European Review for Medical and Pharmacological Sciences 2018; 22: 8367-8373

Analysis on expression level and diagnostic value of miR-19 and miR-21 in peripheral blood of patients with undifferentiated lung cancer

F. QIU, W.-G. GU, C. LI, S.-L. NIE, F. YU

Department of Oncology, The First Affiliated Hospital of Nanchang University, Nanchang, P.R. China

Abstract. – OBJECTIVE: This paper aims to investigate the expression level and diagnostic value of miR-19 miR-21 in peripheral blood of patients with undifferentiated lung cancer.

PATIENTS AND METHODS: 58 patients with undifferentiated lung cancer hospitalized in the oncology department of The First Affiliated Hospital of Nanchang University from September 2014 to May 2017 were selected as the experimental group, and 42 healthy volunteers in the same period as the control group at the same time. General clinical data in the two groups were collected. The expression levels of miR-19 and miR-21 in peripheral blood of the two groups were measured by Real-Time fluorescence quantitative Polymerase Chain Reaction. The expression levels of peripheral blood miR-19 and miR-21 in large and small cell lung cancer of undifferentiated lung cancer were analyzed and compared. ROC curve was used to analyze the diagnostic value of miR-19 and miR-21 in undifferentiated lung cancer.

RESULTS: The expression levels of miR-19 and miR-21 in peripheral blood of the experimental group were significantly higher than those of the control group ($p<0.05$). The AUC of miR-19 in the diagnosis of undifferentiated lung cancer was 0.854; the sensitivity was 98.30%; the specificity was 54.29% and the cut off was 3.54. The AUC of miR-21 in the diagnosis of undifferentiated lung cancer was 0.923; the sensitivity was 86.20%; the specificity was 76.19% and the cut off was 3.89. The AUC of combined detection in the diagnosis of undifferentiated lung cancer was 0.952; the sensitivity was 86.60%; the specificity was 97.62% and the cut off was 0.68. The specificity of combined detection was higher than that of miR-19 and miR-21 ($p<0.05$).

CONCLUSIONS: MiR-19 and miR-21 are highly expressed in peripheral blood of patients with undifferentiated lung cancer; miR-19 and miR-21 are expected to be used as diagnostic markers for undifferentiated lung cancer.

Key Words

MiR-19, MiR-21, Undifferentiated lung cancer, Diagnostic.

Introduction

Primary undifferentiated lung cancer has a high degree of malignancy and poor prognosis, and its incidence is second only to squamous cell carcinoma and adenocarcinoma, accounting for about 1/5 of primary lung cancer[1]. Lung tumor cells have the characteristics of short proliferative cycle, fast growth, wide metastasis, high malignancy, short survival time and poor prognosis in early stage[2]. In China, the morbidity and mortality of lung cancer have been ranked first among all kinds of tumors in men[2]. There are many problems in the early diagnosis of undifferentiated lung cancer. Due to the lack of effective biomarkers for early diagnosis of undifferentiated lung cancer, most of the patients are diagnosed late and missed the best time for surgical treatment[3]. Studies have shown that the 5-year survival rate for early undifferentiated lung cancer is about 76%, while that for advanced lung undifferentiated carcinoma is only 16%[4]. In clinical practice, the diagnosis of lung cancer mostly depends on spiral CT, ultrasonic endotracheal endoscopic biopsy and lung biopsy. Most of the patients were found to be positive, but they were advanced patients because of the low detection rate of spiral CT[5,6]. Pathological biopsy is an invasive examination which is not used if there are other choices, as the staining of surgical specimens requires cytological examination of the tissue, which is a complex and time-consuming process[7]. Therefore, it is very important to search for tumor markers closely related to undifferentiated lung cancer, to differentiate the diagnosis of lung cancer, to evaluate the curative effect, to monitor the recurrence or metastasis and to judge the prognosis of lung cancer[8]. The detection of tumor markers has been widely used in the diagnosis of malignant tumors. Tumor markers are antigens and other bioactive substances produced by the

