Interdiscip Sci Comput Life Sci (2016) 8:209–219
DOI 10.1007/s12539-015-0117-8

ORIGINAL RESEARCH ARTICLE

miRNA–mRNA Interaction Network in Non-small Cell Lung Cancer

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Received: 14 November 2014 / Revised: 3 January 2015 / Accepted: 9 February 2015 / Published online: 4 September 2015

Abstract MicroRNAs (miRNAs) are small RNA molecules, about 20–25 nucleotides in length. They repress or degrade messenger RNA (mRNA) translation, which are involved in human cancer. In this study based on paired miRNA and mRNA expression profiles of non-small cell lung cancer samples, we constructed and analyzed miRNA–mRNA interaction network via several bioinformatics softwares and platforms. This integrative network is comprised of 249 nodes for mRNA, 90 nodes for miRNA and 290 edges that show regulations between target genes and miRNAs. The three miR-1207-5p, miR-1228* and miR-939 are the most connected miRNA that regulated a large number of genes. ST8SIA2, MED1 and HDAC4, SPN, which are targeted by multiple miRNAs and located in the center of the network, are involved in both lung cancer and nervous system via functional annotation analysis. Such a global interaction network of miRNA–mRNA in lung cancer will contribute to refining miRNA target predictions and developing novel therapeutic candidates.

Keywords Lung cancer · miRNA–mRNA interactions · Bioinformatics

1 Introduction

Lung cancer has become the most common cancer among men and women (13.0 % of the total) in the world. In 2012, among men, lung cancer was the highest incidence cancer (16.7 % of the total), and it was also the most common causes of cancer death (23.6 %). The estimated age-standardized rates (ASRs) in the incidence and mortality were 34.2 per 100,000 and 30.0 per 100,000, and both were the highest in cancer [1]. Among women, it was the third most common cancer (8.7 % of the total). Lung cancer was also the second most common cause of cancer death (13.8 %). Because of the poor survival of lung cancer, the 5-year prevalence (1.9 million) is very close to the annual mortality (1.6 million) [1]. Squamous cell lung carcinoma (SCC), lung adenocarcinoma (AD) and large cell lung carcinoma (LCLC) are three main types of non-small cell lung cancers (NSCLCs), which account 83.2 % of lung cancers [2].

miRNAs are small noncoding RNAs (20–25 nucleotides in length) that make gene expression silence posttranscriptionally through binding to their target mRNAs by the 3′ untranslated regions (3′UTRs). Mature miRNAs and argonaute protein are incorporated into an RNA-induced silencing complex (RISC) [3, 4]. RISC guides to its mRNA target in the cytoplasm by the associated miRNA. There

Electronic supplementary material The online version of this article (doi:10.1007/s12539-015-0117-8) contains supplementary material, which is available to authorized users.

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are two primary systems used to control mRNA expression: inhibition of mRNA translation and mRNA degradation. The higher the degree of base pairing between the miRNA and the mRNA, the more likely the target mRNA will be degraded [5]. In some conditions, miRNAs can positively regulate gene expression; however, the underlying mechanisms are not clearly elucidated [4].

miRNAs can modulate many biological processes including development, differentiation, proliferation, cell death, and playing an important role in the pathogenesis of different tumor types [3, 6–9]. In lung cancers, Yanaihara et al. [10] reported that the low overall survival in AD was related to high miR-155 and low let7a-2 expression. Hu et al. [11] reported that a four-miRNA signature (miR-486, miR-30d, miR-1 and miR-499) could predict overall survival for NSCLC independently. Yu et al. [12] found that a five-miRNA signature (miR-137, miR-372, miR-182*, miR-221 and let-7a) was related to disease-free survival in NSCLC. Lebanony et al. [13] discovered that miR-205 was a highly specific biomarker for SCC.

However, there is no global analysis of the miRNA–mRNA interaction network in lung cancers. Such a systems level approach can bring a new way to understand the complex biological processes. In this study, based on pair-matched miRNA–mRNA expression profile of NSCLC samples, we constructed and analyzed the miRNA–mRNA interaction network through a number of bioinformatics tools and platforms.

