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ORIGINAL ARTICLE

Mining featured micro ribonucleic acids associated with lung cancer based on bioinformatics

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Abstract

Background: Few genetic markers useful for the screening of lung cancer risk exist. Although related research has shown that certain expression profiles of micro ribonucleic acids (miRNAs) are different in lung cancer *versus* the normal lung, such as miR-29a and miR-29s, the precise molecular mechanism of lung cancer remains obscure. In order to get a better understanding of the pathogenetic mechanism of lung cancer, we analyzed the differentially expressed genes (DEGs) and identified featured miRNAs in lung cancer tissues.

Methods: We used the gene expression profile GSE10072, including 49 gene chips of non-tumor tissues and 58 gene chips of lung tumor specimens. The DEGs between these two groups were identified by Limma package in R language. The TarBase database was used to construct the networks of miRNA regulating DEGs related to lung cancer. After ordering miRNAs regulating DEGs, we further screened featured miRNAs combined with the miR2Disease database.

Results: A total of 5572 DEGs were obtained between lung cancer and control specimens. After constructing a miRNA regulatory network, a total of 398 regulations between 57 miRNAs and 321 target genes existed. By intergrating the miR2Disease database and using a sorting algorithm, a total of six featured miRNAs related to lung cancer were identified, including miR-520h, miR-133a, miR-34, miR-103, miR-370, and miR-148. They might be involved in lung cancer progression by regulating *ABCG2*, *PKM2*, *VAMP2*, *GPD1*, *MAP3K8*, and *DNMT3B*, respectively.

Conclusion: The top 10 significant miRNAs, such as miR-520h, miR-133a, miR-34, and miR-103 may be potential therapeutic targets for lung cancer.

Introduction

Lung cancer is one of the leading causes of cancer-related deaths in the world, largely because of the genetic and epigenetic damage caused by tobacco smoke.[1] In general, lung cancer is divided into two categories for the purpose of diagnosis and treatment: small cell lung carcinoma and non-small cell lung carcinomas (NSCLCs). Both classifications are difficult to diagnose at an early stage and are always related to poor survival.[2] To improve the patient survival rate, it is critical to investigate the mechanism of tumorigenesis in lung cancer in order to determine effective therapies.[3]

Recently, molecular investigations have provided evidence that the development of lung cancer is involved with genetic alterations, which has contributed to defining the molecular network of lung carcinogenesis. The expressions of Kirsten rat sarcoma viral oncogene homolog (*K-ras*), phosphatase and tensin homolog (*PTEN*), fragile histidine triad gene (*FHIT*) and myosin XVIIIB (*MYO18B*) are frequently altered.[4,5] Studies also show that *p53* and *RB/p16* pathways are usually deficient.[6] In addition, some unknown markers, such as noncoding RNA gene products, may lend insight into lung cancer. Micro ribonucleic acids (miRNAs) are small noncoding RNA gene products considered to be important regulators of gene expression and play a crucial role in cellular growth, differentiation, and death.[7,8] Some miRNA expression levels are closely related with human tumorigenesis. It is reported that miR-29 seconds is inversely correlated to DNA (cytosine-5-)-methyltransferase 3 alpha (*DNMT3A*) and DNA (cytosine-5-)-methyltransferase 3 beta (*DNMT3B*) in lung cancer tissues and plays a role in the epigenetic normalization of NSCLCs.[9] Let-7 microRNA, as a tumor suppressor

gene, has been demonstrated to directly repress cancer growth in the lung.[8] Although numerous studies have contributed to exploring the mechanisms of lung cancer, the role of miRNAs in lung cancer progression is not yet clarified.

In our study, a set of gene expression profiles of lung cancer and controls were analyzed to identify the differentially expressed genes (DEGs). We then applied bioinformatics tools to identify miRNAs regulating DEGs. Using TarBase and the MiR2Disease database, we further screened featured miRNA involved in the occurrence of lung cancer. Our work may help to seek potential targets for lung cancer therapies.