Corresponding Author: Feng Yu, MD; e-mail: c13970007228@126.com

gene expression of cancer cells during canceration, which can be detected in the body fluid and secretions of tumor patients. Generally speaking, there is little or no endophytism in normal and non-malignant patients[9]. Although the research on tumor markers has made rapid progress over the years, the sensitivity and specificity of early lung cancer diagnosis are still unsatisfactory, and there is also a lack of safe and effective methods for screening lung cancer high-risk populations to achieve the goal of early detection and early treatment, and to improve the survival rate of patients with undifferentiated lung cancer[10]. Therefore, to improve the diagnostic rate of undifferentiated lung cancer, it is a hot topic to find new tumor markers and the best combination of tumor markers for the diagnosis. A serum tumor marker with easy operation, small sample size and high detection accuracy is needed in the clinic to improve the specificity and sensitivity of the detection and make it more valuable in clinical.

The abnormal expression of miR-19 in various human tumors is closely related to the occurrence and development of tumors, especially in hematological tumors[11]. MiR-21 is highly expressed in many solid tumors, including gastric, prostate, colon, pancreatic and esophageal cancer[12,13], and it can inhibit the deficiency of human chromosome 10 protein phosphatase and homologous gene, programmed cell death factor 4 and myosin and promote the occurrence and development of tumor-suppressing gene[14]. In this study, patients with undifferentiated lung cancer treated in The First Affiliated Hospital of Nanchang University were analyzed retrospectively, and the expression levels of miR-19 and miR-21 in peripheral blood of patients with undifferentiated lung cancer were compared.

Patients and Methods

Patients

Patients with undifferentiated lung cancer hospitalized in Oncology Department of the First Affiliated Hospital of Nanchang University from September 2014 to May 2017 were analyzed retrospectively. 58 patients were selected as an experimental group, including 36 males and 22 females, with an average age of (64 ± 13.2) years; 42 healthy volunteers in the same period were selected as a control group, including 26 males and 16 females, with an average age of (64 ± 13.2) years.

Inclusion and Exclusion Criteria

Inclusion criteria: age≥18 years, patients diagnosed as undifferentiated lung cancer clinically, patients received no systematic surgical chemotherapy or drug treatment, patients with perfect clinical data. Exclusion criteria: uncooperative patients, patients with severe liver and kidney failure, women in pregnancy, patients with serious immune disease. All patients were informed and agreed to participate in the clinical study with the approval of the Ethics Committee of the First Affiliated Hospital of Nanchang University and signed complete informed consent.

Reagents and Instruments

RT-PCR kit was purchased from Shanghai Haling Biotechnology Co., Ltd (Shanghai, China); TRIzol was purchased from Beijing Blest Technology Development Co., Ltd (Beijing, China). RNA extraction kit was purchased from Beijing Solarbio Science & Technology Co., Ltd (Beijing, China). RNA reverse transcription kit was purchased from Jiangsu Chutian Bioscience Co., Ltd (Nanjing, Jiangsu, China). DNA Polymerase from Haibo Biotechnology Co., Ltd (Shanghai, China). Primer sequence synthesis was purchased from Nanjing Kobio Biotechnology Co., Ltd (Nanjing, Jiangsu, China). Automatic microplate reader was purchased from Shandong Biobase Biological Industry Co., Ltd (Shandong, China). PCR instrument was purchased from Shanghai Shedrock instrument Co., Ltd (Shanghai, China).

Specimen Collection

5 mL fasting peripheral venous blood of the two groups was collected and centrifuged for 15 min at 3500 rpm. The upper serum was collected and stored in -80° C refrigerator for detection.

