

## RESEARCH ARTICLE

# MiRNA Synergistic Network Construction and Enrichment Analysis for Common Target Genes in Small-cell Lung Cancer

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## Abstract

**Background:** Small-cell lung cancer (also known as SCLC) is an aggressive form and untreated patients generally die within about 3 months. To obtain further insight into mechanism underlying malignancy with this cancer, an miRNA synergistic regulatory network was constructed and analyzed in the present study. **Method:** A miRNA microarray dataset was downloaded from the NCBI GEO database (GSE27435). A total of 546 miRNAs were identified to be expressed in SCLC cells. Then a miRNA synergistic network was constructed, and the included miRNAs mapped to the network. Topology analysis was also performed to analyze the properties of the synergistic network. Consequently, we could identify constitutive modules. Further, common target genes of each module were identified with CFinder. Finally, enrichment analysis was performed for target genes. **Results:** In this study, a miRNA synergistic network with 464 miRNAs and 2981 edges was constructed. According to the topology analysis, the topological properties between the networks constructed by LC related miRNAs and LC unrelated miRNAs were significantly different. Moreover, a module cilque0 could be identified in our network using CFinder. The module included three miRNAs (hsa-let-7c, hsa-let-7b and hsa-let-7d). In addition, several genes were found which were predicted to be common targets of cilque0. The enrichment analysis demonstrated that these target genes were enriched in MAPK signaling pathways. **Conclusions:** Although limitations exist in the current data, the results uncovered here are important for understanding the key roles of miRNAs in SCLC. However, further validation is required since our results were based on microarray data derived from a small sample size.

**Keywords:** Small cell lung cancer - mechanism - miRNA synergistic network

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## Introduction

Small cell lung cancer (also known as SCLC), accounts for about 20% of lung cancer, which is a type of malignant cancer, with short cell cycle and a tendency for early metastasis to other body sites (Nasu et al., 2011). If untreated, the patients with SCLC will die in less than 3 months. The disease is highly sensitive to chemotherapy and radiation, and treatment can often relieve the symptom initially. But because of acquisition of drug resistance, the prognosis of SCLC is poor (Guo et al., 2010; Li et al., 2012). Anyway, SCLC is a complex disease, which is associated with various paraneoplastic syndromes, and the processes of the disease are regulated by thousands of regulators. Among these regulators, microRNAs (miRNAs) are attracted more and more attention recently. MicroRNAs (miRNAs) are endogenous, non-coding RNAs (~22 nucleotides) that usually play important roles in a lot of biology processes, including development, cell

proliferation, differentiation and apoptosis (Anglicheau et al., 2010), and aberrant of miRNAs expression is associated with many disease, such as cancer (Krek et al., 2005). Specifically, miRNAs can bind to the 3'-untranslated region (3'-UTR) of the target messenger RNA (mRNA). Perfect complementarity between the microRNA and its target mRNA reduces protein levels due to RNA silencing (Lewis et al., 2005).

The present studies have generated a large list of miRNAs which may play important roles in the development of SCLC, such as miR-502, miR-1827 and miR-92a-2 (Ranade et al., 2010; Xiong et al., 2011; Ding et al., 2012). For example, miR-502 is implicated in cell cycle progression via regulating the expression of SET8 which encodes a histone H4 lysine 20 monomethyltransferase. The single nucleotide polymorphisms (SNPs) at the miR-502 binding site can affect an individual's cancer risk (Ding et al., 2012).

In many cases, multiple miRNAs work synergistically

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to carry out their biological function (Enright et al., 2003; Krek et al., 2005). However, the researches emphasized on the miRNAs synergistic regulations in SCLC remain rare. In this study, we constructed a miRNAs synergistic network and identified the modules in the network. Meanwhile, the common targets of each module were found and further analyzed.

