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Original Article

Diagnostic significance of circulating let-7c-5p and its potential target gene network in non-small cell lung cancers

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Abstract: Background: Variations of the microRNA (miRNA) expression pattern have progressively been related to pathophysiology of cancer cells, which facilitates the interest of miRNAs becoming one of the most explored molecules in the field of cancer research, especially non-small cell lung cancers (NSCLCs). Circulating miRNAs indicate an extraordinary influence as diagnostic biomarkers, since they play an essential role in many cellular processes contributing to carcinogenesis. However, the clinical diagnostic significance of circulating let-7c-5p in NSCLC patients remains unclarified. Hence, the present meta-analysis was performed to evaluate the probability of circulating let-7c-5p as a diagnostic biomarker with microarray datasets for NSCLCs, as well as its potential molecular mechanism. Material and methods: NSCLC-related circulating miRNA microarray datasets were accumulated from two individual public databases: the NCBI Gene Expression Omnibus (GEO) and EBI Array Express, till 30th November 2015. The quality control of the data output was assessed with Limma package and ExiMiR package in R. Standardized mean difference (SMD) and 95% confidence intervals (CIs) of let-7c-5p level was analyzed with STATA 12.0. Cochran's Q test, as well as the I^2 statistic was used to value the probable heterogeneity. Significant heterogeneity was considered when a p value was < 0.05 or I^2 was > 50%. The stability of the pooled results was estimated with sensitivity analysis. In addition, the predicted target genes of let-7c-5p by bioinformatics approaches were evaluated with Gene Ontology (GO) and KEGG pathway. Results: Expression data of circulating let-7c-5p from NSCLC patients (n = 250) and healthy controls (n = 242) was extracted from 6 miRNA datasets (GSE61741, GSE46729, GSE40738, GSE24709, GSE17681 and GSE27486). A remarkable downregulation of circulating let-7c-5p level was achieved in NSCLC cases as compared to that in the control groups (SMD = -0.425; 95% CI, -0.793 to -0.057; P = 0.023), as assessed with random-effects model, since significant heterogeneity existed (P = 0.005, I^2 = 70.3%). Furthermore, 154 validated targeting genes were recruited by literatures screening and in silico prediction via miRWalk. The bioinformatics analysis indicated that let-7c-5p may play an important role in the development of NSCLC through Chronic myeloid leukemia pathway, Adherens junction pathway, ErbB signaling pathway, Pathways in cancer, MAPK signaling pathway and other pathways. Conclusions: Let-7c-5p expression level is significantly reduced in peripheral blood and whole blood cells of NSCLC patients. Let-7c-5p may play a vital role in the tumorigenesis of NSCLC via targeting different genes and influencing variant pathways. However, the diagnostic value of let-7c-5p is still required to be confirmed with larger cohorts. More well-designed experiments are also desired to explore the molecular mechanism of let-7c-5p in NSCLC.

Keywords: Biomarker, non-small cell lung cancer, meta-analysis, microarray datasets, let-7c-5p, bioinformatics

Introduction

MicroRNAs (miRNAs) are small molecules (about 20 nucleotides) with essential power on the function of cells, which regulate the expression level of their target genes generally on the post-transcriptional level via binding to complementary sequences on corresponding mRNAs and lead to gene silencing [1, 2]. Hitherto, the miRNA Database MiRBase (http://www.mirbase.org) presents 1881 precursors and 2588 mature Homo sapiens miRNAs [3, 4]. Some of the miRNAs have been investigated to play important roles in the carcinogenesis and development of different malignancies [5, 6]. Cancer-related tissue miRNAs, as well as circu-

lating miRNAs, have also been proved to provide an encouraged prospect of detecting neoplasia at their early stages [5, 7-9].

Non-small cell lung cancer (NSCLC) is the prominent cause of cancer related deaths worldwide. The 5-year overall survival rate is strongly correlated with the appropriate timing of diagnosis [8, 10-16]. As a non-invasive tool for screening of NSCLCs, blood based analysis approach offers a compatible choice for clinic. Some circulating miRNAs have been validated as constant and prospective biomarkers in early diagnosis of NSCLCs [17-20], such as miR-21, miR-25, miR-126, miR-141, miR-155, etc. However, there is no appropriate biomarker established for NSCLC in a screening setting at early stages as of now. Hence, there is an undisputable requirement for molecular investigations to assist in the early diagnosis of NSCLCs.

Let-7c-5p has been studied in several cancers. Zhao et al. [21] found that the expression of let-7c-5p in NSCLC tissues was significantly lower than that in normal lung tissues. Similar to the downregulating pattern in cancer tissues, Dou et al. [22] reported that the expression of let-7c-5p in plasma was consistently downregulated in the patients with NSCLC and the ROC curve for let-7c-5p showed a solid diagnostic value with AUC of 0.714. However, only the aforementioned two studies have explored the significance of let-7c-5p on NSCLC. The diagnostic effect of circulating let-7c-5p on NSCLC remains still unclear.

Since a single miRNA can regulate lots of target genes, it is supposed that the majority of the human genes (20,000-25,000) can be regulated by particular miRNAs [23]. Silencing of the target genes is evidently the central mechanism of regulation, including via translational repression and mRNA degradation. To abundantly benefit from the huge number of miRNAs and their potential targets, computational algorithms like miRWalk (http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/) has been developed to detect adequate miRNA targets [24, 25], which hosts the predicted and the experimentally validated miRNA-target interaction pairs [24, 25], thus miRWalk can provides a publicly available comprehensive and convenient bioinformatics resource.

Thus, the current study included two parts. The first part was a meta-analysis carried out to assess the possibility of circulating let-7c-5p as a biomarker with microarray datasets for early detection of NSCLCs, followed by the second part of a systematic gene signature of validated targets of let-7c-5p with bioinformatics methods.

Materials and methods

Data acquisition

The 2 well-established databases of microarray were searched for the current meta-analysis up to 30th November 2015: the National Center of Biotechnology Information (NCBI) Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) and the European Bioinformatics Institute (EBI) Array Express (http://www.ebi.ac.uk/arrayexpress/). The following keywords were: bronchi OR lung OR bronchioles OR alveoli OR pulmonary OR respiratory, carcinoma OR tumor OR cancer OR adenocarcinoma OR neoplas* OR malignan*, OR blood ser* OR plasma OR circulating, miRNA OR microRNA OR noncoding RNA.

Inclusion criteria

The inclusion criteria were: (i) NSCLC patients were considered as case group and non-cancerous healthy persons were as controls, and each study should have at least 3 samples; (ii) the original expression profiling data of miRNAs should be offered or could be calculated.

