X-2_HCT2KALXX_L7_2	15803281	15378016	97.31%	150
x_3	X-3_HGVJ2ALXX_L4_1	16598693	16592693	99.96%	150
	X-3_HGVJ2ALXX_L4_2	16598693	16266517	98.00%	150
x_5	X-5_HGVJ2ALXX_L3_1	13859387	13853611	99.96%	150
	X-5_HGVJ2ALXX_L3_2	13859387	13634255	98.38%	150
s21_1	s21-1_HKNMGALXX_L7_1	16542732	16537048	99.97%	150
	s21-1_HKNMGALXX_L7_2	16542732	16154034	97.65%	150
s21_2	s21-2_HKNMGALXX_L8_1	15197794	15190364	99.95%	150
	s21-2_HKNMGALXX_L8_2	15197794	14831723	97.59%	150
s21_3	s21-3_HKNMGALXX_L8_1	15858298	15850436	99.95%	150
	s21-3_HKNMGALXX_L8_2	15858298	15553036	98.08%	150
sp_9	sp-9_HGVJ2ALXX_L3_1	13972891	13966835	99.96%	150
	sp-9_HGVJ2ALXX_L3_2	13972891	13735401	98.30%	150

Statistical Analysis

All the data were presented as the mean ± standard errors of the mean. SPSS 22.0 was applied for the statistical analyses. The difference between groups were compared by *t* test. $P < .05$ was set as a significant difference.

Transcriptome Sequencing

Total RNA was isolated from 10 samples, including 3 in control group (A549 cells), 3 in negative control (NC) group (A549 cells transferred with miRNA-21 negative control), and 4 in SH group (A549 cells transferred with miRNA-21 inhibitor). The sequencing library preparations were performed by using RNA Library Prep Kit (NEB) following the manufacturer's instruction. Then, the library preparations were sequenced on an Illumina Hiseq platform with 25 bp/150 bp paired-end reads generation. Quality control was performed using Trimmomatic (version 3.6).[11]

Clean reads were mapped to human reference genome (hg38, UCSC)[12] by Hisat2 software (version 2.0.5)[13] using default parameter. Based on human genome annotation information provided by Gencode database,[14] the corresponding read count was obtained using StringTie (version 1.2.3).[15] Furthermore, quantification for the results was conducted by counts per millions.

Differentially Expressed Genes Analysis

Differentially expressed genes (DEGs) were screened for NC group versus control group, SH group versus control group, and SH group versus NC group using edgeR in R.[16] |logFC (fold change)|>2 and FDR (false discovery rate) < 0.01 were set as threshold value. Furthermore, VENN analysis was performed for DEGs among 3 groups.

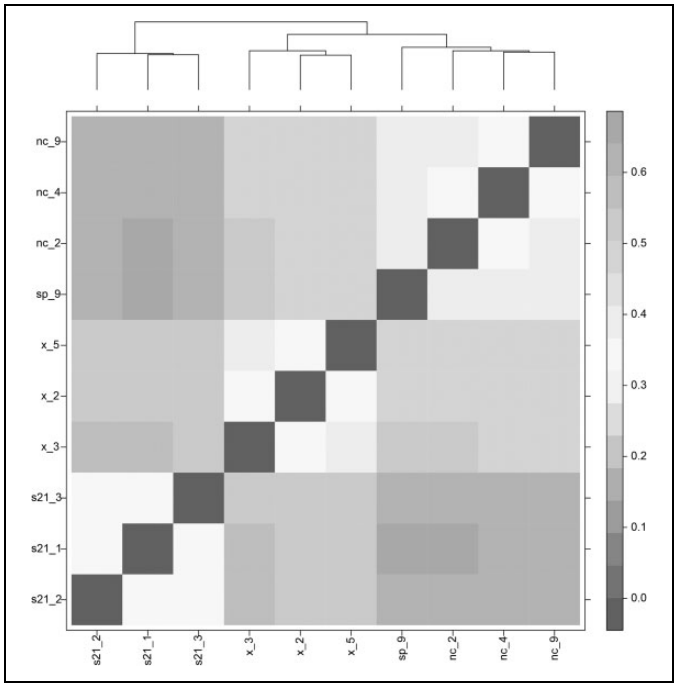

Figure 2. Cluster analysis for samples.

Kyoto Encyclopedia of Genes and Genomes Pathway Enrichment Analyses

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses for 3 groups' DEGs were conducted using clusterprofiler.[17] *P* value <.05 was set as cutoff criteria.

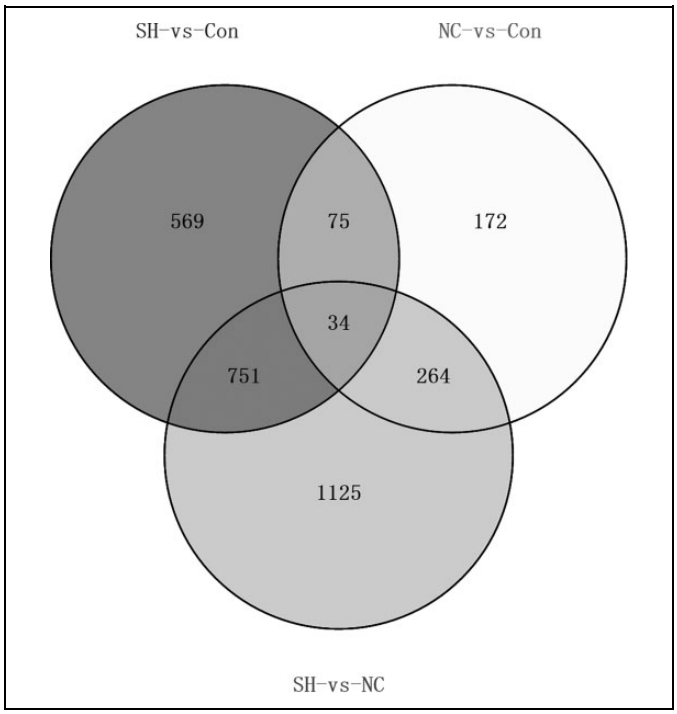

Figure 3. The VENN diagram for differentially expressed genes in negative control (NC) group versus control group, SH group versus control group, and SH group versus NC group.