QRT-PCR Detection

The total RNAs of cells were extracted strictly according to the instructions of TRIzol. All-in-One miRNA qRT-PCR kit was used to detect miR-19 and miR-21 and their expressions strictly according to the instructions with U6 as an internal reference. The upstream primer sequence of miR-19 was 5'-AGUUUUGCAUAGUUGCACUACA-3'; the downstream primer sequences was 5'-GCTCACTGCAACCTCCTC-CTCC-3'. The upstream primer sequence of miR-21 was 5'-UGUG-CAAAUCCAUGCAAAACUGA-3', the downstream primer sequences were 5'-GCTCACTGCAACCTCCTC-CTCC-3'. The upstream primer sequence of internal reference U6 was 5'-GCTTCGGCAGCACATA-TACTAAAAT-3'; the down-

stream primer sequences were 5'-CGCT-TCAC-GAATTTGCGTGTCAT-3'. The amplification reaction was carried out by PCR, and the total volume of PCR reaction system was 20 μL, including 2×miR qPCR Mix (With Sybr Green) 10 μL, forward primer 0.4 μL, reverse primer 0.4 μL, miR 0.5 μL, and the rest was filled with ddH_2O. Wells were set in triplicate for each test. PCR reaction conditions: 40 cycles of predenaturation at 95°C for 8 min, denaturation at 95° C for 30 s, annealing at 53°C for 40 s, elongation at 72°C for 50 s.

Statistical Analysis

SPSS 13.0 software (Beijing Xinmei Jiahong Technology Co., Ltd., Beijing, China) was used for statistical analysis. The measurement data was expressed in the form of mean (x±s). The *t*-test was used for the comparison between the two groups. The categorical variable was expressed as percentage (%) and detected by χ^2-test. When $p<0.05$, the difference was statistically significant.

Results

Basic Information

There was no significant difference in sex, age, alcoholism, residence and nationality between the two groups ($p>0.05$). There was a significant difference in smoking history between the two groups ($p<0.05$) (Table I).

Comparison of Expression Level of MiR-19 and MiR-21 in Undifferentiated Lung Cancer

Compared with the control group, the expression of miR-21 in the experimental group (2.28±0.24) was significantly higher than that in the control group (0.82±0.27), and the difference was statistically significant ($p<0.05$). The expression of miR-19 in the experimental group (1.96±0.62) was significantly higher than that in the control group (0.76±0.43), and the difference was statistically significant ($p<0.05$). (Figure 1, Figure 2).

Table I. Basic information of patients (%).

Group		Experimental group (n=58)	Control group (n=42)	χ^2/t	p
Sex				0.014	0.986
	Male	36 (62.07)	26 (61.90)		
	Female	22 (37.93)	16 (38.10)		
Age				0.246	0.682
	≥55	36 (62.07)	24 (57.14)		
	<55	22 (37.93)	18 (42.86)		
Smoking history				8.520	0.002
	Yes	39 (67.24)	15 (35.71)		
	No	19 (32.76)	27 (64.29)		
Alcoholism				0.038	0.964
	Yes	32 (55.17)	24 (57.14)		
	No	26 (44.83)	18 (42.86)		
Residence				0.037	0.967
	Urban	37 (63.79)	26 (61.90)		
	Rural	21 (36.21)	16 (38.10)		
Nationality				1.175	0.310
	Han	31 (53.45)	27 (64.29)		
	Minority nationality	27 (46.55)	15 (35.71)		
Tumor size					
	≥5 cm	35 (60.34)			
	<5 cm	23 (39.66)			
Pathological classification					
	Large cell cancer	34 (58.62)			
	Small cell cancer	24 (41.38)			
Clinical stage					
	I+II	30 (51.72)			
	III+IV	28 (48.28)			