Quality control and data extraction

Two authors (Dongning Huang and Li Qin) extracted the data from all qualified datasets in line with inclusion criteria independently. Discrepancies were resolved via careful consideration with the third author (Haixin Huang). Quality control was performed with Limma package and ExiMiR package in R, including background correction and normalization processing [26, 27]. Expression values of let-7c-5p and sample size were collected. Means and standard deviations (SD) of these expression values were calculated.

Statistical analysis

The meta package in R was employed to carry out the present meta-analysis [26, 28]. Continuous outcomes were estimated as stan-

Table 1. Characteristics of hsa-let-7c-5p expression profiling datasets included in the current meta-analysis

Series	Tissue	Platform	Lung cancer types	Country	Sample of Lung Cancer Patients	Sample of Healthy Controls	Citation (ref.)	Year
GSE61741	Peripheral blood	GPL9040	NSCLC	Germany	73	109	Keller A.	2014
GSE46729	Serum	GPL8786	NSCLC	USA	24	24	Godrey A, et al.	2014
GSE40738	Whole blood	GPL16016	NSCLC	USA	86	59	Patnaik SK, et al.	2012
GSE27486	Whole blood	GPL11432	NSCLC*	USA	22	12	Patnaik SK, et al.	2011
GSE24709	Peripheral blood	GPL9040	NSCLC	Germany	28	19	Keller A, et al.	2011
GSE17681	Peripheral blood	GPL9040	NSCLC	Germany	17	19	Keller A, et al.	2009

NSCLC: non-small cell lung carcinoma. *Only adenocarcinomas were involved.

Table 2. Forest plot of studies evaluating standard mean difference (SMD) of hsa-let-7c-5p expression between NSCLC and control group (a random-effects model)

	Experimental			Control				
Study	Mean	SD	Total	Mean	SD	Total	Weight	SMD. Random [95% CI]
GSE61741	8.466539463	1.116836837	73	8.210447531	1.758626047	109	21.88%	0.167 [-0.130, 0.464]
GSE46729	4.884751292	0.663548196	24	5.199114083	0.743794157	24	15.76%	-0.446 [-1.019, 0.127]
GSE40738	-1.10066039	0.505776181	86	-0.934552213	0.425154876	59	21.08%	-0.350 [-0.684, -0.016]
GSE24709	8.817995811	1.021785898	28	9.396355936	1.259252917	19	15.36%	-0.515 [-1.107, 0.077]
GSE17681	12.20957075	0.636246072	17	12.94234859	0.61790384	19	13.06%	-1.169 [-1.880, -0.459]
GSE27486	-1.200640093	0.547758744	22	-0.876657818	0.333798332	12	12.86%	-0.668 [-1.390, 0.054]
Total [95% CI]			250			242	100.00%	-0.425 [-0.793, -0.057]

Heterogeneity: $Chi^2 = 16.85$; df = 5 (P = 0.005); $I^2 = 70.3\%$. Test for overall effect: Z = 2.27 (P = 0.023).

dard mean difference (SMD) with 95% confidence interval (CI), and effect sizes were pooled with random- or fixed-effects model according to different conditions. Heterogeneity across studies was assessed with the chi-square test of Q and the I^2 statistic [26, 29, 30]. A *P* value < 0.05 or $I^2 > 50\%$ was considered as heterogeneous and the random-effects model (DerSimonian-Laird method) would be chosen to calculate the pooled SMD. Otherwise, the fixed-effects model (Mantel-Haenszel method) would be selected for the pooling process [31].

To further reveal whether the pooled result was due to one single study with a divergent result, sensitivity analysis was performed to omit one study at a time. Additionally, the potential publication bias was evaluated with Begg's and Egger's tests. When p was less than 0.05, the results would be considered as containing publication bias.

Experimentally validated target genes of let-7c-5p

Firstly, we performed a full-scale literature screening to collect all experimentally validated target genes with the following keywords: let-7c-5p OR let-7c*, lung OR pulmonary OR respiratory OR bronchi OR bronchioles OR alveoli OR pneumocytes, cancer OR carcinoma OR tumor OR neoplas* OR malignan* OR adenocarcinoma. The searching databases included PubMed, Wiley Online Library, Web of Science, Science Direct, Cochrane Central Register of Controlled Trials, Google Scholar, EMBASE, Ovid, LILACS, Chinese CNKI, Chong Qing VIP, Wan Fang, China Biology Medicine disc (up to November 30, 2015). Next, we collected the validated genes provided by miRWalk (http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/ [24, 25]). Two groups of gene clusters were eventually merged together for further analysis.

GO analysis

GO analysis was conducted in DAVID Bioinformatics Resources 6.7 (https://david.ncifcrf.gov/), and visualized with the Bingo plugin of Cytoscape v3.2.1 software. Genes were sorted into three major groups, including biological process (GO-BP), cellular component (GO-CC) and molecular function (GO-MF).

Pathway analysis

The KEGG Pathway data obtained from DAVID Bioinformatics Resources 6.7 (https://david.

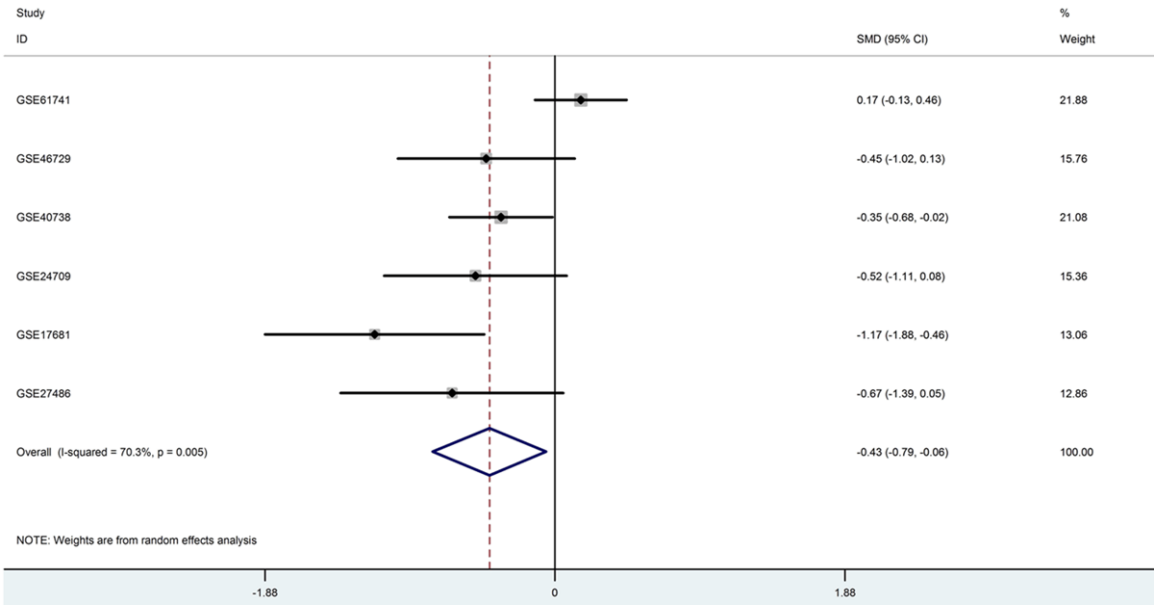

Figure 1. Forest plot of meta-analysis of the diagnostic value of let-7c-5p expression for patients with NSCLCs with six datasets involved. Random effects model was applied when combining standardized mean difference (SMD).