Protein–Protein Interaction Network Analyses

The DEGs in SH group versus control group and SH group versus NC group were integrated, and PPI network analyses were performed by STRING.[18] Required confidence (combined score) >0.7 was regarded as threshold value. Protein–protein interaction (PPI) network was constructed using Cytoscape,[19] and the topologies of networks were analyzed. Based on the ranking of the degree of network nodes, hub nodes in PPI network were obtained.

Modules Analyses

Subnetwork modules analyses were carried out by MCODE.[20] DAVID (version 6.8)[21] was used for gene ontology (GO) and KEGG pathway analysis for the genes in modules. Parameter-enriched count ≥ 2 and P value <.05 were set as significantly enriched results.

Prediction of Target Genes of hsa-miR-21-5p

MiRwalk2 database[22] was used to predict the target genes of hsa-miR-21-5p. The module "predicted target module" was used, and miRNA gene pairs were existed in at least 4 of 6 databases (miRWalk, miRanda,[23] miRDB,[24] miRMap,[25] RNAhybrid,[26] and Targetscan[27]). The DEGs were further screened, and regulatory network between hsa-miR-21-5p and differentially expressed target genes was constructed.

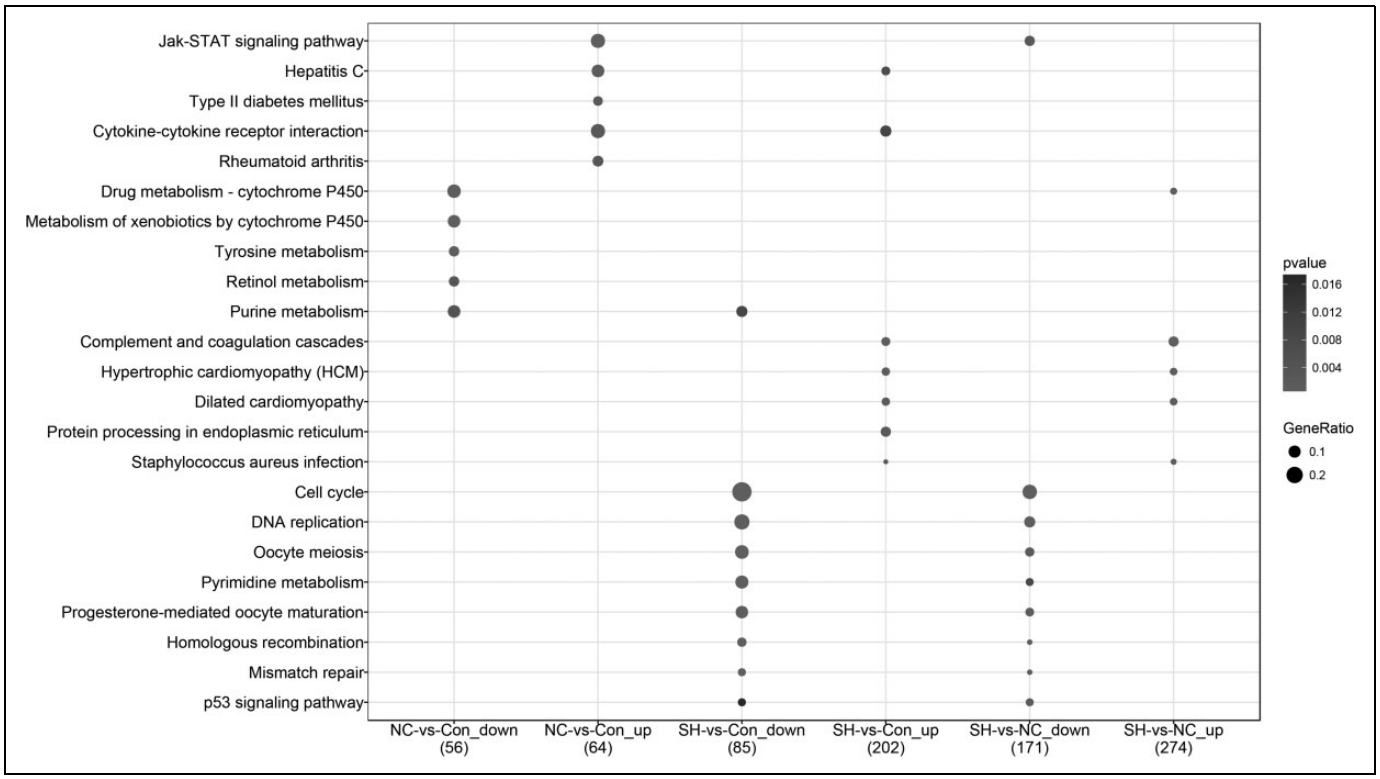

Figure 4. The significant pathways for Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. P value indicates significance enriched in this term; count, the number of genes enriched in this term; GeneRatio, the ratio of the number of DEGs enriched in KEGG term to the number of genes for this pathway recorded in KEGG database.