2 Materials and Methods

2.1 miRNA and mRNA Expression Profiles

In the past dozen years, the growth in number of miRNA and mRNA expression profiles is exponential. Large numbers of expression profiles are available freely from databases Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/geo) [14]. We download the miRNA and mRNA expression profiles from GEO (Accession Number GSE29250 [15]). This expression profile includes 12 pair-matched samples including 6 NSCLC tissues and their matching normal control from adjacent tissues. Genome-wide analysis of miRNA expression and gene expression in NSCLC were parallel measured at Illumina Human v2 MicroRNA expression beadchip and HumanHT-12 V4.0 expression beadchip platforms. When multiple probes for a particular gene, we calculated its signal intensity as the mean of intensities of all these probe sets in this sample. The raw signal was normalized by robust multi-array average (RMA) normalization procedure to produce expression values [16].

2.2 miRNA–mRNA Interactions Analysis and Visualization

We integrated above miRNA–mRNA expression profiles data by MAGIA tool (http://gencomp.bio.unipd.it/magia) [17]. MAGIA first predicts the miRNA target by PITA, miRanda, TargetScan or Boolean combinations of these algorithms. Then, MAGIA combined miRNA targets with different statistical measures, such as Spearman and Pearson correlation and mutual information, to construct miRNA–mRNA bipartite networks. The complete list of identified significant interactions can be imported into other softwares, to allow further visualization and processing. To account for the multiple hypothesis testing, the q value is used to select significant results. Q value is a variant of the traditional p value but correcting for multiple comparisons.

In this study, we chose EntrezGene IDs, Pearson correlation measure and the intersection of PITA (score filter: -10) and miRanda (score filter: 500) target prediction algorithms. Since the data are normally distributed data and medium–large sample size (>5), Pearson correlation measure is used for miRNA–mRNA expression correlation. The miRNAs–mRNAs interaction network was visualized by Cytoscape [18, 19]. This software can get a visualization of nodes and edges as a two-dimensional network and change network factor, such as shape, color and size, based on attribute values. It also supports a number of automated network layout algorithms.

2.3 Functional Analysis Via GO, Pathways and Human Disease Resources

To elucidate the functional significances of identified miRNA–mRNA interactions, we combined GeneDecks, DAVID and Malacard to analyze gene ontology, pathway information and human disease information by list of significant down-regulated target genes in lung cancer miRNA–mRNA interactions network.

DAVID [20] (The Database for Annotation, Visualization and Integrated Discovery) Bioinformatics Resources (http://david.abcc.ncifcrf.gov/) is a tool to gain biological features/meaning through large gene lists. It is useful to understand biological themes. GeneDecks [21] (http://www.genecards.org/genedecks) can analyze the gene through sharing the same descriptors, using the built-in rich annotation of human gene in the GeneCards (a searchable, integrated tool of annotation information about human genes [22]). GeneDecks can provide the information about disorders, drug relationships, pathways, etc, by gene lists. Malacard (http://www.malacards.org/) [23] is an integrated database of human disorder and their annotations. This

database lists the genes related to lung cancer. We can compare these lung cancer-related genes with the down-regulated target genes, which are provided by MAGIA. We always used the default settings in the GeneDecks, DAVID and Malacard analysis.

3 Results

3.1 Construction of miRNA–mRNA Interaction Network

Each patient has four profiling arrays measured the miRNA and mRNA expression together with their adjacent normal tissues. The characteristics of samples are given in Table S1. After normalization, the expression matrix was input into MAGIA pipeline, and an aggregate number of 290, 6230, 31,249 miRNA–mRNA interactions with correlations <-0.75, <-0.50, <-0.25, respectively, were identified. Figure 1, Fig. S1 and Fig. S2 show the global miRNA–mRNA interaction network in lung cancer, respectively based on the correlations <-0.75, <-0.50, <-0.25. We chose the genes whose correlations <-0.75 as highly negative correlated genes for follow-up analysis.

This network consists of 249 nodes for mRNA, 90 nodes for miRNAs and 290 edges that show regulations between these miRNAs and mRNAs. In total, the network involved 61 connected components, including 36 single edges, 11 double edges and 14 triple edges or with more than three edges. These types of connected components, respectively, involve 72, 33 and 234 nodes. Statistics of the connected components is shown in Fig. 2. These results indicated that the majority of miRNAs and their targets can be integrated into a big interlocking network.

In this network, miRNAs (presented in triangle and colored in pink as shown in Fig. 1) usually located in the center of the interaction network or module, while mRNAs (circular and sky blue nodes as shown in Fig. 1) are in the external of the interaction network or module.