Methods and data

Affymetrix microarray data

Gene expression profiles under the accession number GSE10072 were downloaded from the Gene Expression Omnibus (GEO).[10] A total of 107 samples were used for the development of a microarray profile, which contained 58 NSCLC tissues, including 16 from never smokers (NS), 18 from former smokers (FS), 24 from current smokers (CS), and 49 normal samples, which included 15 NS, 18 FS, and 16 CS. There was no significant difference in the number of smokers between the two groups. The raw data were obtained based on the GPL96 Platform.

Data preprocessing and screening of lung cancer related genes

The probe-level data in CEL files were converted into expression profiles; MAS 5.0 performed background correction and standard summarization.[11] For genes corresponding to multiple probe sets, which have a plurality of expression values, the gene expression values of those probe sets were averaged. Eventually, a total of 12 752 gene expression profiles were obtained, including 58 lung cancer and 49 control specimens.

CancerResource (http://bioinformatics.charite.de/cancerresource/) is a database integrating cancer-relevant relationships of compounds and targets.[12] A total of 211 lung cancer-related genes were obtained from CancerResource and 209 genes were involved in gene expression profiles.

Differentially expressed gene (DEG) analysis

A Limma package in R language was used to analyze the DEGs between the 58 lung cancer and 49 control specimens.[13] The *P*-values were adjusted by the Benjamin and Hochberg (BH) method based on the multtest package.[14,15] A fold discovery rate (FDR) of <0.01 was used as the cut-off criterion for DEGs. To get a better understanding of DEGs, the expression values of the DEGs were collected and hierarchical clustering analysis was performed based on Euclidean distance.[16] An enrichment analysis of DEGs corresponding to lung cancer-related genes was then performed using the Fisher test.[17] To facilitate the analysis, we named the DEGs related to lung cancer ($P < 0.01$) as annotated differentially expressed genes (ADEGs).

Micro ribonucleic acid (MiRNA) regulating DEG network analysis

TarBase (http://diana.cslab.ece.ntua.gr/DianaToolsNew/index.php?r=tarbase/index) is a database providing a collection of all experimentally tested miRNA targets.[18] A total of 1094 miRNA-target interactions in humans were selected based on TarBase data. miRNA–targeted DEG regulation networks were constructed and the regulated relations, target genes, and miRNAs were calculated.

Screening of featured miRNAs related to lung cancer

After miRNA-targeted DEG regulation networks were obtained, we aimed to further identify featured miRNAs related to lung cancer. In this study, we applied a ranking approach to obtain featured miRNAs. The Rank value of miRNA regulating DEGs were calculated according to:

$$\text{Rank\_value (i)} = \sum_{j=1}^{n} \frac{\text{DEG}_{\text{Rankj}}}{n}$$

where $\text{DEG}_{\text{Rankj}}$ is the rank of certain DEG in all DEGs. Featured miRNAs were endowed with a smaller rank value. We then tested whether DEGs were related to lung cancer genes by calculating the rank sum of ADEGs and randomly selecting the same amount of DEGs. The procedure was repeated 1000 times. We then calculated the average of the random rank sum, variance, and *P*-value by Z-score test. We also ranked miRNAs regulating DEGs.

The miR2Disease database (http://www.mir2disease.org) is a manually curated database that aims to provide a comprehensive record of miRNA deregulation involved in various human diseases.[19] A total of 42 lung cancer-related genes were stored in the miR2Disease database. We mapped DEGs in miRNA regulating networks into the miR2Disease database to further screen miRNAs.