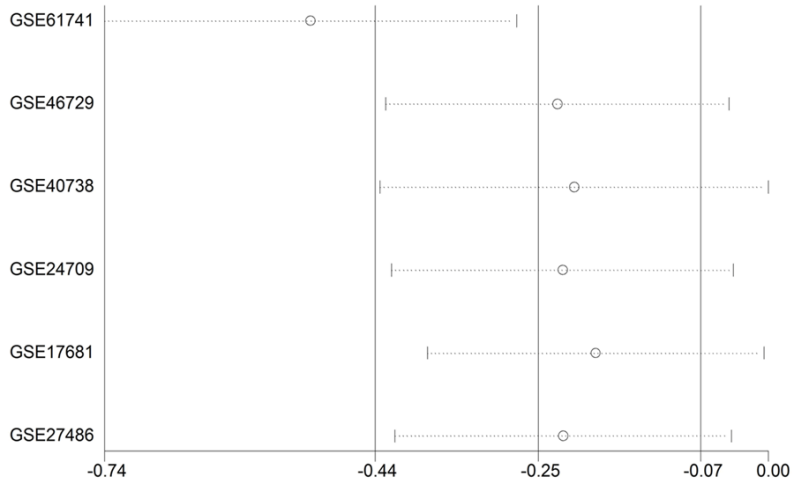

Figure 2. Sensitivity analysis of the value of let-7c-5p on the early diagnosis of NSCLC with six datasets involved.

ncifcrf.gov/) was used in genes interactions. Differentially modified genes were mapped to KEGG pathway database, and the enrichment *P*-value was performed for each pathway.

Results

Features of the included datasets for meta-analysis

Table 1 showed the details of included 6 datasets, including GSE61741 (Germany), GSE46729 (USA), GSE40738 (USA), GSE27486 (USA), GSE24709 (Germany), and GSE17681 (Germany). GSE61741, GSE24709, and GSE17681 were from peripheral blood cells, while GSE46729 was from serum, and GSE40738 and GSE27486 were from whole blood. In sum, 250 NSCLC cases and 242 controls were finally enrolled.

Potential diagnostic significance of circulating let-7c-5p for lung cancer

The pooled means and SD was showed in **Table 2** and the SMD ranged from -1.169 to 0.167 (**Figure 1**). The heterogeneity test showed that significant heterogeneity was found among these individual datasets (P = 0.005, I^2 = 70.3%, **Figure 2**). Thus, the random-effects model was used to calculate the pooled SMD and 95% CI and the result showed that there was markedly difference between NSCLC and non-cancerous control group (SMD = -0.425; 95% CI, -0.793 to -0.057, P = 0.023) and lower circulating let-7c-5p expression was more likely to exist in NSCLC patients as com-

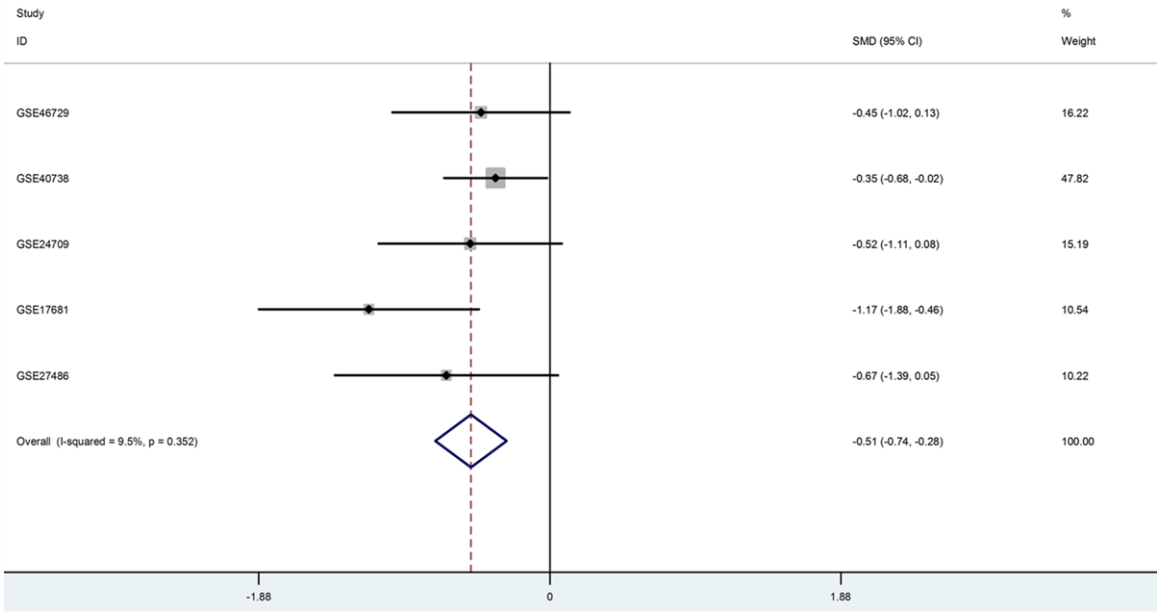

Figure 3. Forest plot of meta-analysis of the diagnostic value of let-7c-5p expression for patients with NSCLCs after removing GSE61741. Fixed effects model was applied when combining standardized mean difference (SMD).

Table 3. Forest plot of studies evaluating standard mean difference (SMD) of hsa-let-7c-5p expression between NSCLC and control group (a fixed-effects model) after removal of GSE61741

	Experimental			Control				
Study	Mean	SD	Total	Mean	SD	Total	Weight	SMD. Random [95% CI]
GSE46729	4.884751292	0.663548196	24	5.199114083	0.743794157	24	17.31%	-0.446 [-1.019, 0.127]
GSE40738	-1.10066039	0.505776181	86	-0.934552213	0.425154876	59	43.50%	-0.350 [-0.684, -0.016]
GSE24709	8.817995811	1.021785898	28	9.396355936	1.259252917	19	16.30%	-0.515 [-1.107, 0.077]
GSE17681	12.20957075	0.636246072	17	12.94234859	0.61790384	19	11.61%	-1.169 [-1.880, -0.459]
GSE27486	-1.200640093	0.547758744	22	-0.876657818	0.333798332	12	11.27%	-0.668 [-1.390, 0.054]
Total [95% CI]			177			133	100.00%	-0.510 [-0.740, -0.279]

Heterogeneity: $Chi^2 = 4.42$; df = 4 (P = 0.352); $I^2 = 9.5\%$. Test for overall effect: Z = 4.33 (P < 0.001).