According to Fig. 1, we found three miRNAs (miR-1207-5p, miR-1228* and miR-939) regulating 105 target mRNA, occupying 42.17 % of the total mRNA (249 target mRNA). In addition, 30 mRNAs are regulated by more than one miRNA. These mRNAs and their interactional miRNA are indicated in Table 1. From expression profile, hsa-miR-1228* and hsa-miR-939 were down-regulated in NSCLC (Table S2), suggesting their protective roles in normal tissues.

3.2 mRNA Analysis in Lung Cancer

MalaCards is an integrated database of human disorders and their annotations, which can quantitatively measure degree of relationship of interested genes to certain disorders. Using MalaCards, we found that total 35 genes had a confirmed role in lung cancer, occupying 14.06 % in the total 249 mRNAs. The results are given in Table 2.

HDAC4, MED1, SPN and ST8SIA2 appear in both Tables 1 and 2. These four genes are regulated by more than one miRNA in lung cancer (Table 1) and their relationships to lung cancer were confirmed in previous reports (Table 2). Compared with the adjacent normal tissue, these mRNAs increase up to 2.5-fold changes, indicating that they potentially play an important role in miRNA–mRNA interaction network in lung cancer (Table S2).

3.3 Functional Annotation Analysis

Using DAVID and GeneDecks, we studied the functional annotations for mRNAs in the miRNA–mRNA interaction network. The results are given in Table 3.

Using DAVID, the result shows that the significant category of Gene Ontology (GO)-molecular function includes seven terms. The most important molecular function is transferase activity (35 genes). The other identified categories, which ranked 2–6, are subsets of transferase activity (Table 3). The most important biological processes are cellular process (143 genes), localization (49 genes) and regulation of gene expression (47 genes). The core of cellular components of genes is intracellular part (147 genes), plasma membrane part (38 genes) and organelle membrane (23 genes). The complete GO categories and included genes are given in Table S3.

Utilizing GeneDecks to analyze these 249 target miRNA, the miRNAs are classified into seven types of descriptors in phenotype attribution. The most three important descriptors are mortality/aging (67 genes), nervous system phenotype (63 genes) and growth/size /body phenotype (54 genes). Only one pathway—axon guidance—was found in significantly in our analysis (six genes). The complete analytical results are given in Table S4.

4 Discussion

4.1 Significant miRNA and mRNA in the Interaction Network

Many miRNAs are abnormally expressed in lung cancer and play a core role in the process of malignant transformation, angiogenesis and tumor metastasis [10–13, 24]. Individual miRNA may regulate and control multiple target mRNA involved in different oncogenic or tumor suppressor pathways. Analyzing the miRNA–mRNA interaction network at systems level may open a new chapter in lung