Results

Screening of DEGs and annotated DEGs

We obtained the publicly available microarray dataset GSE10072 from the GEO database. A Limma package in R language was used to analyze the DEGs between 58 lung cancer tissues and 49 non-tumor samples. According to threshold criterion (FDR < 0.01) for DEGs, there were 5572

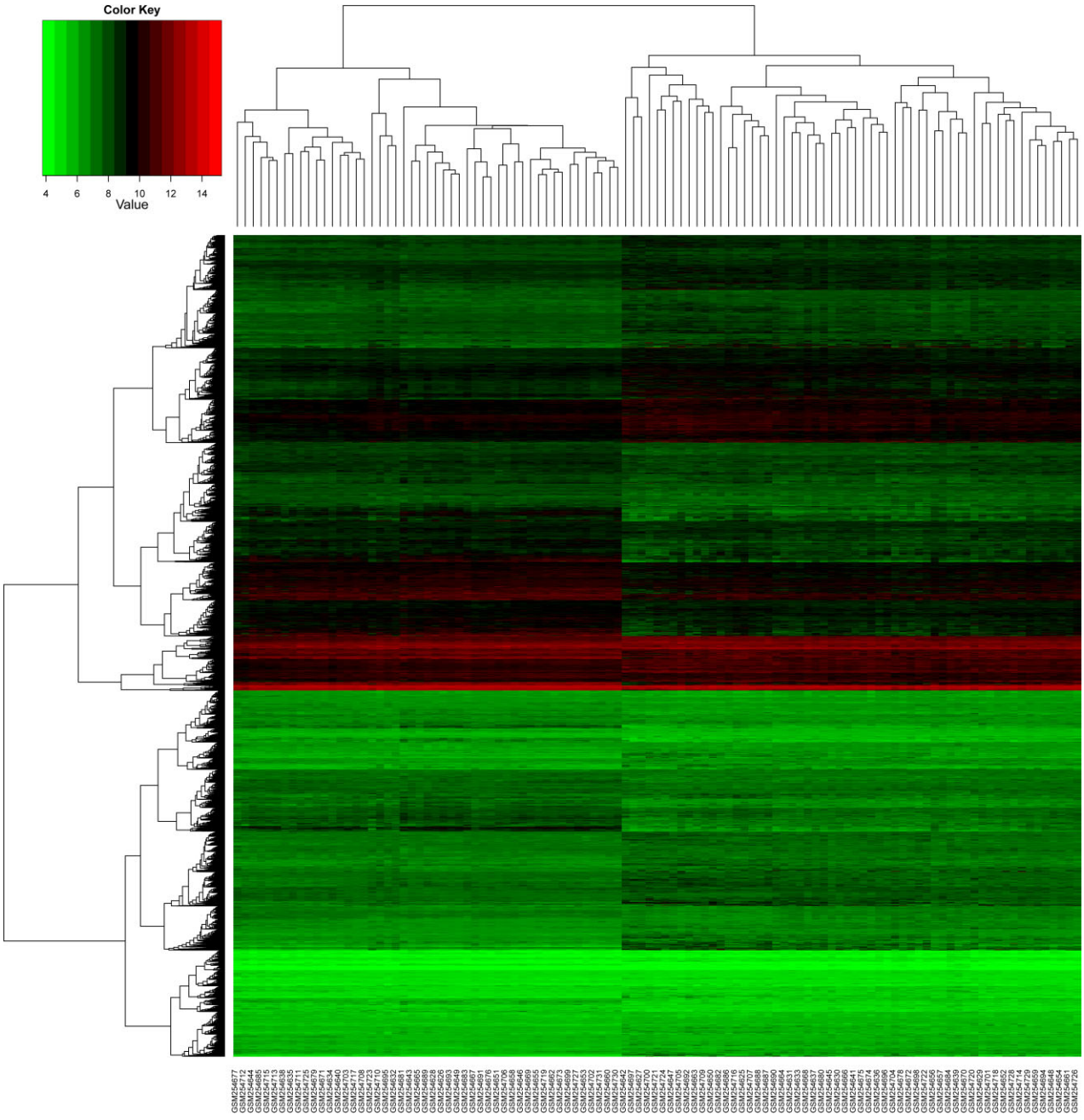

Figure 1 Bi-directional clustering analysis groups samples into lung cancer and control clusters. The green specimens are controls and the red specimens are lung cancer.

