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Integrated bioinformatics analyses identify dysregulated miRNAs in lung cancer

J. WANG, Y.-C. ZHAO, Y.-D. LU, C.-P. MA

Department of Thoracic Surgery, Zhangjiagang First People's Hospital, Zhangjiagang, Jiangsu, China

Abstract. – BACKGROUND: Lung cancer is the most common cause of cancer-related death worldwide. MicroRNAs (miRNAs) play important roles in various biological processes, including cell development, proliferation, differentiation and apoptosis.

MATERIALS AND METHODS: In the current study, using miRNA expression profiles from the Gene Expression Omnibus (GEO) database, we used three independent tests: Wilcox test, *t*-test and Fisher's exact test to investigate miRNA's involvement in lung carcinogenesis.

RESULTS: Ten differentially expressed miRNAs were identified. Among them, miR-675 drew specific attention. Ingenuity pathway analysis of its target genes revealed its impact on cell death and cell cycle. It is possible that miR-675 contributes to the pathogenesis of lung cancer through its down regulation of the tumor suppressor gene RB1.

CONCLUSIONS: Our results suggest miR-675 may serve as a potential therapeutic target of lung cancer.

Keyword:
miRNA, Expression, Target genes, Ingenuity pathway analysis.

Introduction

Lung cancer is the most common cause of cancer-related death worldwide[1]. Its five-year survival rate is the lower than other cancer types[2]. Most patients are at late stage when diagnosed[3]. So, it is necessary to find new biomarkers for early diagnoses.

MicroRNAs (miRNAs) are small non-coding RNAs with the length of 20-25 nt[4]. By complementarily binding to their target mRNA molecules in the 3'-untranslated region (3' UTR), the translation of the mRNA molecules will be inhibited[5]. In a few cases the binding areas are in the 5'-UTR or coding region. MiRNA genes can exist in genomes as either single copy, multiple copies or appear as gene clusters or other forms. The vast majority of them are located in the intergenic regions. Its transcription is independent of the other gene, and will not be translated into proteins. MiRNAs play important roles in various biological processes, including cell development, proliferation, differentiation and apoptosis. Several studies have reported that miRNAs are involved in the initiation and progression of cancer[6]. The pathogenesis of lung cancer and its associated miRNAs has been the research hotspot[7,8]. The results indicate that miRNAs are candidate diagnostic and prognostic markers of lung cancer.

In this study, using microarray data from the Gene Expression Omnibus (GEO) database, we aim to find the miRNAs in pathogenesis of lung cancer. To get credible results, differentially expressed miRNAs were identified by combining the results of three methods: Wilcox test, *t*-test and Fisher's exact test. Target genes of the selected miRNAs were predicted. Ingenuity Pathway Analysis (IPA) was carried out for the targets of especially interesting miRNAs to explore the underlying impacts on biological pathways.

Materials and Methods

Microarray data

From the GEO database, we got the microarray data of miRNA: GSE24709, which represents miRNA expression profile of 28 lung cancer patients and 19 normal controls. All RNAs were extracted from peripheral blood. The data set was based on the platform GPL9040: febit Homo Sapiens miRBase 13.0. All raw data including original CEL files and the SOFT formatted family file were obtained.

Detection of differently expressed miRNAs

Normalization of the raw data was performed in R software (version 3.0.0) with the Robust Multi-array Analysis (RMA)[9]. The resulting

Corresponding Author: Chunping Ma, MD; e-mail: zjgmcp@sina.com

log2-transformed RMA expression values were, then, used for further analysis. Three tests: *t*-test, Wilcox test and Fisher's exact test were used to identify differential expressed miRNAs between lung cancer and normal control samples. miRNAs supported by all three tests were considered to be involved in the pathogenesis of the disease.

MiRNA target gene prediction

Target gene prediction for the differentially expressed miRNAs was carried out with current available methods, including microT[10], miRanda[11], mircode[12] and Targetscan[13]. In order to reduce the false positive target prediction, the results supported by at least two methods were remained as reliable miRNA targets. Besides, target genes of miRNAs with experimental support in the TarBase 6.0 database[14] were also included in the final lists.

Ingenuity Pathway Analysis

The most significantly differentially expressed miRNAs were considered for Ingenuity Pathway Analysis (IPA). We exploited the Ingenuity Pathways Knowledge Base to look for enrichments in cellular functions, pathways or disease related genes among putative target genes.