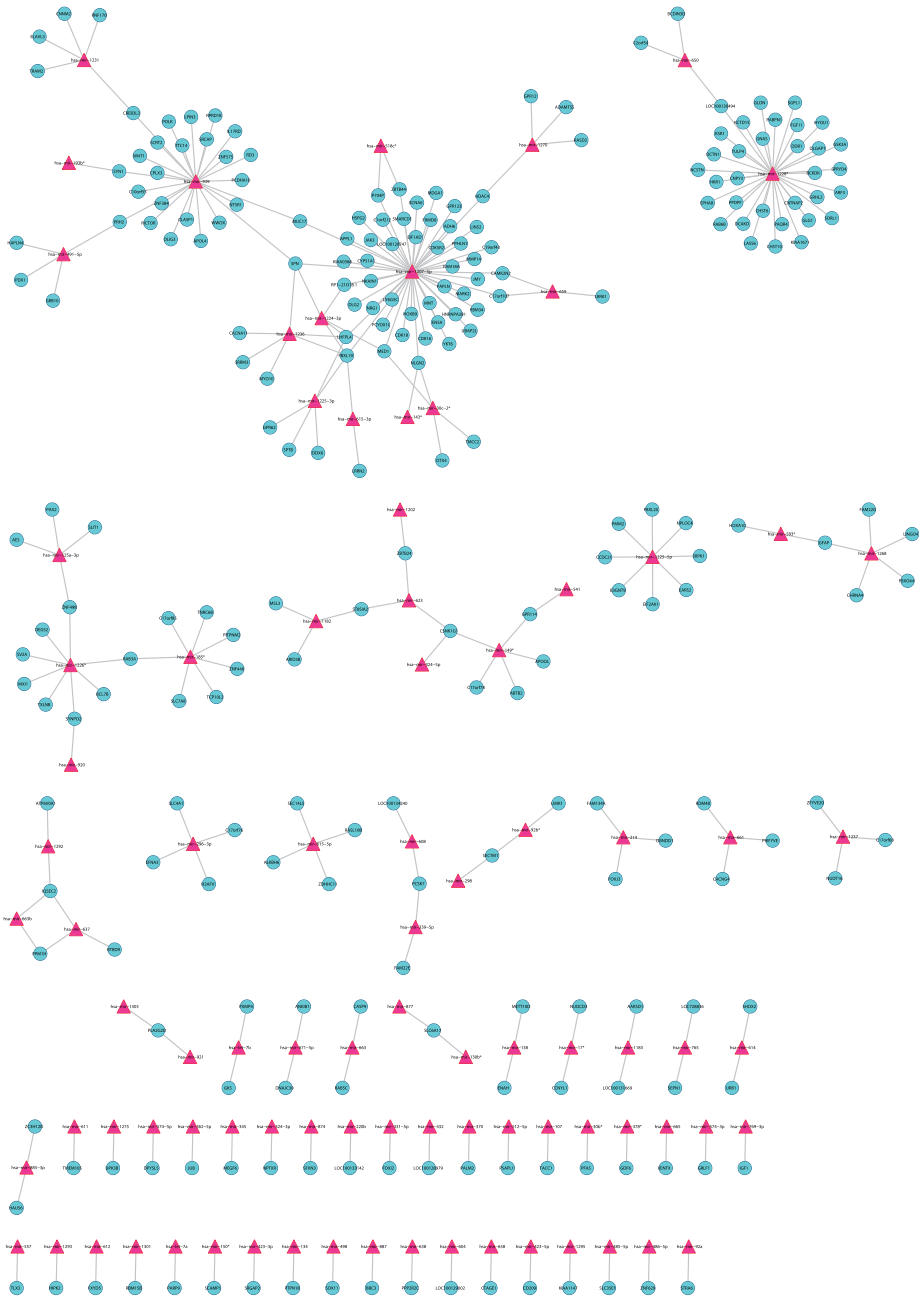

Fig. 1 Interaction network of miRNAs–mRNAs in lung cancer by cytoscape 3 software. MiRNAs are presented in *triangle* and colored in *pink*, while mRNAs are expressed by *circular* and in *sky blue*

cancer, which will be helpful in elucidating disease mechanisms, providing better targeted agents and finding sensitive early biomarkers [6].

In this study, we constructed and analyzed the interaction network of miRNA and their target miRNA in lung cancer by using bioinformatics tools and matched mRNA, miRNA expression profiles data. This study showed that 105 of 249 mRNA in the network are down-regulated by three miRNA, miR-1207-5p, miR-1228* and miR-939. miR-1207-5p regulated the most number of mRNA in the interaction network. Chen et al. [25] reported that the expression of miR-1207-5p was significantly decreased in gastric cancer tissues compared with the adjacent tissues. miR-1207-5p could bind to the human telomerase reverse transcriptase (hTERT) 3′ UTR and down-regulate expression of hTERT. hTERT can reverse telomere shortening, as the catalytic subunit of telomerase, and then prevent proliferation, reduce invasion and induced cell cycle arrest in

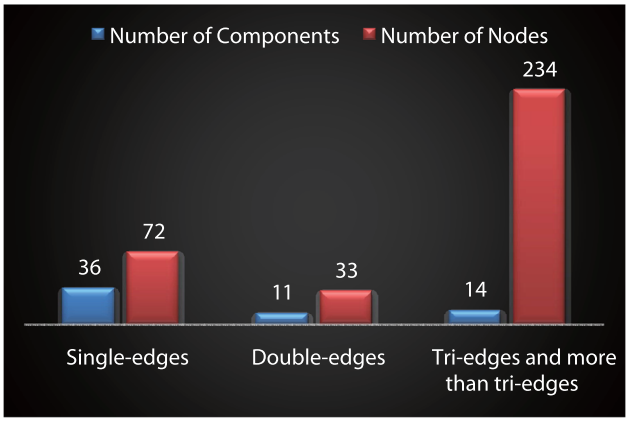

Fig. 2 The statistics of connected components. There are 36 single edges, 11 double edges and 14 triple edges or with more than three edge components. These types of connected components, respectively, involve 72, 33 and 234 nodes

gastric cancer cells in vitro. Loss of miR-1207-5p may boost hTERT protein expression and elevate the development of gastric cancer. Previous reports indicated that hTERT gene amplification causes hTERT overexpression in lung adenocarcinoma and is an independent poor prognostic marker in NSCLC [26]. But there is no clear evidence on the relationship between miR-1207-5p and hTERT in NSCLC. Our analysis firstly pointed out miR-1207-5p as an important regulator in miRNA–mRNA interaction network.