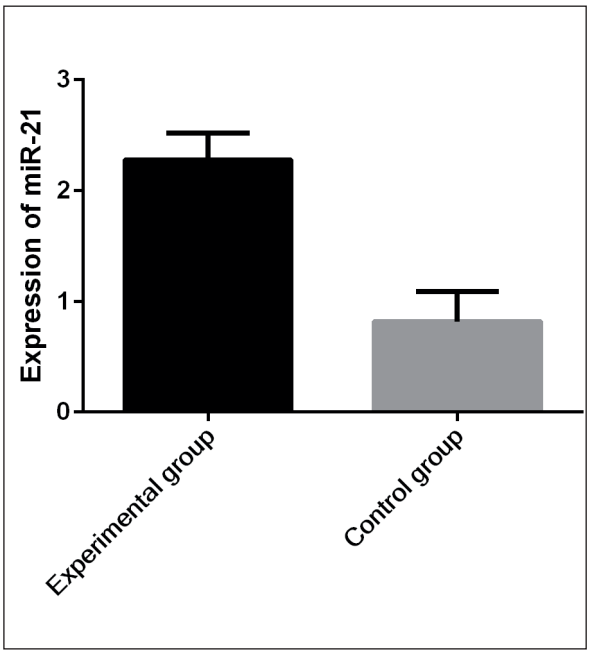

Figure 1. Comparison of expression of miR-21 in undifferentiated lung cancer. qRT-PCR showed that the expression of miR-21 in peripheral blood of the experimental group was significantly higher than that of the control group (p<0.05).

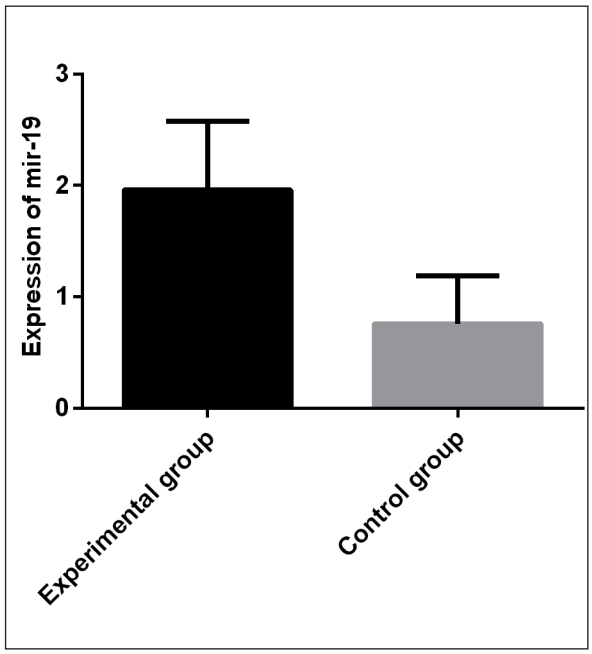

Figure 2. Comparison of expression of miR-19 in undifferentiated lung cancer. qRT-PCR showed that the expression of miR-19 in peripheral blood of the experimental group was significantly higher than that of the control group (p<0.05).

Comparison of the Expression Level of MiR-19 and MiR-21 in Large and Small Cell of Undifferentiated Lung Cancer

There was no significant difference between the expression of miR-19 and miR-21 in large cell undifferentiated lung cancer (2.36±0.23, 1.36±0.23) and small cell undifferentiated lung cancer (2.23 ±0.36, 1.98 ±0.26) (p>0.05). (Figure 3, Figure 4).

ROC Curve Analysis

The AUC of miR-19 in diagnosis of undifferentiated lung cancer was 0.854; the sensitivity was 98.30%; the specificity was 54.29% and the cut off was 3.54. The AUC of miR-21 in diagnosis of undifferentiated lung cancer was 0.923; the sensitivity was 86.20%; the specificity was 76.19% and the cut off was 3.89. The AUC of combined detection in the diagnosis of undifferentiated lung cancer was 0.952; the sensitivity was 86.60%; the specificity was 97.62% and the cut off was 0.68. The specificity of the combined detection was higher than that of miR-19 and miR-21, and the difference was statistically significant (p<0.05) (Table II) (Figure 5).