DEGs. The results of hierarchical clustering are shown in a heat map (Fig 1). Genes with similar expression levels were collected together and samples (case and control) were relatively distinguished based on the gene expression profiles. By integrating the CancerResource database, DEGs were significantly enriched in ADEGs (P-value = 0.001171), which suggested that the DEGs identified in this work were efficient.

miRNA regulating DEG network analysis

We constructed miRNA-targeted DEG networks using the TarBase database and obtained 398 regulations between 57 miRNAs (such as miR-520h, miR-133a, miR-103, miR-34, miR-370, and miR-148) and 321 DEGs (such as SMAD family member 6 [*SMAD6*], adenosine 5′-triphosphate-binding cassette, sub-family G [WHITE], member 2 [*ABCG2*], pyruvate

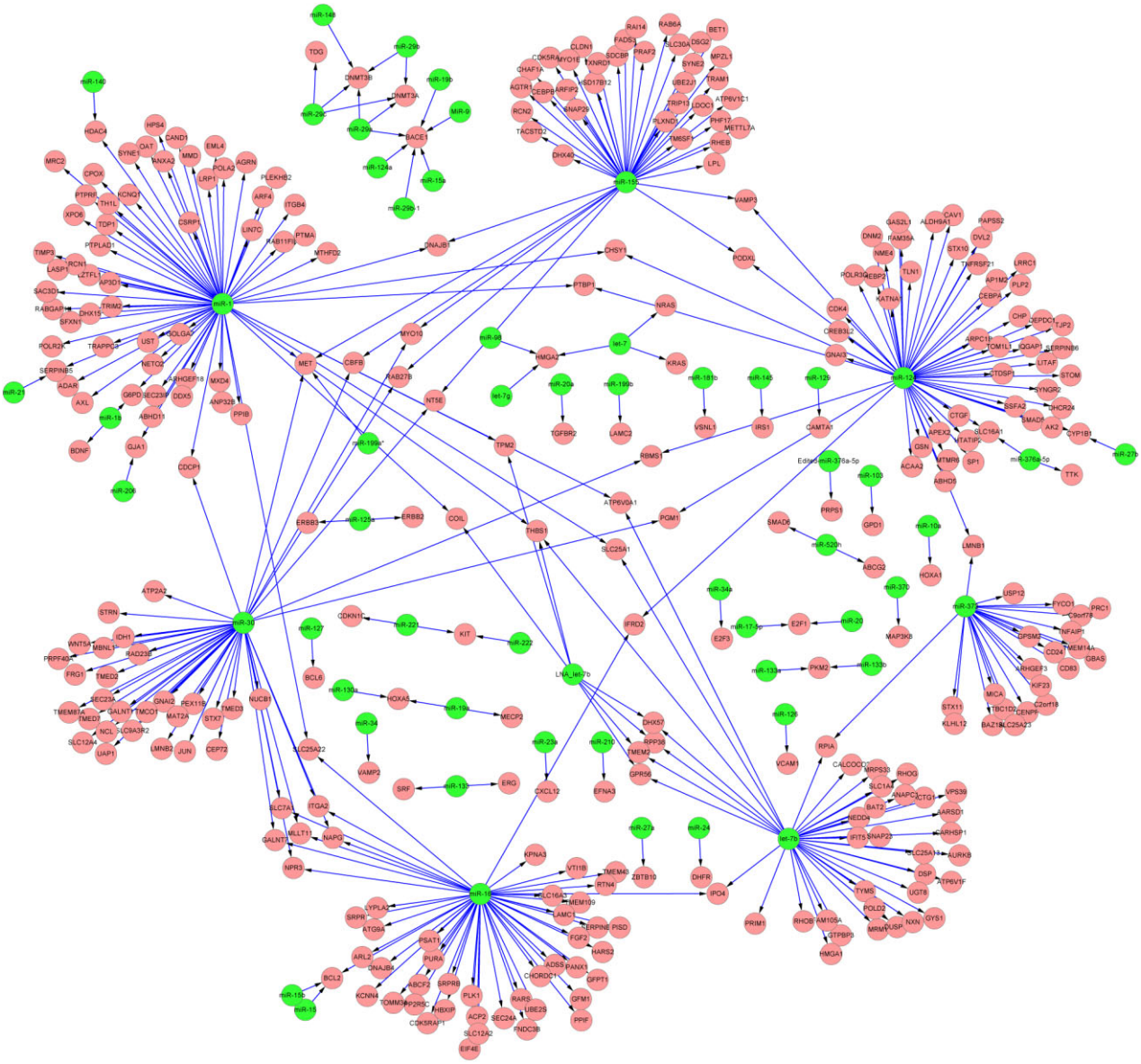

Figure 2 Micro ribonucleic acid (miRNA)-targeted differentially expressed gene regulation networks. The green nodes are miRNAs and the pink nodes are differentially expressed target genes.

kinase, muscle [*PKM2*], mitogen-activated protein kinase 8 [*MAP3K8*], vesicle-associated membrane protein 2 [*VAMP2*], glycerol-3-phosphate dehydrogenase 1 [*GPD1*] and DNA [cytosine-5-]-methyltransferase 3 beta) [*DNMT3B*]) (Fig 2).

Screening of featured miRNAs related to lung cancer

There were 115 ADEGs among the DEGs identified in our paper. Their rank sum was 300251. We randomly selected 115 DEGs and calculated rank sum, variance, and *P*-value. Their value was 321423.8, 16512.12, and 0.09 respectively. These data showed that lung cancer-related genes ranked highest among the DEGs. We screened the significant miRNAs associated with lung cancer by ordering 