miR-1228* was significantly decreased in human gastric cancer tissues compared with normal tissues. Overexpression of miR-1228* prevented xenograft tumor formation in vivo using the tumor xenograft model. Selective restoration of miR1228* might be advantageous for therapy of gastric cancer [27]. miR-939 is substantially highly expressed in ADC compared with their age- and gender-matched control sera [28]. miR-939 may have relevance for early diagnostic biomarker of ADC. But its mRNA targets and molecular mechanism are unknown. Both miR-1228* and miR-939 are found as essential nodes in our network analysis. Furthermore, our analysis provided the refined mRNA targets and potential mechanistic understanding of the two miRNAs.

4.2 Significant mRNAs in the Interaction Network

HDAC4, MED1, SPN and ST8SIA2 were identified as the most important controlled genes in this interaction network because they are regulated by more than one miRNA in lung cancer (Table 1) and their contribution to lung cancer were confirmed in previous reports (Table 2).

SPN (sialophorin) is known as CD43. In the interaction network, it linked to four miRNAs (miR-939, miR-1224-3p, miR-1236 and miR-1207-5p). Normally, SPN is produced by white blood cells as a transmembrane sialoglycoprotein. Its main cellular functions are intercellular adhesion, intracellular signaling, apoptosis, migration and proliferation [29]. But abnormal expression has been found in cancers, including adenoid cystic carcinoma [30, 31], SCLC and NSCLC. Fu et al. [29] found that SPN could cause lung cancer pathogenesis by various ways, including preventing malignant cells from NK attack and apoptosis, driving metastasis by mechanisms of anti-adhesion, pro-adhesion and migration. MED1 (mediator complex subunit 1) is regulated by three miRNA (miR-1207-5p, miR-1224-3p and miR-30c-2*). Kim et al. [32] demonstrated that the loss of MED1 expression was highly correlated with increased rates of invasion and metastasis in NSCLC. Knockdown of MED1 in NSCLC cell lines results in the change of mRNA levels of the metastasis-related genes. These results indicate that MED1 regulate the invasion and metastasis of NSCLC by regulating the expression of multifold metastasis-related genes. HDAC4 (histone deacetylase 4) gene is linked to two miRNA (miR-1270 and miR-1207-5p). HDAC4 encodes a histone deacetylase and represses gene transcription by influencing transcription factor access to DNA in cells [33]. Previously, HDAC4 is reported to influence cell differentiation in other types of cancer, such as leukemia [34, 35]. But its role in lung development of HDAC4 is unclear. ST8SIA2 (ST8 alpha-*N*-acetyl-neuraminide alpha-2, 8-sialyltransferase 2) is regulated by two miRNA (miR-623 and miR-1182). ST8SIA2 can synthesize polysialic acid (PSA) independently and transfer sialic acid through alpha-2, 8-linkages to the alpha-2, 3-linked and alpha-2, 6-linked sialic acid of *N*-linked oligosaccharides of glycoproteins [36]. Tanaka et al. [37, 38] indicated that PSA played an important role in tumor development, particularly formation of metastatic foci, and was associated with a poor postoperative prognosis and specifically expressed in advanced-stage NSCLC.

Our analysis of miRNA–mRNA provided many potential miRNA regulators of these four genes, uncovering another layer of modulation on lung cancer initiation and development at global level. It should be noted that all of these genes are targeted by more than one miRNA modulator, indicating that the combined effects of multiple miRNAs may play an important role in lung cancers.