## Materials and Methods

### Data selection and processing

The miRNAs dataset on SCLC was obtained from the GEO (GSE27435). The microarray platform (Capitalbio mammal microRNA V3.0) was used to analyze the miRNAs expression profiles in 42 SCLC samples, and then background correction and linear standardization were performed for these expression profiles. After preprocessing, probe sets were mapped to miRBase10.0 (Griffiths-Jones et al., 2008). If there were multiple probe sets that correspond to the same miRNA, the expression values of those probe sets were averaged. Finally, 546 miRNAs expressed in SCLC were identified.

### Resource of lung cancer related miRNA

The information about disease related miRNAs were downloaded from miR2Disease (Jiang et al., 2009). MiR2Disease is a manually curated database, including a comprehensive relationship between miRNA and various human diseases from reference and the diseases related miRNA are defined as the miRNA deregulated in particular diseases. According to the data we downloaded from miR2Disease, 117 miRNAs were found to be associated with lung cancer.

### Construction of miRNA synergistic network

The Pearson Correlation Coefficient ( $r$ ) was calculated for 546 miRNAs. In this study, we set the threshold as  $r \geq 0.8$ . In the criterion, the miRNA synergistic network was constructed.

### Topology analysis for miRNAs synergistic network

Cytoscape (Killcoyne et al., 2009) and its plugin networkAnalysis (Assenov et al., 2008) were used to analyze the topological properties of the miRNAs synergistic network. Here, the degree is a numerical invariant which describes how many miRNAs interact with a specific miRNAs in the network. Clustering coefficient is a measure of degree to which nodes in a graph tend to cluster together. The average shortest pathway length was defined as the average values of all shortest length.

### Analysis of Modules in the miRNA synergistic network

Many present studies have demonstrated that miRNAs are synergistic in many biology processes and diseases as well as regulating genes with the same or similar functions in most conditions. In order to further study the mechanism of lung cancer processes, we analyzed the miRNAs in our synergistic network from point of view of module. Several modules were identified by CFinder (Adamcsek et al., 2006), according to the definition that module was

the fully connected subgraph.

### Function and pathway annotation

The common target genes of each module were found by using Targetscan. To demonstrate the function of these target genes, functional enrichment analysis was performed by DAVID for GO (Ashburner et al., 2000), and KEGG (Kanehisa, 2002) pathways annotation terms. DAVID (Huang da et al., 2009) is a multifunction tools, and its basic principle is based on the Fisher's exact test.

## Results

### Identified miRNAs related to lung cancer

Using the dataset (GSE27435), a total of 546 miRNAs were identified to be expressed in SCLC cells, and a total of 117 miRNAs were identified to be related to lung cancer according to the miR2Disease base. The 117 miRNAs deregulated in lung cancer may play important roles in the regulatory network of lung cancer development.

### Construction of miRNA synergistic network

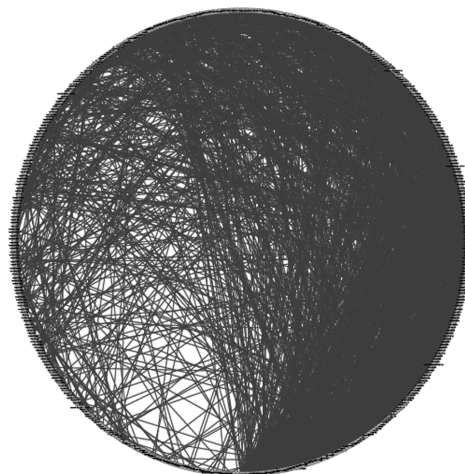
The expression values of 546 miRNAs were calculated by the Pearson Correlation Coefficient. In this study, we set the threshold as  $r \geq 0.8$ . Finally, a miRNA synergistic network with 2981 edges including 464 miRNAs was constructed in this criterion (Figure 1).