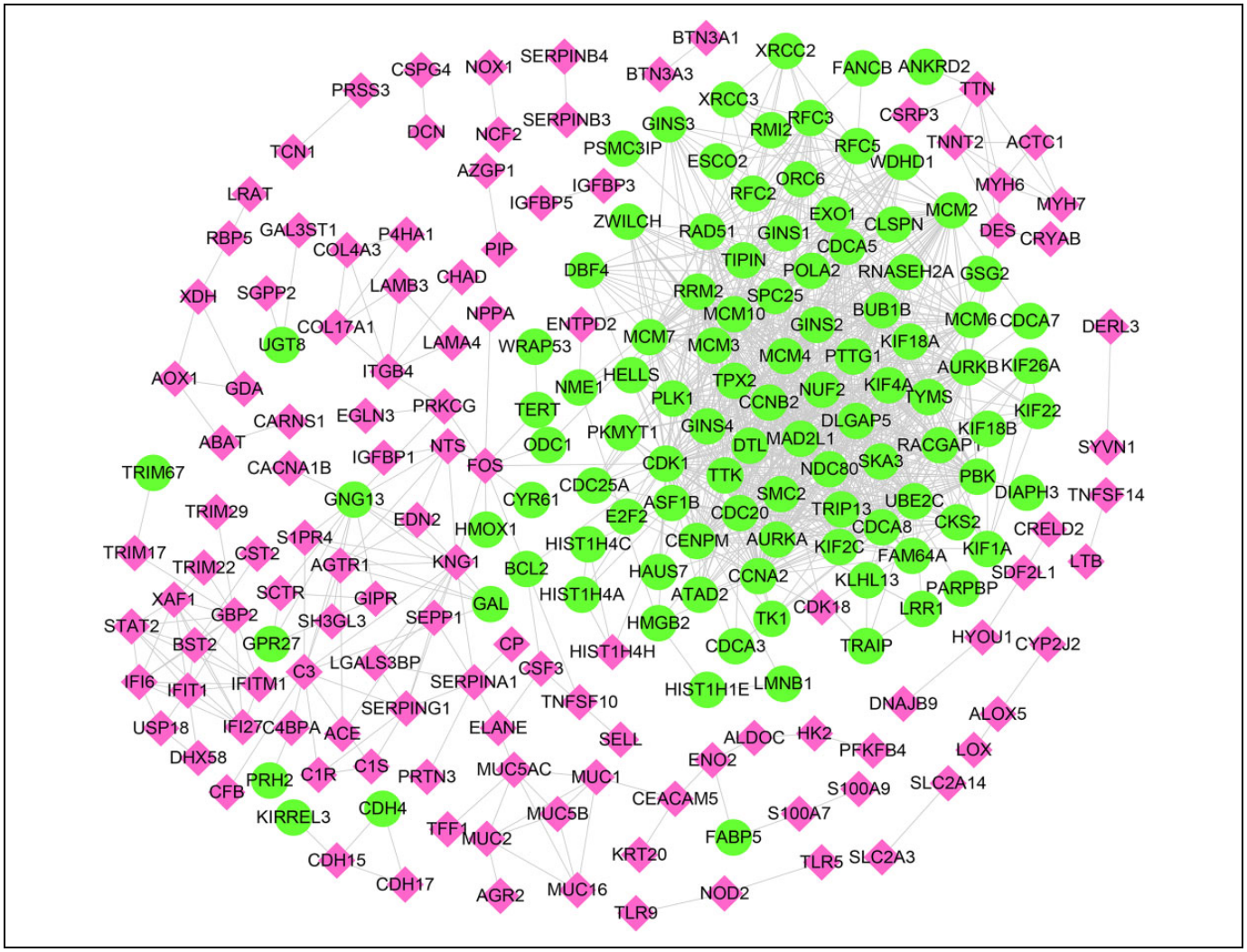

Figure 5. The protein–protein interaction (PPI) network for differentially expressed genes (DEGs) in SH group vs control group and SH group vs negative control (NC) group. Light diamond nodes indicates upregulated genes; deep circle nodes, downregulated genes.

The differentially expressed targets of hsa-miR-21-5p were subjected to GO function and KEGG pathway analysis. P value <.05 and count ≥ 2 were set as the cutoff values.

Transcription Factor Analysis

IRegulon plugin[28] in cytoscape is used to predict transcription factors (TFs) that regulated target genes of hsa-miR-21-5p. IRegulon was used to predict TFs by calculating TFs and genes binding motif enrichment analysis. Motif enrichment analysis used several position weight matrix to sort and score for each motif. Preferred motif was used for predicting final TFs. Minimum identity between orthologous genes = 0.05 and maximum false discovery rate on motif similarity = 0.001. The TF-target gene pairs with normalized enrichment score >4 were captured. The collected TFs could be the targets for hsa-miR-21-5p or regulators for DEGs.

Results

Quantitative PCR Validation of Transfection

The results for qPCR validation of transfection are presented in Figure 1. When we performed quality testing for the first validated successful samples, some samples were unqualified, and library sequencing could not be conducted. Thus, we added 3 miR-21 inhibitor transfection groups in the second experiments. Eventually, in total, 3 groups containing 10 samples were included in the present study as follows: control group, A549 cells (x_2, x_3, and x_5); NC group, A549 cells + miRNA-21 negative control (nc_2, nc_4, and nc_9); and SH group, A549 cells + miRNA-21 inhibitor (s21_1, s21_2, s21_3, and sp_9). The expression of miRNA-21 was significantly lower in SH group compared to NC group (P <0.01), which suggested that the miRNA-21 expression was effectively inhibited by miRNA-21 inhibitor.

Sequencing Analysis

A total of 10 samples were subjected to RNA sequencing. In order to obtain the clean reads with high quality, quality control was conducted and Q20 was calculated. As shown in Table 1, more than 2.7 million clean reads were obtained for each sample. In addition, more than 97.31% clean reads had Q20 quality, suggesting that the reads obtained had high quality. The raw sequencing data were deposited in NCBI Sequence Read Archive database with the accession number of SRP199405.

Gene Expression Analysis

The correlations among samples were calculated by Euclidean distance method, and cluster analysis for samples is presented in Figure 2. The figure showed that the correlation between sp_9 and other samples was relatively low; thus, we deleted this sample in the following analysis.

Analysis of DEGs

In total, 2990 DEGs were identified, including 545 DEGs in the NC group versus control group, 1429 DEGs in SH group versus control group, and 2174 DEGs in SH group versus NC group. The VENN diagram for 3 comparison groups is shown in Figure 3. There were 34 overlapped DEGs among 3 groups.