4.3 Significant Gene Ontology Annotations

The result of gene ontology annotation manifested the molecular function is concentrated in transferase activity. Five terms of molecular function are subsets of transferase activity. The smallest subset is protein serine/threonine kinase activity, and it is a subset of all other terms about

Table 1 The genes in the miRNA–mRNA network, which are regulated by more than one miRNA

No	Gene name	Explanation	MiRNA	Correlation	*q* value
1	C17orf107	Hypothetical protein LOC100130311	miR-1207-5p	−0.857	0.295
			miR-659	−0.782	0.552
2	CAMK2N2	Calcium-/calmodulin-dependent protein kinase II inhibitor 2	miR-659	−0.860	0.278
			miR-1207-5p	−0.797	0.515
3	CREB3L3	cAMP responsive element binding protein 3-like 3	miR-1231	−0.824	0.416
			miR-939	−0.772	0.578
4	GFAP	Glial fibrillary acidic protein	miR-1268	−0.799	0.507
			miR-593*	−0.773	0.576
5	GPR114	G protein-coupled receptor 114	miR-541	−0.770	0.587
			miR-149*	−0.766	0.598
6	HDAC4	Histone deacetylase 4	miR-1270	−0.920	0.057
			miR-1207-5p	−0.780	0.558
7	LOC100130494	Hypothetical LOC100130494	miR-650	−0.814	0.446
			miR-1228*	−0.785	0.548
8	MUC17	Mucin 17, cell surface associated	miR-939	−0.806	0.479
			miR-1207-5p	−0.780	0.555
9	P704P	Prostate-specific P704P	miR-518c*	−0.754	0.626
			miR-1207-5p	−0.751	0.628
10	PCSK7	Proprotein convertase subtilisin/kexin type 7 (pseudogene)	miR-339-5p	−0.800	0.505
			miR-608	−0.751	0.628
11	PLA2G2D	Phospholipase A2, group IID	miR-921	−0.874	0.203
			miR-1303	−0.752	0.628
12	PPM1H	Protein phosphatase 1H (PP2C domain containing)	miR-663b	−0.872	0.208
			miR-637	−0.751	0.628
13	PRH2	Proline-rich protein HaeIII subfamily 1; proline-rich protein HaeIII subfamily 2	miR-491-5p	−0.823	0.417
			miR-939	−0.765	0.598
14	RAB3A	RAB3A, member RAS oncogene family	miR-185*	−0.862	0.270
			miR-1226*	−0.775	0.571
15	RP121O18.1	Kazrin	miR-1207-5p	−0.824	0.416
			miR-1224-3p	−0.777	0.566
16	SECTM1	Secreted and transmembrane 1	miR-298	−0.787	0.546
			miR-92b*	−0.760	0.613
17	SLC6A17	Solute carrier family 6, member 17	miR-130b*	−0.835	0.378
			miR-877	−0.778	0.564
18	ST8SIA2	ST8 alpha-*N*-acetyl-neuraminide alpha-2, 8-sialyltransferase 2	miR-623	−0.853	0.311
			miR-1182	−0.751	0.629
19	SYN1	Synapsin I	miR-939	−0.815	0.442
			miR-193b*	−0.756	0.622
20	SYNPO2	Synaptopodin 2	miR-920	−0.789	0.541
			miR-1226*	−0.785	0.548
21	ZBTB24	Zinc finger and BTB domain containing 24	miR-623	−0.777	0.566
			miR-1202	−0.765	0.598
22	ZBTB44	Zinc finger and BTB domain containing 44	miR-1207-5p	−0.896	0.131
			miR-518c*	−0.850	0.329
23	ZNF498	Zinc finger protein 498	miR-125a-3p	−0.827	0.407
			miR-1226*	−0.784	0.548

Table 1 continued

No	Gene name	Explanation	MiRNA	Correlation	q value
24	CSNK1G1	Casein kinase 1, gamma 1	miR-623	−0.800	0.504
			miR-149*	−0.784	0.548
			miR-324-5p	−0.776	0.567
25	IQSEC2	IQ motif and Sec7 domain 2	miR-637	−0.824	0.416
			miR-1292	−0.784	0.548
			miR-663b	−0.764	0.601
26	MED1	Mediator complex subunit 1	miR-1207-5p	−0.833	0.387
			miR-1224-3p	−0.818	0.433
			miR-30c-2*	−0.784	0.548
27	NLGN2	Neuroligin 2	miR-1207-5p	−0.823	0.416
			miR-30c-2*	−0.790	0.537
			miR-143*	−0.775	0.571
28	LHFPL4	Lipoma HMGIC fusion partner-like 4	miR-1225-3p	−0.845	0.337
			miR-1207-5p	−0.793	0.530
			miR-1236	−0.793	0.530
			miR-1224-3p	−0.756	0.621
29	SPN	Sialophorin	miR-939	−0.872	0.209
			miR-1224-3p	−0.813	0.446
			miR-1236	−0.794	0.530
			miR-1207-5p	−0.790	0.538
30	FBXL19	F-box and leucine-rich repeat protein 19	miR-1236	−0.837	0.370
			miR-1207-5p	−0.806	0.481
			miR-615-3p	−0.794	0.528
			miR-1224-3p	−0.793	0.530
			miR-1225-3p	−0.788	0.541