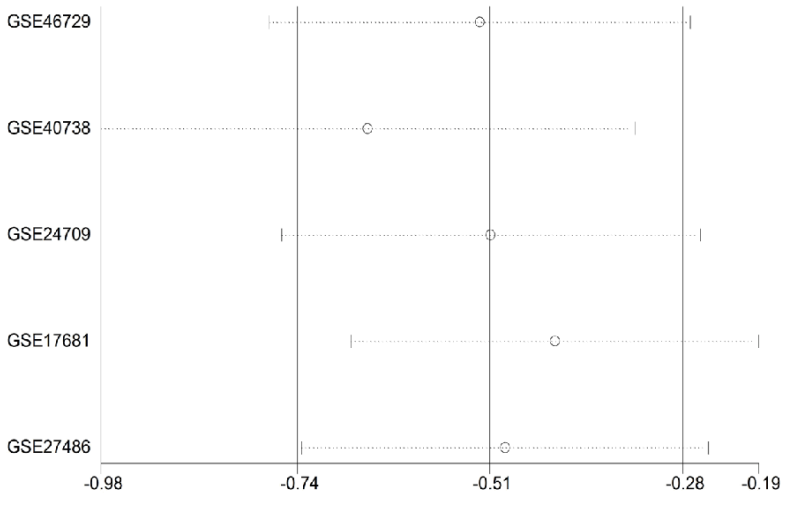

Figure 4. Sensitivity analysis of the value of let-7c-5p on the early diagnosis of NSCLC after removing GSE61741.

pared to non-cancer controls.

Sensitivity analysis and publication bias assessment

According to sensitivity analysis, results indicated that the study of GSE61741 could have the most negative influence on the pooled SMD (**Figures 1** and **2**). Thus, we re-calculated the pooled SMD by removing the data of GSE61741. Since no heterogeneity existed among the left 5 databases (P = 0.352, $I^2 = 9.5\%$), fixed-

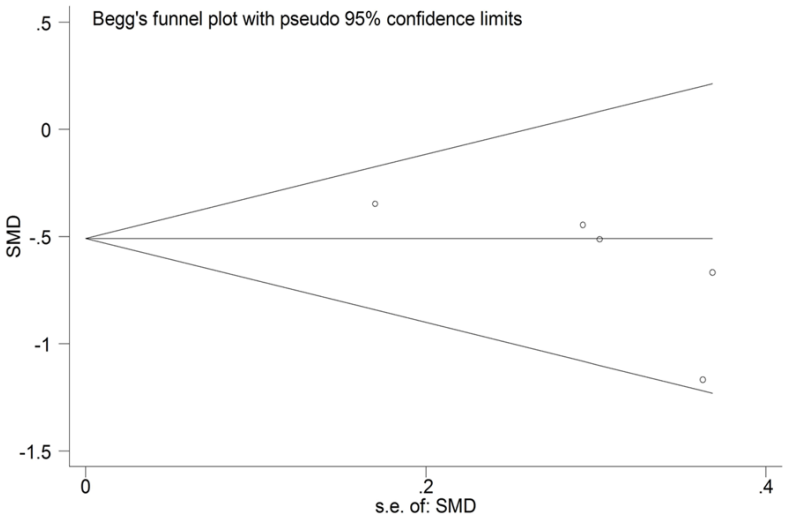

Figure 5. Begg's funnel plot for the assessment of potential publication bias. No potential publication bias was found.

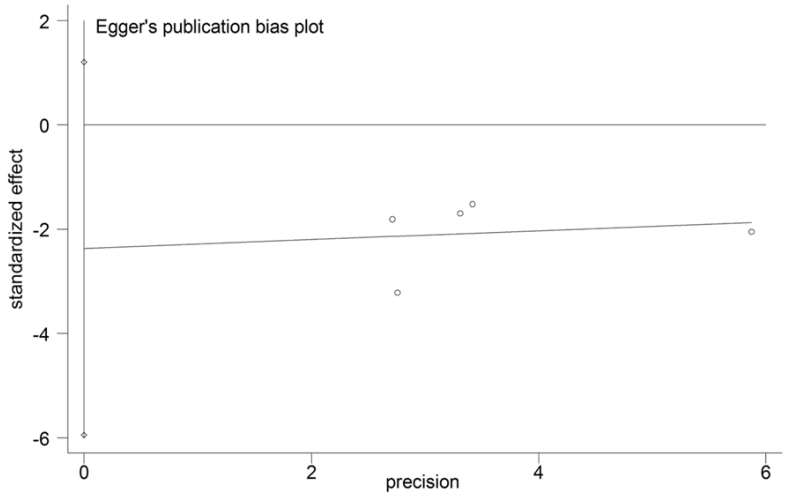

Figure 6. Egger's funnel plot for the assessment of potential publication bias. No potential publication bias was observed.

effects model could be performed for the pool SMD, which showed similar, but more evident trend compared to that before GES61741 being omitted (SMD = -0.510; 95% CI, -0.74 to -0.279, P < 0.001, **Figure 3**; **Table 3**). This founding indicated that the study of GSE61741 was the source of heterogeneity (**Figure 4**). Consistently, symmetry distribution was present by the Begg's funnel plot and no noticeable publication bias was found after the removal of GSE61741 (**Figure 5**, P = 0.086). In addition, constant no indication of potential publication bias was revealed with Egger's test (**Figure 6**, P = 0.125).

Validated targets, GO analysis and pathway analysis

After proving the downregulation of let-7c-5p in NSCLC, we then further investigated its potential role and targets in NSCLC development. Altogether, 154 target genes were finally gained from literatures searching and miRWalk extraction. In order to investigate the potential mechanism of let-7c-5p, the integrative results of let-7c-5p targets in GO were categorized according to three parts: biological process (BP), cellular component (CC) and molecular function (MF) (**Table 4**; **Figures 7**-**10**). Subsequent to the pathway analysis, there were 10 pathways available with P < 0.05, out of which four signaling pathways were remarkably significant (P < 0.01, **Table 5**): chronic myeloid leukemia pathway (NRAS, ACVR1B, TGFBR1, CBL, BCL2L1, MYC; P = 0.001098827), arrhythmogenic right ventricular cardiomyopathy (ARVC) pathway (ACTB, ATP2A2, ACTN4, CACNG8, RYR2, ITGA10; P = 0.001166809), ribosome pathway (RPL18A, RPL3, RPS4Y1, RPS13, UBA52, RPS24; P = 0.002136602), and adherens junction pathway (ACTB, ACVR1B, CSNK2A1, ACTN4, TGFBR1; P = 0.008671443).