57 miRNAs (Table 1). Using the miR2Disease to search for the 57 miRNAs, we found that there were 11 overlapping miRNAs. These 11 miRNAs were ranked highest among all of the miRNAs (Table 2). We found that four miRNAs (miR-130a, miR-20a, miR-19a and miR133b) existed in the miR2Disease in the top 10 miRNAs (Table 1). Using PubMed, four miRNAs – miR-520h, miR-133a, miR-34 and miR-103 – were reported to have an intimate relationship with lung cancer.

Table 1 The rank of miRNAs related to lung cancer

miRNA	avg_Target rank_sum	Rank
miR-130a	123	1
miR-20a	193	2
miR-520h	475.5	3
miR-19a	566	4
miR-133a	606	5
miR-133b	606	6
miR-103	749	7
miR-34	878	8
miR-370	1217	9
miR-148	1707	10
miR-23a	1774	11
miR-133	1808.5	12
miR-19b	1855	13
miR-9	1855	14
miR-15a	1855	15
miR-29b-1	1855	16
miR-124a	1855	17
miR-373	2019	18
miR-24	2051	19
miR-210	2300	20
miR-29a	2312	21
miR-125a	2350.5	22
miR-221	2395	23
miR-34a	2403	24
miR-29b	2540.5	25
miR-140	2620	26
miR-376a-5p	2759	27
miR-155	2766	28
miR-124	2820	29
miR-129	2836	30
miR-16	2868	31
let-7b	2876	32
miR-1b	2949	33
miR-30	2985	34
let-7g	3072	35
miR-98	3072	36
miR-1	3148	37
let-7	3225	38
miR-127	3242	39
miR-27a	3297	40
miR-126	3332	41
LNA_let-7b	3440	42
miR-29c	3462	43
miR-206	3955	44
miR-21	4144	45
miR-222	4250	46
miR-145	4349	47
miR-181b	4510	48
miR-376a-5p	4560	49
miR-199a	4696	50
miR-17-5p	4904	51
miR-20	4904	52
miR-15	5006	53
miR-15b	5006	54
miR-10a	5082	55
miR-27b	5381	56
miR-199b	5398	57

miRNA – micro ribonucleic acid.

Table 2 Known 11 miRNAs related to lung cancer rank

miRNA	Rank
miR-130a	1
miR-20a	2
miR-19a	4
miR-133b	6
miR-210	20
miR-29a	21
miR-125a	22
let-7g	35
miR-1	37
miR-126	41
miR-21	45

miRNA – micro ribonucleic acid.