### Topology analysis for miRNAs synergistic network

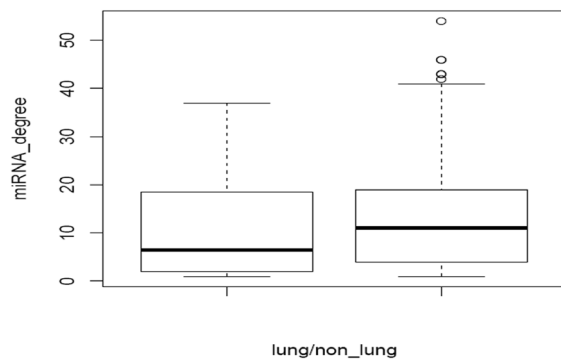
To analyze the topological properties of the miRNAs synergistic network, the Topology analysis was performed. Through the analysis, we found that the topological properties between the networks constructed by LC related miRNAs and LC unrelated miRNAs were significantly different. The degrees of the network constructed by LC

**Table 1. Topological Properties of the miRNAs Synergistic Network**

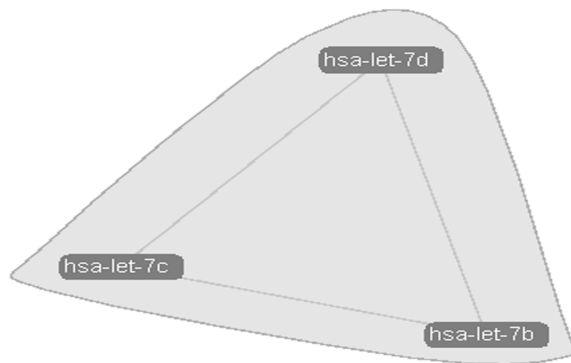
	Lung cancer related	Lung cancer unrelated	P
Degree	10.67647	13.30693	0.01355
Clustering coefficient	0.532144	0.4800167	0.05882
The average shortest pathway	6.88532	5.74078	2.20E-09



**Figure 1. Synergistic Network of miRNAs Related to Lung Cancer**



**Figure 2. Degrees of miRNAs Synergistic Network. Left: LC Related; Right: LC Unrelated Samples**



**Figure 3. The Module Cilque0 Including Three miRNAs**

related miRNAs were much smaller, while the clustering coefficient was significantly larger. These results were corresponding with module identification, indicating that miRNAs may work together to carry out their biological function (Table 1 and Figure 2).

#### Analysis of Modules in the miRNA synergistic network

According to the references, miRNAs work synergistically to carry out their biological function in many biology processes (Xu et al., 2011). To further analyze the structure of our synergistic network in the point view of module, we used Cfinder to identify the modules in our network. Our criterion was that the fully connected subgraph would be defined as module. Finally several modules were found. Among them, a module named clique0, which included three miRNAs (hsa-let-7c, hsa-let-7b and hsa-let-7d), was attracted our attention. All of these three miRNAs belong to a same miRNA family, let-7. Moreover, these three miRNAs are all proved to be related to lung cancer (Johnson et al., 2007; Agirre et al., 2008). Our results were corresponding with the former researches, which indicated that the deregulation of miRNAs belonging to the same family may lead to the similar abnormal phenotype (Lu et al., 2008). What's more, these three miRNAs were predicted to be co-expressed according to our synergistic network. Combining with these results, we are likely to indicate that hsa-let-7c, hsa-let-7b and hsa-let-7d may work together to carry out their biological function in the development of lung cancer (Figure 3)

#### Pathway enrichment analysis

To identify the biological pathways that module clique0 involved in, pathway enrichment analysis was performed.

Firstly, we used TargetsCan to find the common target genes of the miRNAs (hsa-let-7c, hsa-let-7b and hsa-let-7d) in clique0. Then the functional enrichment of these three targets was assessed based on GO and KEGG pathways annotation terms. At last, through the pathway enrichment analysis, most of the common target genes of cilque0 including c-myc, FGF5 and TGFBR1, were found to be enriched in MAPK signaling pathway. The results were consistent with the previous studies that miRNAs are trend to be enriched in signal transduction pathway (Cui et al., 2006). Interestingly, we also found these target genes were also found to be enriched in pathways in cancer. It indicated that miRNAs in clique0 may play important roles in progression of SCLC.