Discussion

The different expression pattern of miRNAs in human lung cancers has improved the understandings of the pathomechanisms of this life-threatening disease, and has also offered new

Table 4. GO analysis of the predicted target genes

GO Category	Term	*P* Value	Targets
Biological Process	GO:0006325: chromatin organization	0.000000069	18
Biological Process	GO:0016568: chromatin modification	0.000000231	15
Biological Process	GO:0051276: chromosome organization	0.000002260	18
Biological Process	GO:0006396: RNA processing	0.000156000	16
Biological Process	GO:0006338: chromatin remodeling	0.000163000	6
Biological Process	GO:0008380: RNA splicing	0.000301000	11
Biological Process	GO:0045884: regulation of survival gene product expression	0.000907000	4
Biological Process	GO:0006414: translational elongation	0.002430000	6
Biological Process	GO:0046456: icosanoid biosynthetic process	0.002870000	4
Biological Process	GO:0006397: mRNA processing	0.002930000	10
Biological Process	GO:0000375: RNA splicing, via transesterification reactions	0.002950000	7
Biological Process	GO:0000377: RNA splicing, via transesterification reactions with bulged adenosine as nucleophile	0.002950000	7
Biological Process	GO:0000398: nuclear mRNA splicing, via spliceosome	0.002950000	7
Biological Process	GO:0006636: unsaturated fatty acid biosynthetic process	0.003740000	4
Biological Process	GO:0001516: prostaglandin biosynthetic process	0.004410000	3
Biological Process	GO:0046457: prostanoid biosynthetic process	0.004410000	3
Biological Process	GO:0016570: histone modification	0.005450000	6
Biological Process	GO:0006633: fatty acid biosynthetic process	0.006120000	5
Biological Process	GO:0016569: covalent chromatin modification	0.006240000	6
Biological Process	GO:0022613: ribonucleoprotein complex biogenesis	0.006500000	7
Biological Process	GO:0040007: growth	0.007030000	7
Biological Process	GO:0030728: ovulation	0.007170000	3
Biological Process	GO:0016071: mRNA metabolic process	0.007350000	10
Biological Process	GO:0006690: icosanoid metabolic process	0.009290000	4
Cellular Component	GO:0031981: nuclear lumen	0.000000091	34
Cellular Component	GO:0031974: membrane-enclosed lumen	0.000000109	39
Cellular Component	GO:0070013: intracellular organelle lumen	0.000000116	38
Cellular Component	GO:0043233: organelle lumen	0.000000209	38
Cellular Component	GO:0008180: signalosome	0.000071200	4
Cellular Component	GO:0030529: ribonucleoprotein complex	0.000131000	15
Cellular Component	GO:0043232: intracellular non-membrane-bounded organelle	0.000153000	40
Cellular Component	GO:0043228: non-membrane-bounded organelle	0.000153000	40
Cellular Component	GO:0005654: nucleoplasm	0.000173000	20

Cellular Component	GO:0005730: nucleolus	0.000300000	17
Cellular Component	GO:0022626: cytosolic ribosome	0.000657000	6
Cellular Component	GO:0005829: cytosol	0.000819000	24
Cellular Component	GO:0044451: nucleoplasm part	0.000945000	14
Cellular Component	GO:0022627: cytosolic small ribosomal subunit	0.004860000	4
Cellular Component	GO:0033279: ribosomal subunit	0.004930000	6
Cellular Component	GO:0033276: transcription factor TFTC complex	0.006240000	3
Cellular Component	GO:0000123: histone acetyltransferase complex	0.009060000	4
Molecular Function	GO:0003723: RNA binding	0.000168000	18
Molecular Function	GO:0000166: nucleotide binding	0.001370000	34
Molecular Function	GO:0030528: transcription regulator activity	0.005740000	24
Molecular Function	GO:0032555: purine ribonucleotide binding	0.008260000	27
Molecular Function	GO:0032553: ribonucleotide binding	0.008260000	27
Molecular Function	GO:0005524: ATP binding	0.008840000	23

Notes: GO analysis was categorized according to three parts: biological process, cellular component and molecular function. Only those *P* value was less than 0.01 were presented in the table.