Kyoto Encyclopedia of Genes and Genomes Pathway Enrichment Analyses

The results for KEGG pathway enrichment analyses are presented in Figure 4. The downregulated genes for SH group versus control group and SH group versus NC group were mainly enriched in pathways associated with cancers, and upregulated genes for these 2 comparison groups were mainly enriched in pathways related to cardiomyopathy.

Protein–Protein Interaction Network Analysis

The PPI network for DEGs in SH group versus control group and SH group versus NC group is presented in Figure 5. A total of 222 nodes and 1112 interaction pairs were included in this network. The top 15 nodes with higher degrees in the network were cyclin dependent kinase 1, cyclin A2, mitotic arrest deficient 2 like 1, cyclin B2, aurora kinase B, cell division cycle 20, minichromosome maintenance complex component 4, ribonucleotide reductase regulatory subunit M2, minichromosome maintenance complex component 2, kinesin family member 2C, TTK protein kinase, minichromosome maintenance 10 replication initiation factor (MCM10), cell division cycle associated 8 (CDCA8), NDC80, kinetochore complex component, and denticleless E3 ubiquitin protein ligase homolog (Table 2). The hub nodes in PPI may play key roles in lung cancer development.

Table 2. The Top 15 Nodes With Higher Degrees in the Network.

Gene	Degree	Regulator
CDK1	57	Down
CCNA2	49	Down
MAD2L1	48	Down
CCNB2	47	Down
AURKB	45	Down
CDC20	45	Down
MCM4	45	Down
RRM2	44	Down
MCM2	43	Down
KIF2C	42	Down
TTK	41	Down
MCM10	40	Down
CDCA8	40	Down
NDC80	39	Down
DTL	39	Down

Abbreviations: AURKB, aurora kinase B; CCNA2, cyclin A2; CCNB2, cyclin B2; CDCA8, cell division cycle associated 8; CDC20, cell division cycle 20; CDK1, cyclin dependent kinase 1; DTL, denticleless E3 ubiquitin protein ligase homolog; KIF2C, Kinesin family member 2C; MAD2L1, mitotic arrest deficient 2 like 1; MCM2, minichromosome maintenance complex component 2; MCM4, minichromosome maintenance complex component 4; MCM10, minichromosome maintenance 10 replication initiation factor; NDC80, kinetochore complex component; RRM2, ribonucleotide reductase regulatory subunit M2; TTK, TTK protein kinase.

Modules Analyses

Genes with similar function can be clustered in 1 module. The results for modules analyses showed that the score of cluster 1 was 29.062, which was higher than other modules. There were 33 nodes, and 465 interaction pairs were included in cluster 1 (Figure 6A). The GO and KEGG pathway enrichment analyses showed that genes in cluster 1 were significantly enriched in protein binding (eg, MCM10, CDCA8), nucleus (eg, MCM10, CDCA8), cell division (eg, CDCA8), and cell cycle pathway (Figure 6B).

MiRNA-TF-Target Genes Regulating Network

A total of 43 miRNA-target genes pairs were obtained. We obtained 3 TFs (TFDP3, RAD21, and IL24) by predicting TFs of these target genes; TFDP3 and RAD21 were target genes of hsa-miR-21-5p (Figure 7), and MCM10 and CDCA8 were 2 target genes of hsa-miR-21-5p.

In order to explore the biological function involved with target genes of hsa-miR-21-5p, 43 target genes were subjected to GO and KEGG pathway analysis. The target genes were significantly enriched in 42 GO BP terms, 14 GO MF terms, and 7 KEGG pathways. Figure 8 exhibits the top 10 terms for GO BP, GO MF and pathways. The target genes were closely related to anion binding, cell growth, response to transition metal nanoparticle, focal adhesion, and p53 signaling pathway.

Discussion

Lung cancer has been a health threat for the high morbidity and mortality. The miRNAs played pivotal roles in gene expression