Results

Differentially expressed miRNAs

Based on the results of the three tests, 10 miRNAs were identified to be differentially expressed in lung cancer and normal control samples (Figure 1 and Table I), including eight upregulated miRNAs and two downregulated ones. Among them, miR-675 showed the most significant dysregulation.

Target gene prediction

Since miRNAs function through targeting mRNAs, we retrieved the putative target genes of differentially expressed miRNAs. The most significant dysregulated miRNA, miR-675, attracted our attention since one of its target genes with experimental support is the tumor suppressor gene *RB1*. *RB1* is involved in the lung cancer pathway (hsa05222 and hsa05223).

Ingenuity Pathway Analysis

Putative target genes of miR-675 were imported into the IPA Tool to investigate their possible biological interactions. Table II represents the list of top 5 networks identified by IPA. Of these networks, Cell death and survival was the highest rated network with 109 focus molecules and the significance score of 44 (Table II, Figure 2). The score is the probability that a collection of genes equal to or greater than the number in a network could be achieved by chance. The IPA analysis also groups the miR-675 target genes into biological mechanisms that are related to cell cycle, cellular development, RNA post-transcriptional modification, molecular transport and carbohydrate metabolism. Sulfate activation for sulfonation and glutamate receptor signaling came out to be the significant pathways (Figure 3).

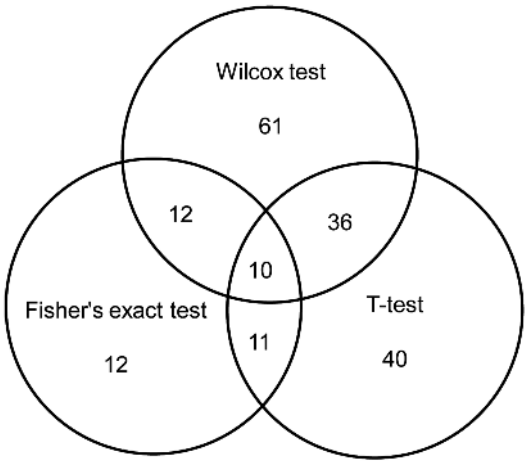

Figure 1. VENN diagram of the numbers of differentially expressed miRNAs identified by the three tests. The intersection of the three tests was selected for further analysis.

Discussion

In this study, using three tests (Wilcox test, T-test and Fisher's exact test), we found 10 differentially expressed miRNAs that may play crucial regulatory roles in the pathogenesis of lung cancer (Table I). By retrieving the target genes of these miRNAs, the most significantly dysregulated miRNA, miR-675, attracted our attention since one of its target genes with experimental support is the tumor suppressor gene *RB1*. The protein encoded by *RB1* is a negative regulator of the cell cycle and was the first tumor suppressor gene found. In addition, RB1 is involved in the lung cancer pathway (http://www.kegg.jp/kegg/pathway.html, hsa05222 and hsa05223). Therefore, we further carried out IPA for the putative target genes of miR-675. IPA program is based on the Ingenuity Pathway Knowledge Base

Table I. Differentially expressed miRNAs from the three tests.

MiRNA	t-test	Wilcox test	Fisher's exact test	Log (Fold change)
hsa-miR-675	1.30E-04	4.49E-03	2.21E-03	1.69
hsa-miR-18a*	7.15E-04	1.23E-03	1.46E-02	1.40
hsa-miR-183	1.43E-03	1.70E-03	2.48E-02	1.28
hsa-miR-140-3p	3.05E-03	2.99E-02	3.58E-02	0.94
hsa-miR-182	5.90E-03	4.33E-03	4.15E-02	0.88
hsa-miR-21	6.88E-03	7.38E-03	4.56E-02	1.40
hsa-let-7e	1.32E-02	1.54E-02	3.73E-02	-1.17
hsa-miR-31	4.63E-02	3.25E-02	3.10E-02	0.92
hsa-miR-574-5p	4.68E-02	4.94E-02	4.57E-02	0.51
hsa-miR-126	4.78E-02	2.75E-02	4.28E-02	-1.20