Discussion

In this study, our experimental design primarily found featured miRNAs associated with lung cancer based on bioinformatics. We identified 5572 DEGs between lung cancer and control specimens. After constructing miRNA regulating DEG networks, we obtained 398 miRNA-target interactions, 57 miRNAs, and 321 DEGs. Using the CancerResource and MiR2Disease databases, we finally obtained the top 10 significantly featured miRNAs related to the development of lung cancer; using PubMed, four miRNAs – miR-520h, miR-133a, miR-34 and miR-103 – were reported to have an intimate relationship with lung cancer. As shown in Figure 2, miR-520h shows interaction with *SMAD6* and *ABCG2*. *PKM2* is a direct target for miR-133a. *VAMP2* and *GPD1* are targets for miR-34 and miR-103, respectively.

A recent study reports that miR-520h is a mediator in suppressing the progression of lung cancer.[20] Resveratrol, served as a component in Chinese herbs, can suppress various tumor activities, such as lung cancer.[21] Resveratrol can suppress the migratory ability of tumor cells to lungs by regulating the miRNA-520h-mediated signal cascade.[20] It has been demonstrated that *ABCG2* is overexpressed in human cancers and hsa-miR-520h can downregulate *ABCG2* in pancreatic cancer to inhibit migration and invasion.[22–24]

Another recent study indicates that the expression of miR-133a significantly declined in lung squamous cell carcinoma compared with normal tissues.[25] miR-133a, as a tumor suppressor, shows a significant effect in inhibiting tumor cell proliferation. *PKM2*, as a protein kinase and a transcriptional coactivator, represents an attractive target for cancer therapy.[26] Increased expression of *PKM2* can provide advantages for diverse cancer cell growth and survival.[27] Recent studies show that miR-133a is a targeting transcriptor of *PKM2* and the overexpression of *PKM2* is associated with the downregulation of miR-133a.[28] We can infer that miR-133a may play an important role in lung cancer by regulating *PKM2*.

In addition, in mammalians, the miR-34 family comprises three processed miRNAs, including miR-34a, which is highly expressed in the brain, and miR-34b/c, which is mainly expressed in the lung.[29,30] miRNA-34 has a tumor suppression function in lung cancer.[31] Exogenous miRNA-34 can reduce the proliferation and invasion of lung cancer epithelial cells. Moreover, the aberrant expression of miR-103 is found in metastasis-associated gene 1 silencing lung cancer cells.[32] The differential expression of miR-103 is closely related with lung cancer progression. *VAMP2* is thought to participate in neurotransmitter release. However, little is known about miR-34 regulating *VAMP2* in lung cancer.

Four miRNAs (miRNA-130a, miRNA-20a, miRNA-19a and miR-133b) are related with lung cancer based on the miR2Disease database. miRNA-130a has been suggested as a novel prognostic marker for NSCLC patients.[33] miRNA-130a is differentially expressed in smoking patients with NSCLC compared with non-smoking ones. A previous study has also shown that miRNA-130a was able to inhibit tumor migration of NSCLC.[34] The expression of miRNA-19a was found to be downregulated in lung cancer tissues compared with controls.[35] The low expression of miRNA-19a is related to a poor prognosis in patients with lung cancer. Numerous studies have suggested that the expression of miRNA-133b is involved in various cancer progressions.[36–38] The expression of miRNA-133b is decreased in lung cancer cells and functions in inducing apoptosis in tumor cells.[39] Although evidence of miRNA-20a regulating lung cancer development is insufficient, previous studies have shown that miRNA-20 is differentially expressed in cervical cancer and has been considered to be a prognostic marker for oral squamous tumors.[40,41] The relationship between miRNA-20a and lung cancer needs to be further investigated.

Conclusion

Our study more intuitively shows the relationship between miRNA and DEGs in lung cancer than previous reports. We ordered miRNAs based on reasonable indicators and obtained four miRNAs related to lung cancer reported in the literature. The featured miRNAs identified in our paper play key roles in the initiation and progression of lung cancer and may be potential targets for lung cancer treatment. Further research is required to more closely investigate the exact mechanism of lung cancer.

Disclosure

No authors report any conflict of interest.

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