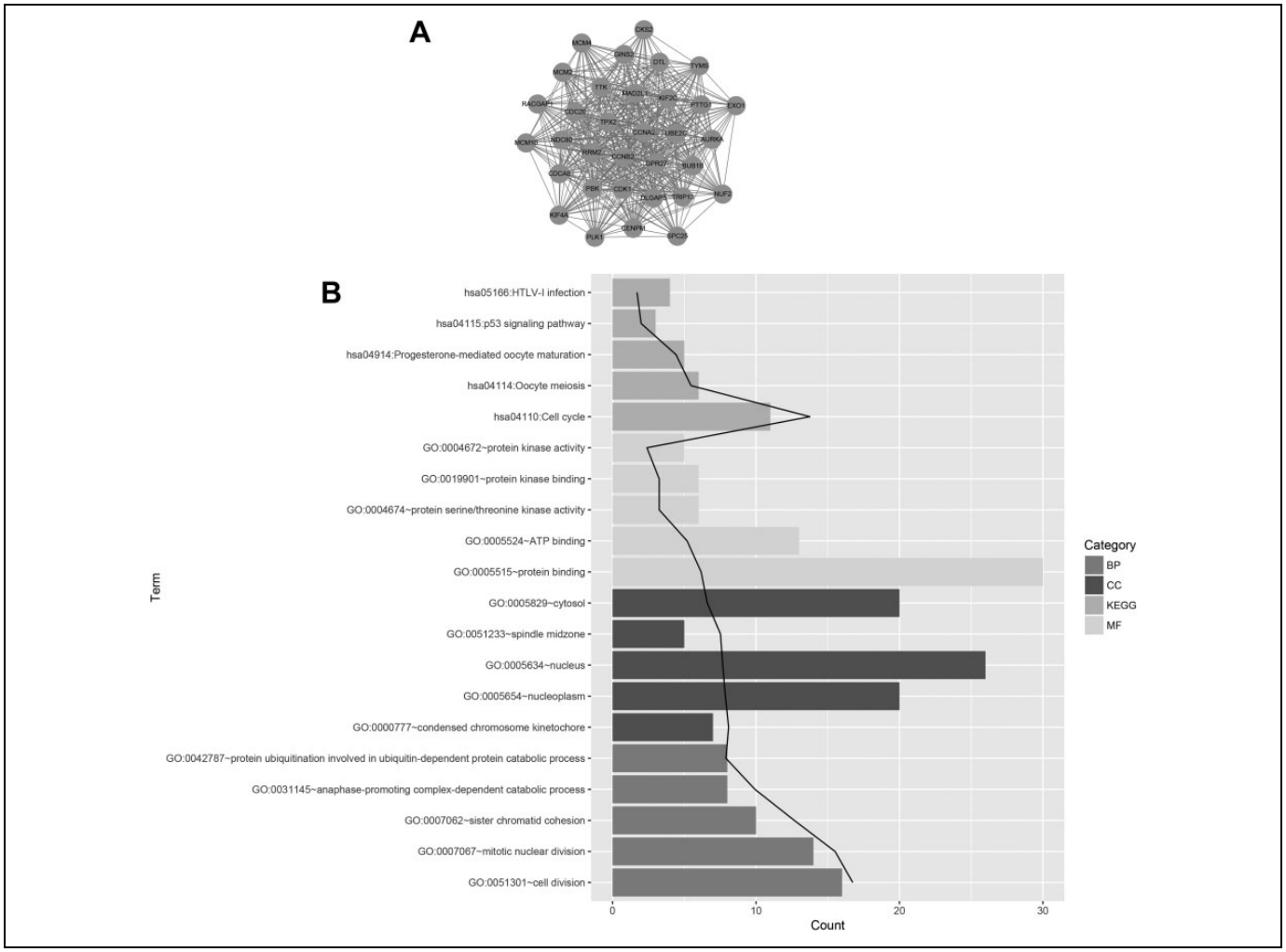

Figure 6. Subnetwork module, cluster 1, (A) and functional enrichment analyses for it (B). Category: gene ontology (GO) category and Kyoto Encyclopedia of Genes and Genomes (KEGG); BP indicates biological process; CC, cellular component; MF, molecular function; Term, GO function term or KEGG pathway; Count, the number of genes enriched in this term; trend line, −log10 (*P* value).

regulation, and their aberrant expression is implicated in tumorigenesis and progression.[29] MiRNA-21 has been proposed to be a biomarker for diagnosis, prognosis, and response to therapy.[30] However, the mechanism related to the role of miRNA-21 in lung cancer has not been clarified clearly. In the present study, the DEGs related to miRNA-21 inhibition were identified by RNA sequencing. Our data suggested that MCM10 and CDCA8 were significantly affected by miRNA-21 inhibition in A549 cells, which were hub nodes in PPI network with higher degrees; MCM10 was significantly enriched in G1/S transition of mitotic cell cycle (GO-BP), cell proliferation (GO-BP), and DNA replication (GO-BP). Cell division cycle associated 8 was closely related to cell division (GO-BP). Furthermore, these 2 nodes were significantly enriched in cluster 1 and miRNA-TF-target genes regulating network. In addition, TFDP3 (TF of MCM10 and CDCA8) and RAD21 (TF of MCM10) were target genes of hsa-miR-21-5p.

Minichromosome maintenance 10 replication initiation factor, chromatin-associated protein, is important for efficient DNA replication origin firing in human cells.[31,32] Minichromosome maintenance 10 replication initiation factor contributes to genetic diseases related to genome instability and aberrant proliferation, such as cancer.[33] Increased transcript levels of MCM10 are related to the development of some cancers, indicating that MCM10 could be the target of specific oncogenes.[34,35] A previous study indicated that the overexpression of MCM10 was associated with adverse prognosis in urothelial carcinoma.[36] Das et al suggested that the expression level of MCM10 was correlated with stages of cervical carcinogenesis.[37]

Multiple genetic changes underlying the evolution of cancer cell development drive the deregulation of cell cycle, cell proliferation, and DNA replication. Uncontrolled cell cycle is the most common characteristic for human cancers.[38] The mitogenic signaling and responses to antimitogenic signals were altered in tumor cells, which results in unscheduled proliferation of tumor cells.[39,40] G1 is one phase of cell cycle, in which there are a flood of cell signals that determine the cell division,