(IPKB) which is derived from known functions and interactions of genes in published articles. IPA allows the identification of networks, functions and pathways of the miR-675 target genes. The results showed that cell death and survival was the highest rated network with 109 focus molecules and the significance score of 44 (Table II, Figure 2). Cell cycle was the most enriched cellular function as shown in Table II. These results suggested the involvement of miR-675 in cancer through its impact on cell death or cell cycle. In addition, glutamate receptor signaling came out to be a significant pathway (Figure 3) and accumulating evidences have suggested the importance of this pathway in lung cancer growth[15,16]. All these results supported that miR-675 may play important roles in the pathogenesis of lung cancer through its regulation of its target genes, especially the target gene, *RB1*. To date, no reported about the relationship between miR-675 and lung cancer has been proposed. However, previous study[17] has reported that miR-675 regulates colorectal cancer development through down regulation of *RB1*, and may serve as a potential target for therapy. Moreover, miR-675 overexpression in hepatoma cells revealed a pre-

Table II. Network, diseases and functions related to miR-675 target genes.

Top Networks		
ID	**Associated Network Functions**	**Score**
1	Cell death and survival, Behavior, Nervous system development and function	43
2	Carbohydrate metabolism, Small molecule biochemistry, Skeletal and muscular disorders	32
3	Cell cycle, Cell morphology, Cellular function and maintenance	30
4	Cancer, Gastrointestinal disease, Auditory disease	27
5	Cell cycle, Cell death and survival, Tumor morphology	24
Diseases and Disorders		
Name	***p*-value**	**#Molecules**
Neurological disease	3.66E-04 - 3.62E-02	18
Organismal Injury and abnormalities	3.66E-04 - 4.79E-02	13
Cancer	6.07E-04 - 4.88E-02	74
Gastrointestinal disease	6.07E-04 - 2.93E-02	44
Respiratory disease	5.38E-03 - 3.34E-02	11
Molecular and cellular functions		
Name	***p*-value**	**#Molecules**
Cell cycle	4.70E-05 - 4.79E-02	14
Cellular development	1.37E-03 - 4.79E-02	17
RNA Post-transcriptional modification	4.21E-03 - 3.62E-02	5
Molecular transport	5.91E-03 - 4.46E-02	8
Carbohydrate metabolism	6.12E-03 - 2.43E-02	1

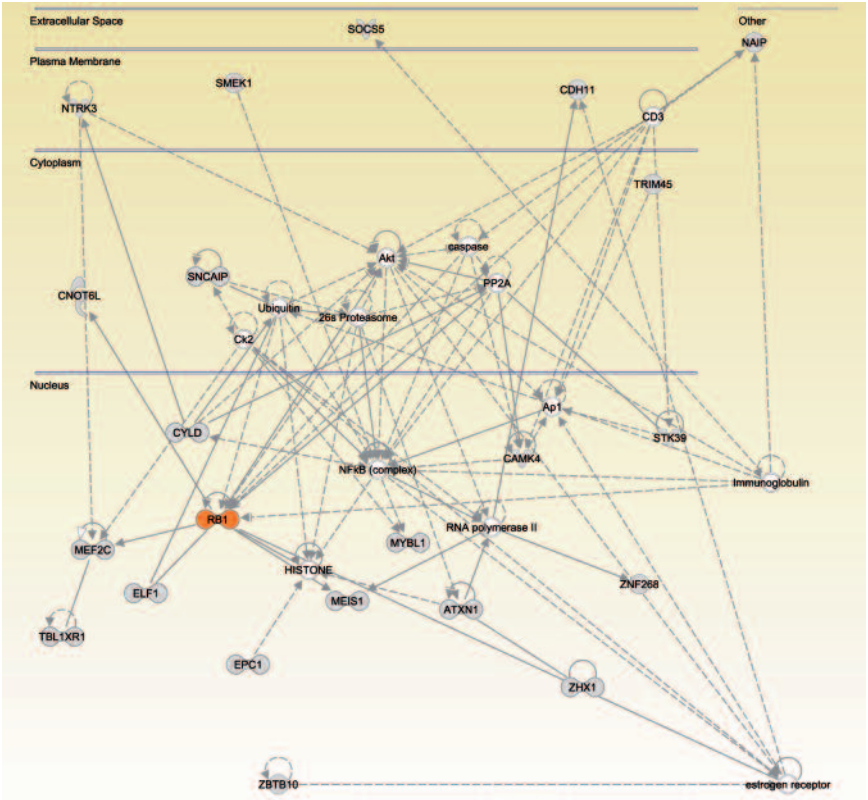

Figure 2. Most highly rated network in IPA analysis. A solid line represents a direct interaction between the two gene products and a dotted line means there is an indirect interaction.