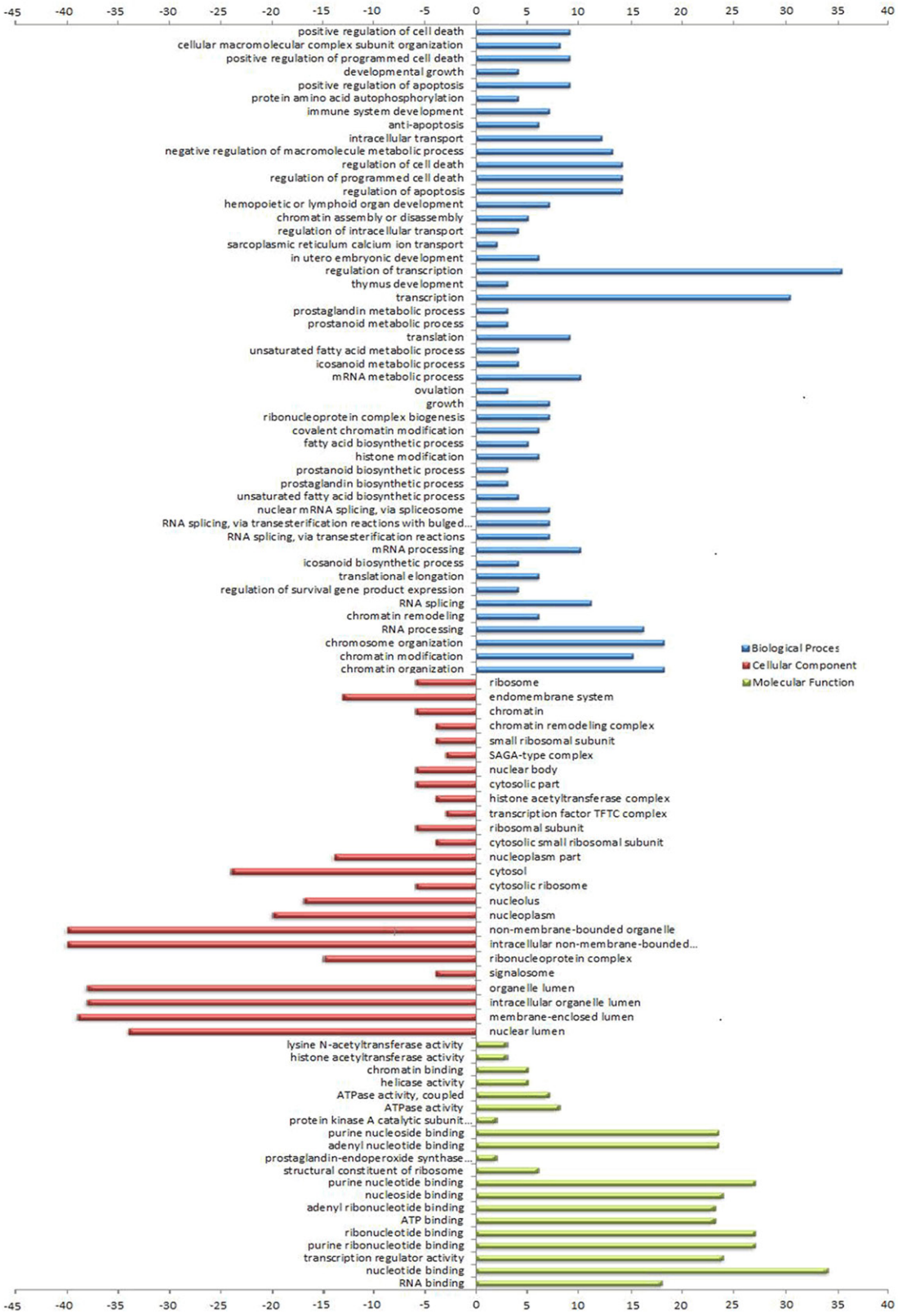

Figure 7. GO analysis of validated target genes of let-7c-5p.

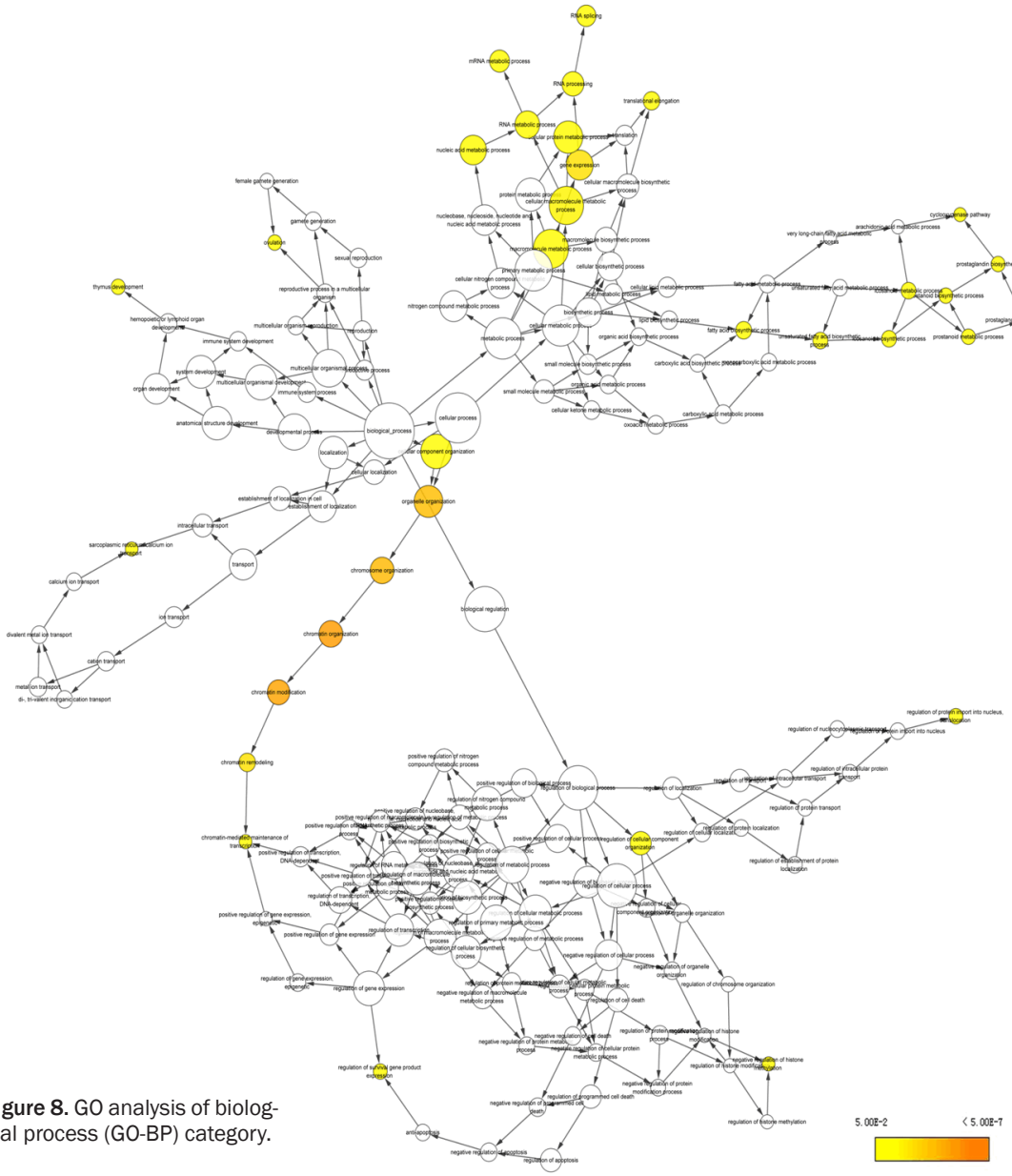

Figure 8. GO analysis of biological process (GO-BP) category.

approaches for diagnose, disease monitoring and even molecular therapy [20]. In clinic, the application of miRNAs as molecular biomarkers is principally attractive due to the outward stability in both formalin fixed paraffin embedded (FFPE) and straightforwardly manageable bodily fluid samples such as blood, serum, sputum, or pulmonary lavage. The invasive diagnostic biopsies are restrained due to the age and comorbidities of some lung cancer patients, hence the minimally invasive techniques are predominantly appropriate for this group of patients [7, 8, 20, 32]. However, no specific miRNA as biomarker has been discovered in clinic for the early diagnosis of NSCLC.