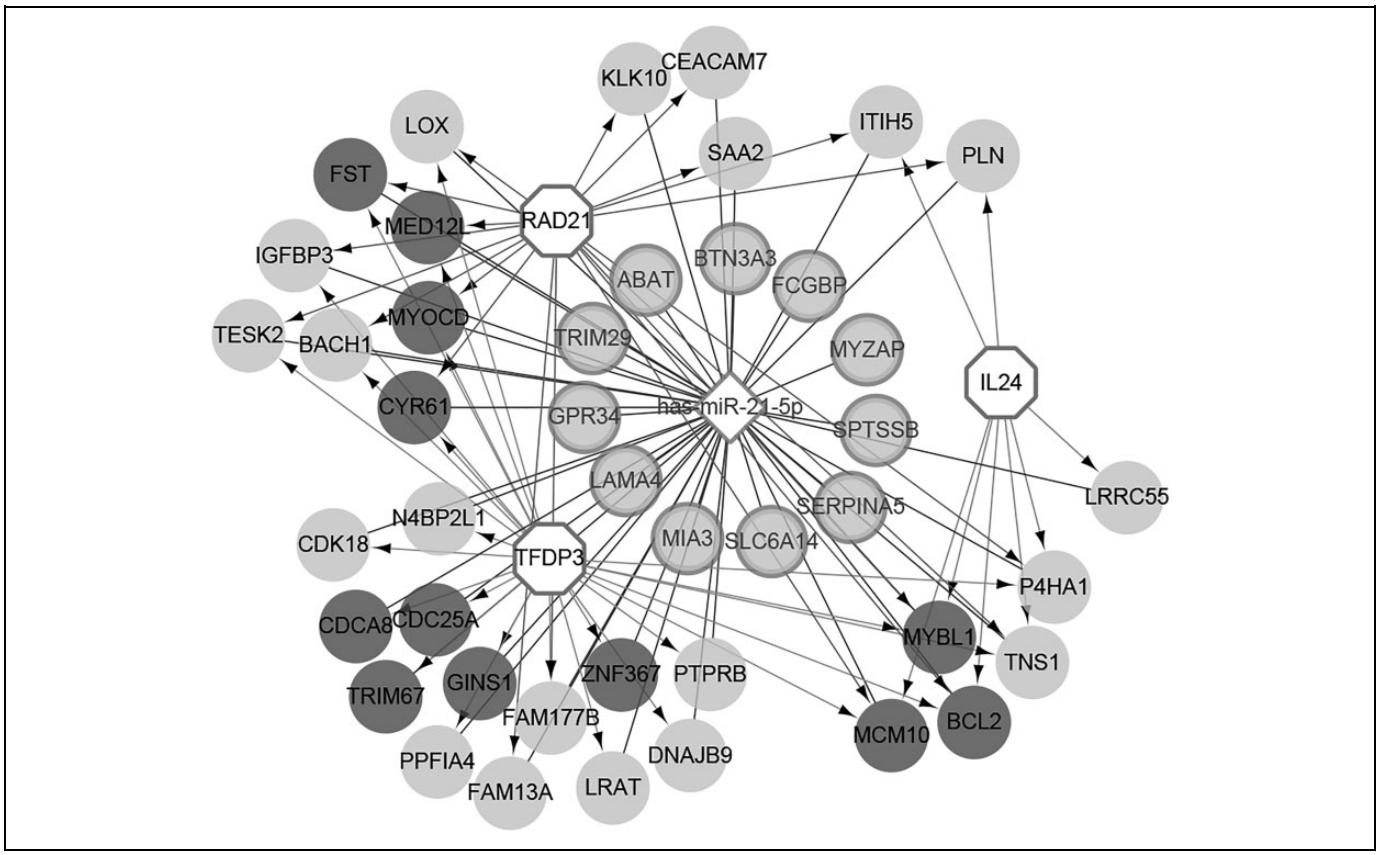

Figure 7. MicroRNA (miRNA)-transcription factor (TF)-target genes regulating network. Diamond nodes in the middle: hsa-miR-21-5p; polygon nodes: TF; light round nodes: up-regulated genes; deep round nodes: downregulated genes; round nodes with frame: genes only regulated by miRNA; arrow: TF-target genes regulatory relationship; straight line: miRNA-target genes regulatory relationship.

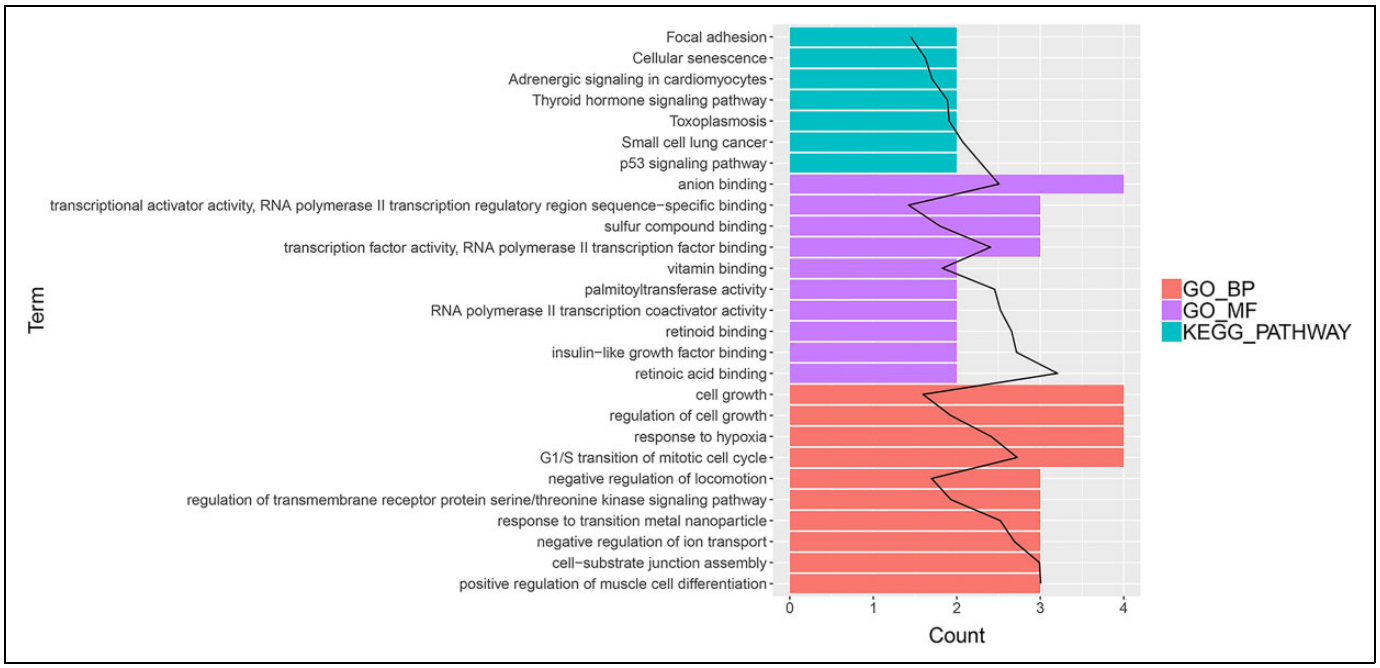

Figure 8. The top 10 GO function terms and pathways for target genes of hsa-miR-21-5p.