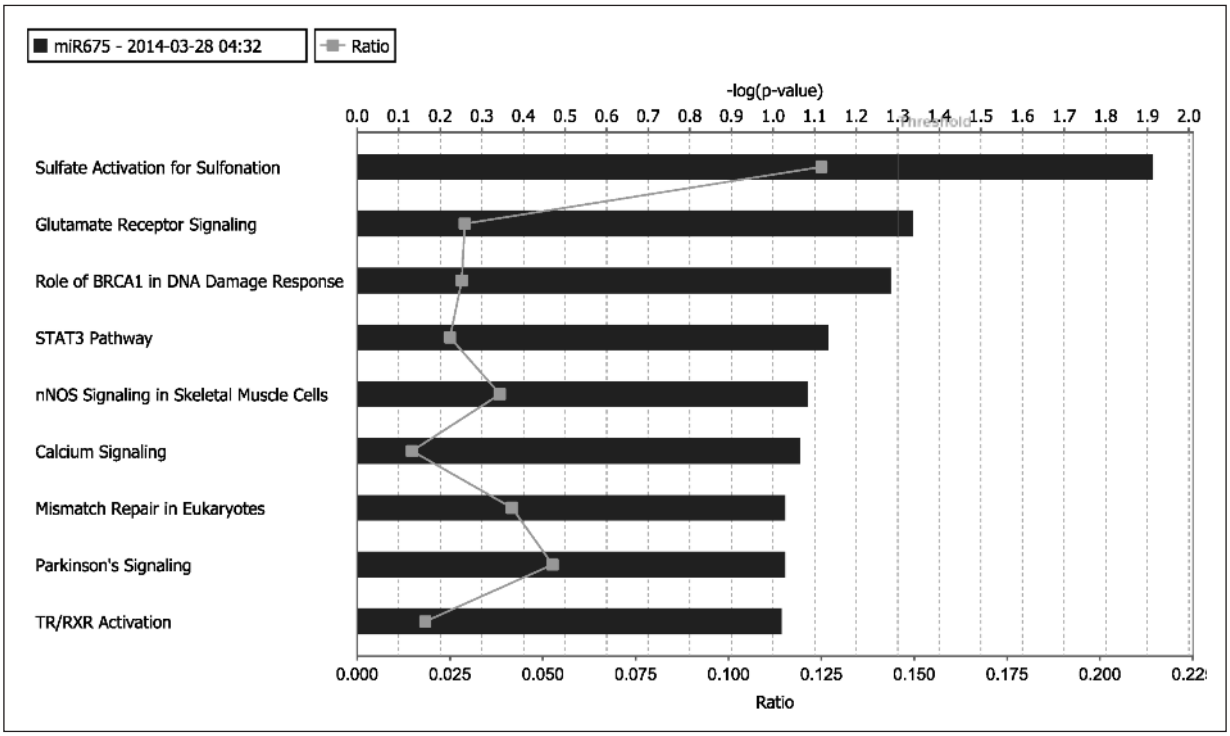

Figure 3. Pathway analysis of miR-675 target genes. The y-axis represents the top pathways as calculated by IPA and the x-axis represents the ratio of number of genes from the dataset that map to the pathway and the number of all known genes in the pathway.

dominant up regulation of cell adhesion and cell cycle initiation pathways and miR-675 mediated increases in proliferation with a repression of *RB1*[18]. Combined with our results, we speculate that miR-675 may contribute to the pathogenesis of lung cancer through its regulation of *RB1*.

Conclusions

We identified ten differentially expressed miRNAs by integrating three independent tests. Among them, miR-675 drew specific attention. IPA analysis revealed its impact on cell death and cell cycle. It is possible that miR-675 contributes to the pathogenesis of lung cancer through its down regulation of the tumor suppressor gene *RB1*.

Conflict of interest

The Authors have no conflict of interests of declare.

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