transferase activity in this analysis. Some members of serine/threonine kinases, such as protein kinase C (PKC), are involved in NSCLC [39]. They play a key role in cancer cell proliferation, polarity and survival [40].

There are seven GO terms involved in biological processes regulation. The term of regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process and the term of regulation of nitrogen compound metabolic process shared the completely same genes. The term of regulation of transcription is a subset of above two terms. The genes of regulation of cellular carbohydrate catabolic process and regulation of carbohydrate catabolic process are the same. The other regulation involves gene expression and macromolecule biosynthetic process. Most genes of the above terms are attached to the term of cellular process. Overall, these results suggested that lung cancer is derived from systemic dysfunction of various cellular aspects.

Lung cancer is a type of respiratory system disorder. Interestingly, we found that 63 network refined genes were classified as the nervous system phenotype and 25 genes were classified as the nervous system development (Table 3). Combining above two lists, we get 67 non-redundant genes associated with the nervous system. Among them, 21 genes have been associated with lung cancer in other reports (given in Table 2 by adding an asterisk (*) in Gene name column). It is worth mentioning that the genes ST8SIA2, MED1 and HDAC4, which are regulated by more than one miRNA in this miRNA–mRNA interaction network, appeared again here. Thus, the three genes may influence both lung cancer and nervous system.

The relationship between nervous system and lung cancers has been noticed in other's studies. For example, [41] and Stephanie [42] found that NSCLC often affects the central nervous system (CNS). CNS frequently causes the decline of the life quality and the shortened survival as a complication. But at the same time, the available treatment options are limited. So focusing on the genes that both are related to NSCLC and nervous system may provide a novel approach to treat these complications.

Table 2 The genes in the miRNA–mRNA network, which are related to lung cancer (provided by Malacards)

No.	Gene name	Malacards score	MiRNA	Correlation	q value
1	HOXB9	19.68	miR-1207-5p	−0.853	0.310
2	ELAVL3*	18.91	miR-1231	−0.757	0.619
3	SHOX2*	17.35	miR-614	−0.829	0.404
4	CASP9*	17.02	miR-663	−0.792	0.531
5	WNT1*	16.02	miR-939	−0.880	0.181
6	HNRNPA2B1	15.87	miR-1207-5p	−0.765	0.600
7	EIF1AD	15.08	miR-1207-5p	−0.816	0.442
8	BBC3*	14.16	miR-887	−0.810	0.458
9	FXYD5	14.12	miR-612	−0.786	0.548
10	HOXA10*	11.11	miR-593*	−0.757	0.620
11	MNT	10.8	miR-1207-5p	−0.790	0.537
12	HKR1	10.56	miR-1228*	−0.757	0.620
13	FAM134A	10.49	miR-214	−0.925	0.046
14	DPYSL5*	9.97	miR-574-5p	−0.753	0.627
15	PDX1	8.93	miR-491-5p	−0.812	0.446
16	NPTXR*	8.24	miR-324-3p	−0.758	0.618
17	IGF1*	8.06	miR-769-3p	−0.773	0.576
18	MMP14*	8.03	miR-1207-5p	−0.777	0.566
19	JAK3	7.81	miR-1207-5p	−0.801	0.502
20	DDR1	7.72	miR-1228*	−0.763	0.601
21	CHRNA4*	7.6	miR-1268	−0.879	0.181
22	NTSR1*	7.34	miR-939	−0.788	0.541
23	ST8SIA2*	7.13	miR-623	−0.853	0.311
			miR-1182	−0.751	0.629
24	MXI1	7.1	miR-1226*	−0.751	0.628
25	LIMK1*	5.77	miR-92b*	−0.752	0.627
26	SGPL1	5.37	miR-1228*	−0.816	0.442
27	POLK	5	miR-939	−0.837	0.370
28	MED1*	4.73	miR-1207-5p	−0.833	0.387
			miR-1224-3p	−0.818	0.433
			miR-30c-2*	−0.784	0.548
29	GNAS*	4.42	miR-1228*	−0.751	0.628
30	HSPG2*	3.99	miR-1207-5p	−0.769	0.591
31	ENAH*	2.55	miR-138	−0.781	0.555
32	NRG1*	1.95	miR-1207-5p	−0.816	0.442
33	HDAC4*	1.93	miR-1270	−0.920	0.057
			miR-1207-5p	−0.780	0.558
34	WWOX*	1.75	miR-939	−0.842	0.346
35	SPN	1.08	miR-939	−0.872	0.209
			miR-1224-3p	−0.813	0.446
			miR-1236	−0.794	0.530
			miR-1207-5p	−0.790	0.538