cell survival, and cell growth. The mistakes in G1 signaling can cause cancer development and progression. The GO function enrichment analysis showed that MCM10 was significantly enriched in G1/S transition of mitotic cell cycle (GO-BP), cell proliferation (GO-BP), and DNA replication (GO-BP). In our present study, MCM10 was found to be one important node in PPI network with higher degrees and miRNA- TF-target genes regulating network. Taken together, MCM10 plays a key role in cell cycle–related biological process. Furthermore, in the present study, CDCA8 was also one important node in PPI network with higher degrees and in miRNA-TF-target genes regulating network. It is reported that CDCA8, also known as borealin, is a component of a chromosomal passenger complex essential for stability of the bipolar mitotic spindle.[41] The study of Hayama et al showed that CDCA8 overexpression was correlated with poor prognosis of lung cancer, and inhibition of the expression of this gene could suppress the growth of lung cancer cells.[42] Furthermore, Hayama et al suggested that CDCA8 was regarded as a promising target for the finding of novel therapeutics. Besides, Chang et al reported that nuclear accumulation of CDCA8 was linked to poor prognosis of gastric cancer.[43] In the study of Jiao et al, they suggested that forkhead box M1-CDCA8 signature might be associated with the progression of breast cancer.[44] Therefore, we infer that CDCA8 is one of the important genes involved in lung cancer. In addition, in this study, CDCA8 was enriched in cell division (GO-BP). Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. As mentioned earlier, CDCA8 may be one of the important genes involved in lung cancer. Thus, CDCA8 may play a key role in lung cancer via cell division.

Interestingly, our study showed that MCM10 and CDCA8 were 2 target genes of hsa-miR-21-5p. Furthermore, as previous studies stated, miRNA-21 played significant roles in lung cancer. Combined with abovementioned discussions, we infer that miRNA-21 may play significant roles in lung cancer partly via MCM10 and CDCA8. Therefore, miRNA-21 inhibition in A549 cells may affect the cell cycle-related biological processes and cell division by targeting MCM10 and CDCA8.

Besides, TFDP3 (TF of MCM10 and CDCA8) and RAD21 (TF of MCM10) were target genes of hsa-miR-21-5p. Some previous studies showed that RAD21 played significant roles in several cancers, such as breast cancer,[45] and oral squamous cell carcinoma.[46] Some studies indicated that TFDP3 was involved in prostate cancer cell survival[47] and regulated epithelial–mesenchymal transition in breast cancer.[48] Thus, we infer that TFDP3 and RAD21 are important TFs in miRNA-21-interfered lung cancer.

Although significant findings are determined in this study, lacking experimental validation is a limitation in this study. In this article, the role of miRNA-21 was just explored in A549 cells, hence clinical samples are warranted for validation. The targets predicted for miRNA-21 was not validated by luciferase assays. Therefore, studies with large clinical samples size are warranted in the near future.

Conclusion

In conclusion, miRNA-21 may play a significant role in cell cycle, cell proliferation, and cell division related to biological process in lung cancer partly via MCM10 and CDCA8. Both TFDP3 and RAD21 are important TFs in miRNA-21-interfered lung cancer. However, no verifications were performed for the results of the present study, which would be conducted in future studies.

Authors' Note

Our study did not require an ethical board approval because it did not contain human or animal trials.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Linyou Zhang https://orcid.org/0000-0002-7713-2963

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin*. 2011;61(2):69-90.
2. Travis WD. *Pathology and Genetics of Tumours of the Lung, Pleura, Thymus and Heart*. Lyon, France: IARC Press; 2004: 532-534.
3. Iyer S, Roughley A, Rider A, Taylor-Stokes G. The symptom burden of non-small cell lung cancer in the USA: a real-world cross-sectional study. *Support Care Cancer*. 2014;22(1):181-187.
4. Alberg AJ, Brock MV, Samet JM. Epidemiology of lung cancer. In: Mason RJ, Slutsky A, Murray JF, Nadel JA, Gotway M. *Murray & Nadels Textbook of Respiratory Medicine*. 5th ed. Amsterdam, the Netherlands: Saunders Elsevier; 2010: 927-939.
5. Yoong J, Park ER, Greer JA, et al. Early palliative care in advanced lung cancer: a qualitative study. *JAMA Intern Med*. 2013;173(4):283-290.
6. Gao W, Shen H, Liu L, Xu J, Xu J, Shu Y. MiR-21 overexpression in human primary squamous cell lung carcinoma is associated with poor patient prognosis. *J Cancer Res Clin Oncol*. 2011; 137(4):557-566.
7. Kim HS, Lee KS, Bae HJ, et al. MicroRNA-31 functions as a tumor suppressor by regulating cell cycle and epithelial-mesenchymal transition regulatory proteins in liver cancer. *Oncotarget*. 2015;6(10):8089-8102.
8. Lim L, Balakrishnan A, Huskey N, et al. MicroRNA-494 within an oncogenic microRNA megacluster regulates G1/S transition in liver tumorigenesis through suppression of mutated in colorectal cancer. *Hepatology*. 2014;59(1):202-215.
9. Yang M, Shen H, Qiu C, et al. High expression of miR-21 and miR-155 predicts recurrence and unfavourable survival in non-small cell lung cancer. *Eur J Cancer*. 2013;49(3):604-615.