## Discussion

MiRNAs regulate the gene expression through binding the 3'-UTR of the target genes, thereby participating in the regulatory network of cancer processes. Several studies have confirmed that the miRNAs control the larger set of genes through synergism, in which multiple miRNAs work synergistically to control individual genes (Enright et al., 2004; Krek et al., 2005). Analysis of the synergism of miRNAs is an important step for further determining miRNA functions at a system-wide level.

In this study, using the dataset of GSE27435, we constructed a synergistic network with 2981 edges including 464 miRNAs. The results of topology analysis demonstrated that the topological properties between the networks constructed by LC related miRNAs and LC unrelated miRNAs were significantly different. It indicated that the synergistic network in this study was optimal.

Moreover, a module cilque0 was identified in our network. The module included three miRNAs (hsa-let-7c, hsa-let-7b and hsa-let-7d). These three miRNAs are all belong to the let-7 microRNA family. Let-7 is the first known human microRNA. Members of this family are highly conserved in sequence and function. The deregulation of miRNAs in let-7 family often leads to abnormal cell differentiation and development cell-based diseases, such as cancer. What's more, many let-7 family members are located in the genomic regions which are frequently deleted in lung cancer patients (Roush and Slack, 2008; Boyerinas et al., 2010). It is also reported that all of these three miRNAs' expression are altered in lung cancer cell compared to normal controls (Johnson et al., 2007; Boyerinas et al., 2010). Especially the hsa-let-7c was proved to be significantly down-regulated in lung cancer and low levels of let-7c were associated with metastasis by experimental verification (Navarro et al., 2009).

For further investigating the synergistic network, the common target genes of clique0 were predicted by TargetsCan, and then the enrichment analysis was performed for these genes. Several genes were found, which were predicted to be the common targets of cilque0. The enrichment analysis demonstrated that most of the target genes were enriched in MAPK signaling pathways. It is expected, because most of the miRNAs involve in signal transduction pathway (Cui et al., 2006). The MAPK pathway is constituted by a serial of proteins, which transduce signal from the receptor on cell surface to DNA in the nucleus.



The signal starts when a signaling molecule binds to the receptor on the cell surface and ends when the DNA in the nucleus expresses a protein and produces some change in the cell, such as cell division (Manning et al., 2002). Much evidence indicated that overexpression and activation of MAPK can result in progression of various cancer (Xue et al., 2012). In this study, many genes involved in MAPK pathway were predicted to be regulated synergistically by miRNAs in cilque0. Moreover some of them are proved to be associated with lung cancer by experimental verification. For example, Myc (c-Myc) is a regulator gene that codes for a transcription factor, which can be phosphorylated and activated by MAPKs. Altered structure and regulation of the c-myc proteins have been associated with a variety of human tumours and derivative cell lines, including Burkitt's lymphoma, promyelocytic leukaemia and SCLC (Nau et al., 1985). Furthermore, let-7 family members can functionally inhibit the mRNAs of c-myc in lung cancer (Garzon et al., 2009). Interestingly, recent researches suggest that myc also negatively regulates transcription of let-7 family members (-a, -b, and -c) (Garzon et al., 2009). According to our results and together with the previous studies, we were likely to suppose that three genes in cilque0 could regulate the expression of myc synergistically, while myc can make response to that regulation. There was a feedback control between the cilque0 between and myc in SCLC. However, more experimental verifications are still needed to prove this hypothesis.

In conclusions, we constructed the miRNA synergistic network via computational analysis, and a module cilque0 was identified in our network. The three miRNAs in the module were all involved in MAPK signaling pathway. What's more, the module and its common target genes are proved to be associated with the lung cancer by previous studies. Combining with all evidence, we even build hypothesis that cilque0 regulates the expression of myc synergistically. Moreover, there is a feedback control between the cilque0 and myc. Although limitations exist in the current data, the results uncovered here are important for understanding the key roles of miRNAs in SCLC. However, further validation is required since our results were based on microarray data derived from a small sample size.

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