To date, only two groups have performed relevant studies on the clinical significance of let-7c-5p on lung cancers. Zhao et al. [21] reported that the expression of let-7c-5p was reduced in NSCLC tissues as compared to that in normal lung tissues. They also observed a noteworthy relationship between low expression of let-7c and tumor metastasis, venous invasion, advanced clinical TNM stages and poor prognosis of NSCLC patients. Constantly, Dou et al. [22]

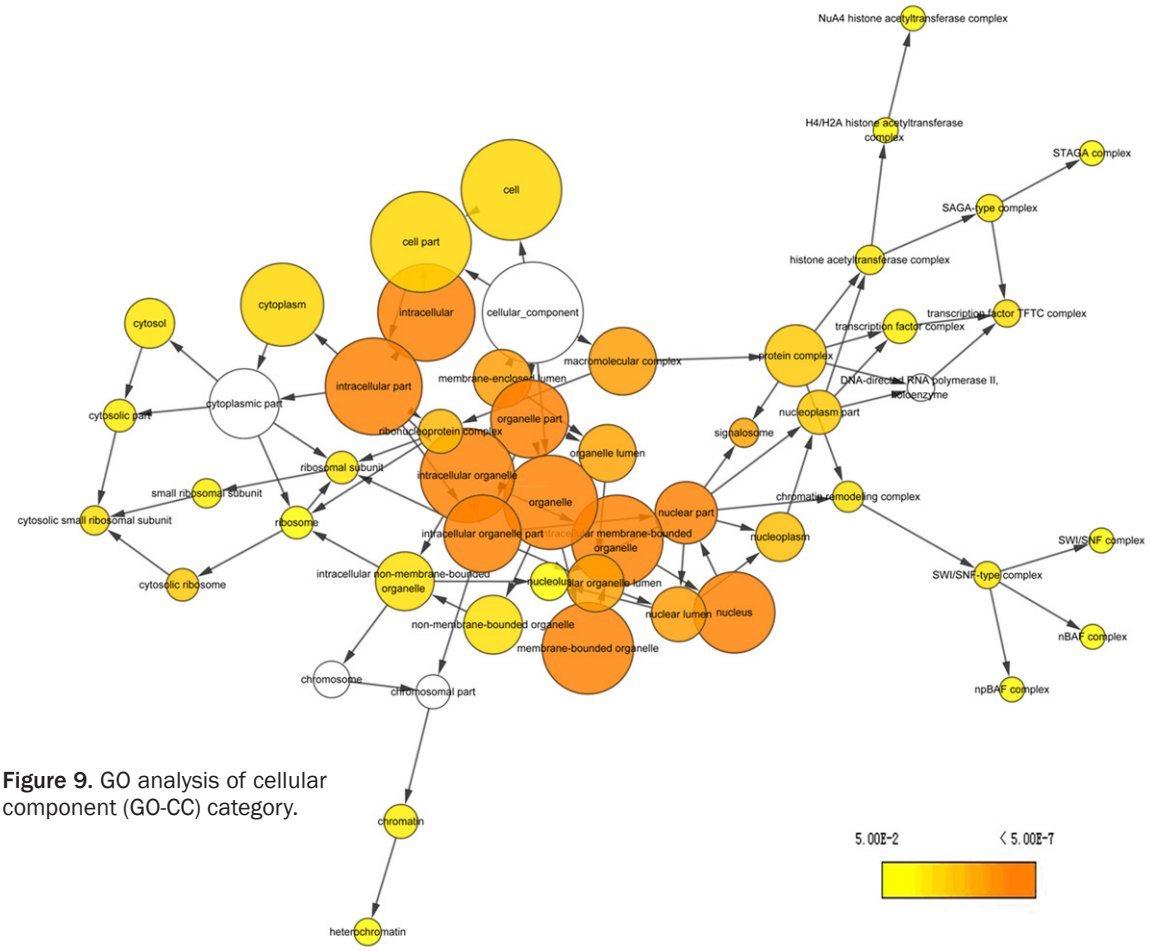

Figure 9. GO analysis of cellular component (GO-CC) category.

found that the plasma let-7c-5p level was also downregulated in NSCLCs. Furthermore, Dou et al. [22] drew the ROC curve for let-7c-5p and achieved a moderate diagnostic value (AUC = 0.714). However, the precise diagnostic effect of circulating let-7c-5p on NSCLC remains still unclear due to the only one available publication. Thus, we performed the current meta-analysis based on GEO microarray datasets, to further confirm if let-7c-5p could be a potential biomarker for the diagnosis of lung cancer or not. Herein, six miRNA profiling datasets with 250 NSCLC samples and 242 healthy controls were finally included (GSE61741, GSE46729, GSE40738, GSE24709, GSE17681 and GSE27486) for analysis. The summary SMD showed that let-7c-5p expression in patients' whole blood, peripheral blood cells and serum was significantly reduced in NSCLC patients than that of the healthy controls (SMD = -0.425; 95% CI, -0.793 to -0.057; P = 0.023), as evaluated with random-effects model, since significant heterogeneity existed (P = 0.005, I^2 = 70.3%). To exclude the influence of heterogeneity, we then re-calculated the pooled SMD by removing the data of GSE61741, which may be the source to produce heterogeneity. The pooled SMD became -0.510 (P < 0.001, 95% CI, -0.74 to -0.279) with fixed-effects model (P = 0.352, I^2 = 9.5%), which presented the similar but more powerful value as that with GSE61741 remaining within the statistics. Thus, the result from current meta-analysis displayed the concordant trend with the report of Dou et al. [22] that markedly lower expression of circulating let-7c-5p occurred in NSCLC patients, as compared to non-cancerous controls.

The biological function and molecular mechanisms of let-7c-5p on NSCLC cells have been investigated by several groups. Zhan et al. [33] performed a series of in vitro and in vivo experi-