These genes by adding asterisk (*) in Gene name column represent that are related to nervous system. The details will be discussed in Sects. 4.2 and 4.3. The correlation score and q value were given by MAGIA

Table 3 GO categories for the target genes that are participate in miRNAs–mRNAs interactions in cancer

Category/attribution	Term/descriptors	Count	*p* value
GO-molecular function (by DAVID)	Transferase activity	35	0.012
	Transferase activity, transferring phosphorus-containing groups	23	0.008
	Kinase activity	22	0.003
	Phosphotransferase activity, alcohol group as acceptor	19	0.007
	Protein kinase activity	16	0.014
	Protein serine/threonine kinase activity	12	0.026
	Transcription cofactor activity	10	0.049
GO-cellular component (by DAVID)	Intracellular part	147	0.043
	Plasma membrane part	38	0.041
	Organelle membrane	23	0.021
	Golgi apparatus	19	0.028
	Endomembrane system	18	0.021
	Vesicle	15	0.045
	Synapse	10	0.037
	Anchored to membrane	8	0.022
	Synapse part	8	0.037
	Vesicle membrane	6	0.043
	Clathrin-coated vesicle membrane	4	0.029
	Synaptic vesicle membrane	3	0.034
GO-biological process (by DAVID)	Cellular process	143	0.049
	Localization	49	0.035
	Regulation of gene expression	47	0.037
	Regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	46	0.043
	Regulation of macromolecule biosynthetic process	46	0.047
	Regulation of nitrogen compound metabolic process	46	0.049
	Regulation of transcription	43	0.045
	Nervous system development	25	0.005
	Phosphorylation	17	0.044
	Protein amino acid phosphorylation	16	0.021
	Small GTPase-mediated signal transduction	10	0.015
	Skeletal system development	10	0.019
	Enzyme-linked receptor protein signaling pathway	10	0.029
	Secretion by cell	7	0.047
	Neurotransmitter transport	5	0.0207
	Establishment or maintenance of cell polarity	4	0.0237
	Mammary gland development	4	0.045
	Regulation of cellular carbohydrate catabolic process	3	0.017
	Regulation of carbohydrate catabolic process	3	0.017
Phenotype (by GeneDecks)	Mortality/aging	67	2.18E−11
	Nervous system phenotype	63	7.77E−16
	Growth/size/body phenotype	54	1.20E−09
	Behavior/neurological phenotype	50	4.90E−11
	Homeostasis/metabolism phenotype	50	1.01E−06
	Cellular phenotype	48	1.13E−08
	Craniofacial phenotype	23	9.45E−07
Kegg pathway (by DAVID)	Axon guidance	6	0.012

5 Conclusion

The expression profiles of miRNAs and mRNAs in matched samples provided a good opportunity to construct miRNA–mRNA interaction network via bioinformatics tools. In this system-level analysis, we found a number of master miRNAs and mRNAs, which are potentially important for lung cancer initiation and development. This global interaction network of miRNA–mRNA will contribute to refine miRNA target predictions and developing novel therapeutic candidates.

Acknowledgments This work was supported by the National High Technology Research and Development Program of China (863 Program, Grant No. 2014AA021102), the National Natural Science Foundation of China (U1304311, 31100901), China Postdoctoral Science Foundation funded Project (2013M531064, 2014T70363), Natural Science Foundation of Heilongjiang Province of China (QC2010012), Heilongjiang Postdoctoral Foundation (LBH-Z12171).

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