10. Zhang J, Zhang C, Hu L, et al. Abnormal expression of miR-21 and miR-95 in cancer stem-like cells is associated with radioresistance of lung cancer. *Cancer Invest*. 2015;33(5): 165-171.
11. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for illumina sequence data. *Bioinformatics*. 2014;30(15): 2114-2120.
12. Kent WJ, Sugnet CW, Furey TS, et al. The human genome browser at UCSC. *Genome Res*. 2002;12(6):996-1006.
13. Kim D, Langmead B, Salzberg SL. HISAT: a fast spliced aligner with low memory requirements. *Nat Methods*. 2015;12(4): 357-360.
14. Harrow J, Frankish A, Gonzalez JM, et al. GENCODE: the reference human genome annotation for The ENCODE Project. *Genome Res*. 2012;22(9):1760-1774.
15. Pertea M, Pertea GM, Antonescu CM, Chang TC, Mendell JT, Salzberg SL. StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nat Biotechnology*. 2015;33(3): 290-295.
16. Robinson MD, Oshlack A. A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biol*. 2010;11(3):R25.
17. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS*. 2012; 16(5):284-287.
18. Szklarczyk D, Franceschini A, Wyder S, et al. STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res*. 2015;43(Database issue):D447-D452.
19. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. 2003;13(11):2498-2504.
20. Bader GD, Hogue CW. An automated method for finding molecular complexes in large protein interaction networks. *Bmc Bioinformatics*. 2003;4:2.
21. Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc*. 2009;4(1):44-57.
22. Dweep H, Sticht C, Pandey P, Gretz N. miRWalk–database: prediction of possible miRNA binding sites by "walking" the genes of three genomes. *J Biomed Inform*. 2011;44(5):839-847.
23. Enright AJ, John B, Gaul U, Tuschl T, Sander C, Marks DS. MicroRNA targets in drosophila. *Genome biology*. 2003;5(1): R1.
24. Wong N, Wang X. miRDB: an online resource for microRNA target prediction and functional annotations. *Nucleic Acids Res*. 2014;43(D1):D146-D152.
25. Vejnar CE, Zdobnov EM. MiRmap: comprehensive prediction of microRNA target repression strength. *Nucleic Acids Res*. 2012; 40(22):11673-11683.
26. Krüger J, Rehmsmeier M. RNAhybrid: microRNA target prediction easy, fast and flexible. *Nucleic Acids Res*. 2006;34(Web Server Issue):W451-W454.
27. Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB. Prediction of mammalian microRNA targets. *Cell*. 2003;115(7): 787-798.
28. Janky RS, Verfaillie A, Imrichová H, et al. iRegulon: from a gene list to a gene regulatory network using large motif and track collections. *PLoS Comput Biol*. 2014;10(7):e1003731.
29. Pereira DM, Rodrigues PM, Borralho PM, Rodrigues CM. Delivering the promise of miRNA cancer therapeutics. *Drug Discov Today*. 2013;18(5-6):282-289.
30. Markou A, Zavridou M, Lianidou ES. miRNA-21 as a novel therapeutic target in lung cancer. *Lung Cancer (Auckl)*. 2016;7: 19-27.
31. Homesley L, Lei M, Kawasaki Y, Sawyer S, Christensen T, Tye BK. Mcm10 and the MCM2–7 complex interact to initiate DNA synthesis and to release replication factors from origins. *Genes Dev*. 2000;14(8):913-926.
32. Kliszczak M, Sedlackova H, Pitchai GP, Streicher WW, Krejci L, Hickson ID. Interaction of RECQ4 and MCM10 is important for efficient DNA replication origin firing in human cells. *Oncotarget*. 2015;6(38):40464-40479.
33. Thu YM, Bielinsky A-K, eds. *MCM10: One Tool for All-Integrity, Maintenance and Damage Control. Seminars Cell Dev Biol*. Amsterdam, Netherlands: Elsevier; 2014.
34. Wu C, Zhu J, Zhang X. Integrating gene expression and protein-protein interaction network to prioritize cancer-associated genes. *Bmc Bioinformatics*. 2012;13:182.
35. Thu YM, Bielinsky AK. Enigmatic roles of Mcm10 in DNA replication. *Trends Biochem Sci*. 2013;38(4):184-194.
36. Li WM, Huang CN, Ke HL, et al. MCM10 overexpression implicates adverse prognosis in urothelial carcinoma. *Oncotarget*. 2016;7(47):77777-77792.
37. Das M, Prasad SB, Yadav SS, et al. Over expression of minichromosome maintenance genes is clinically correlated to cervical carcinogenesis. *Plos One*. 2013;8(7):e69607.
38. Evan GI, Vousden KH. Proliferation, cell cycle and apoptosis in cancer. *Nature*. 2001;411(6835):342-348.
39. Malumbres M, Barbacid M. Milestones in cell division: to cycle or not to cycle: a critical decision in cancer. *Nat Rev Cancer*. 2001;1(3):222-231.
40. Massagué J. G1 cell-cycle control and cancer. *Nature*. 2004; 432(7015):298-306.
41. Gassmann R, Carvalho A, Henzing AJ, et al. Borealin: a novel chromosomal passenger required for stability of the bipolar mitotic spindle. *J Cell Biol*. 2004;166(2):179-191.
42. Hayama S, Daigo Y, Yamabuki T, et al. Phosphorylation and activation of cell division cycle associated 8 by aurora kinase B plays a significant role in human lung carcinogenesis. *Cancer Res*. 2007;67(9):4113-4122.
43. Chang JL, Chen TH, Wang CF, et al. Borealin/Dasra B is a cell cycle-regulated chromosomal passenger protein and its nuclear accumulation is linked to poor prognosis for human gastric cancer. *Exp Cell Res*. 2006;312(7):962-973.
44. Jiao DC, Lu ZD, Qiao JH, Yan M, Cui SD, Liu ZZ. Expression of CDCA8 correlates closely with FOXM1 in breast cancer: public microarray data analysis and immunohistochemical study. *Neoplasma*. 2015;62(3):464-469.
45. Atienza JM, Roth RB, Rosette C, et al. Suppression of RAD21 gene expression decreases cell growth and enhances cytotoxicity

of etoposide and bleomycin in human breast cancer cells. *Mol Cancer Ther*. 2005;4(3):361-368.

46. Yamamoto G, Irie T, Aida T, Nagoshi Y, Tsuchiya R, Tachikawa T. Correlation of invasion and metastasis of cancer cells, and expression of the RAD21 gene in oral squamous cell carcinoma. *Virchows Arch*. 2006;448(4):435-441.
47. Ma Y, Xin Y, Li R, et al. TFDP3 was expressed in coordination with E2F1 to inhibit E2F1-mediated apoptosis in prostate cancer. *Gene*. 2014;537(2):253-259.
48. Yin K, Liu Y, Chu M, Wang Y. TFDP3 Regulates epithelial-mesenchymal transition in breast cancer. *PLoS One*. 2017; 12(1):e0170573.