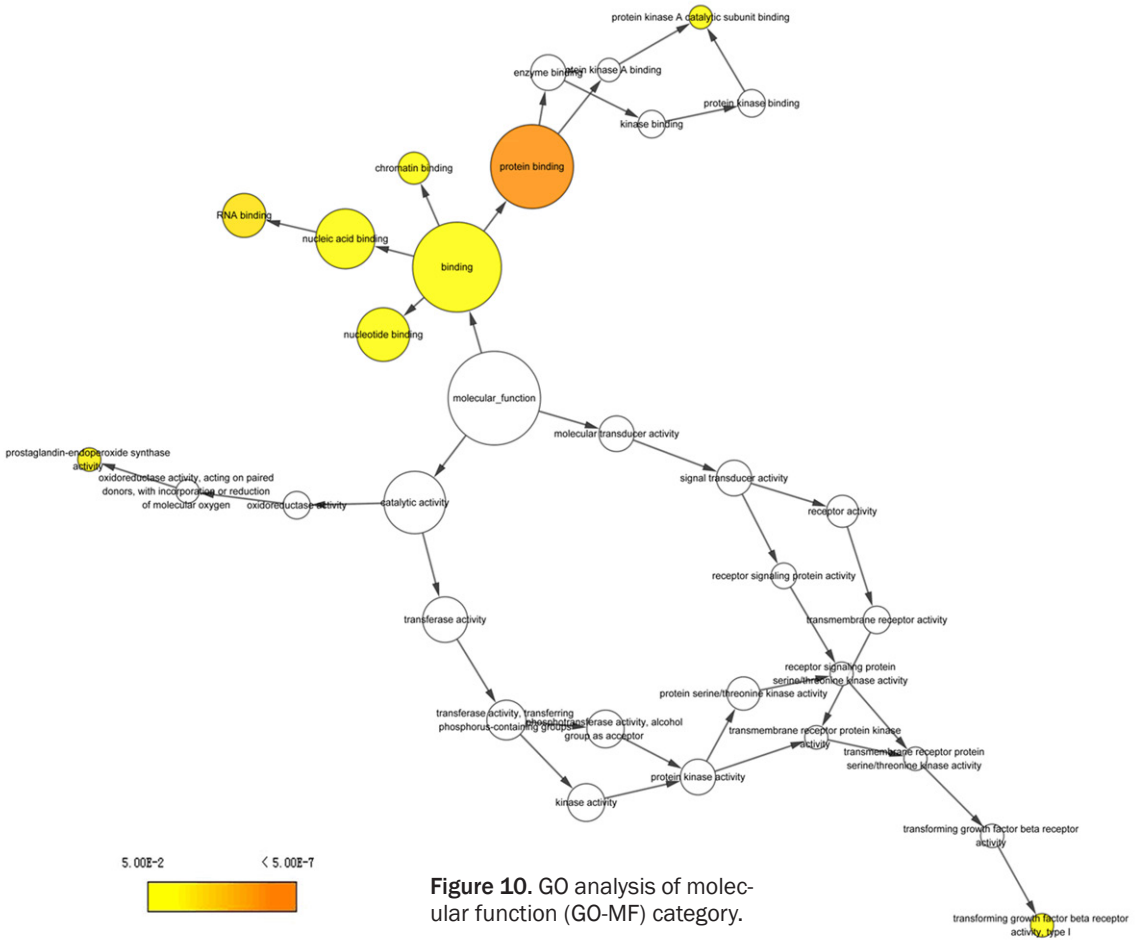

Figure 10. GO analysis of molecular function (GO-MF) category.

ments, including MTS, colony formation, flow cytometry assays and mouse model, which demonstrated that forced overexpression of let-7c could inhibit NSCLC cell proliferation by inducing G1 arrest and suppress tumorigeneses. This cell growth inhibitory function was achieved via targeting different genes, such as HOXA1 [33], ITGB3, MAP4K3 [21], ABCC2, Bcl-XL [34] and TRIB2 [35]. Since one single miRNA can target hundreds of genes, the exploration of a single target gene cannot reveal a widespread molecular mechanism of let-7c-5p. Hence, we collected 154 validated targeting genes via literatures screening and in silico prediction by miRWalk for a comprehensive target gene network analysis. The GO and KEGG pathway analysis indicated that let-7c-5p may play an essential role in the development of NSCLC through different pathways, such as Chronic myeloid leukemia pathway, Adherens junction pathway, ErbB signaling pathway, Pathways in cancer and MAPK signaling pathway. This could lead to novel direction for the future investigation of the molecular mechanisms of let-7c-5p on NSCLC.

Besides the clinical role and bio-function, let-7c-5p also gains the potential in the therapeutic intervention strategy in NSCLC, especially in chemotherapy [36] and molecular therapy [37]. Let-7c-5p could increase the sensitivity of gefitinib, an EGFR-targeting drug, via inhibiting RAS and deactivating the phosphoinositide 3-kinase (PI3K)/AKT and mitogen-activated extracellular signal-regulated kinase (MEK)/extracellular signal-regulated kinase (ERK) signaling pathways. And let-7c-5p plays an essential role in fulvestrant-induced upregulation of gefitinib sensitivity in lung cancer H1975 cells with T790M resistant mutation [37].

To the best of our knowledge, this is the first meta-analysis so far to evaluate the suitability of let-7c-5p as a blood-based marker for early

Table 5. KEGG pathway of validated target genes of let-7c-5p

KEGG Pathway	Count	*P* Value	Genes
hsa05220: Chronic myeloid leukemia	6	0.001098827	NRAS, ACVR1B, TGFBR1, CBL, BCL2L1, MYC
hsa05412: Arrhythmogenic right ventricular cardiomyopathy (ARVC)	6	0.001166809	ACTB, ATP2A2, ACTN4, CACNG8, RYR2, ITGA10
hsa03010: Ribosome	6	0.002136602	RPL18A, RPL3, RPS4Y1, RPS13, UBA52, RPS24
hsa04520: Adherens junction	5	0.008671443	ACTB, ACVR1B, CSNK2A1, ACTN4, TGFBR1
hsa05410: Hypertrophic cardiomyopathy (HCM)	5	0.012191074	ACTB, ATP2A2, CACNG8, RYR2, ITGA10
hsa04012: ErbB signaling pathway	5	0.013195175	NRAS, PTK2, PAK4, CBL, MYC
hsa05414: Dilated cardiomyopathy	5	0.015932867	ACTB, ATP2A2, CACNG8, RYR2, ITGA10
hsa00590: Arachidonic acid metabolism	4	0.021116706	PTGES2, PTGS2, PTGS1, LTA4H
hsa05200: Pathways in cancer	9	0.021160803	FZD8, NRAS, ACVR1B, PTK2, PTGS2, TGFBR1, CBL, BCL2L1, MYC
hsa04010: MAPK signaling pathway	8	0.021701553	MEF2C, NRAS, ACVR1B, CACNG8, TGFBR1, MYC, FLNB, DUSP7

Ten pathways were available, among which, four signaling pathways were significant ($P < 0.01$).

diagnosis of NSCLCs based on the microarray datasets. However, several limitations should be mentioned in the current study. Firstly, the small sample size of six datasets restricted the robustness of the current meta-analysis. Further studies with more samples included should be deliberated to study the role of circulating let-7c-5p in NSCLCs. Secondly, the let-7c-5p expression was only based on microarray assays. Future confirmation with more precise techniques like real time RT-qPCR should be performed. Thirdly, extra source of a relevant bias might be due to the only two regions involved in the meta-analysis (USA and Germany). Fourthly, only validated target genes were collected to analyze the potential gene-network of let-7c-5p. Other in silico predictions from different software could be gathered to have a more expansive insight of the molecular mechanism of let-7c-5p in NSCLC. Therefore, the current result needs to be interpreted cautiously.

Conclusion

Collectively, our data show that circulating let-7c-5p is reduced in blood of lung cancer patients as compared to that of non-cancerous controls. Let-7c-5p can be used to detect human lung cancers from blood cells as a promising biomarker candidate. Further studies of high quality and large cohort in the future are preferred to support the predictive power of circulating let-7c-5p with validated detection techniques. Furthermore, our prediction bioinformatics data may assist researchers to explore the molecular mechanisms of let-7c-5p in the tumorigenesis and progression of lung cancer comprehensively.

Disclosure of conflict of interest

None.

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