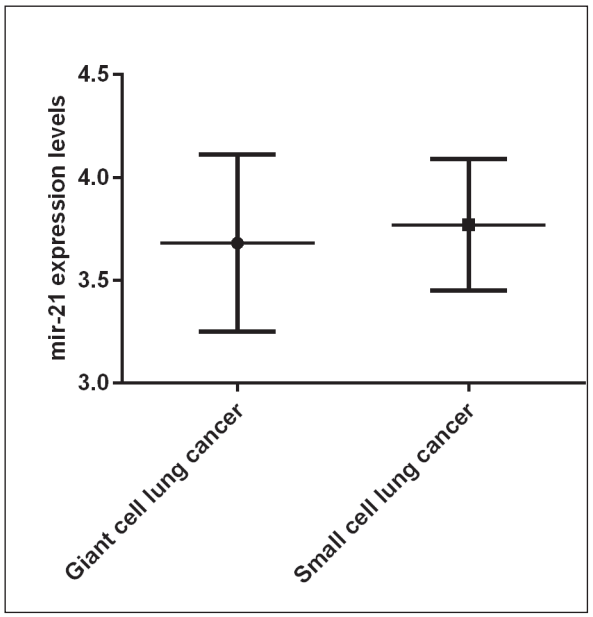

Figure 3. Comparison of the expression level of miR-21 in large and small cell of undifferentiated lung cancer. qRT-PCR showed that there was no significant difference in the expression of peripheral blood miR-21 in large and small cell of undifferentiated lung cancer (p>0.05).

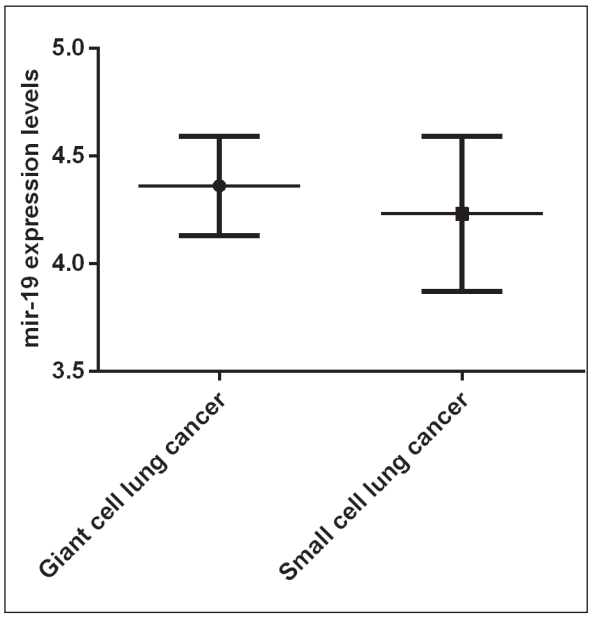

Figure 4. Comparison of the expression level of miR-19 in large and small cell of undifferentiated lung cancer. qRT-PCR showed that there was no significant difference in the expression of peripheral blood miR-19 in large and small cell of undifferentiated lung cancer (p>0.05).

Discussion

As one of the most common and fatal tumors in the world, the morbidity and mortality of undifferentiated lung cancer still rank the first of malignant tumors in China[15]. However, the high mortality of undifferentiated lung cancer is not only limited in China, but also the highest in many countries and regions[16]. In recent years, although the level of diagnosis and treatment and the 5-year survival rate of undifferentiated lung cancer has been improved, it is still one of the malignant tumors with the poorest prognosis[17]. With the development of medical technology, some progress has been made in the research and treatment of tumors. However, the pathogenesis of undifferentiated lung cancer has not been fully elucidated, and there are no effective biological indicators for the early diagnosis, treatment guidance, therapeutic evaluation and prognosis of undifferentiated lung cancer[18].

In this study, the detection of the expression level of miR-19 and miR-21 in peripheral blood of patients with undifferentiated lung cancer

Table II. ROC curve analysis of miR-19 and miR-21.

Indicator	AUC	OR	95% CI	P	Sensitivity (%)	Specificity (%)	Cut off
miR-19	0.854	0.037	0.781-0.927	0.00	98.30	54.29	3.54
miR-21	0.923	0.028	0.869-0.977	0.00	86.20	76.19	3.89
Combined detection	0.952	0.02	0.912-0.991	0.00	86.60	97.62	0.68

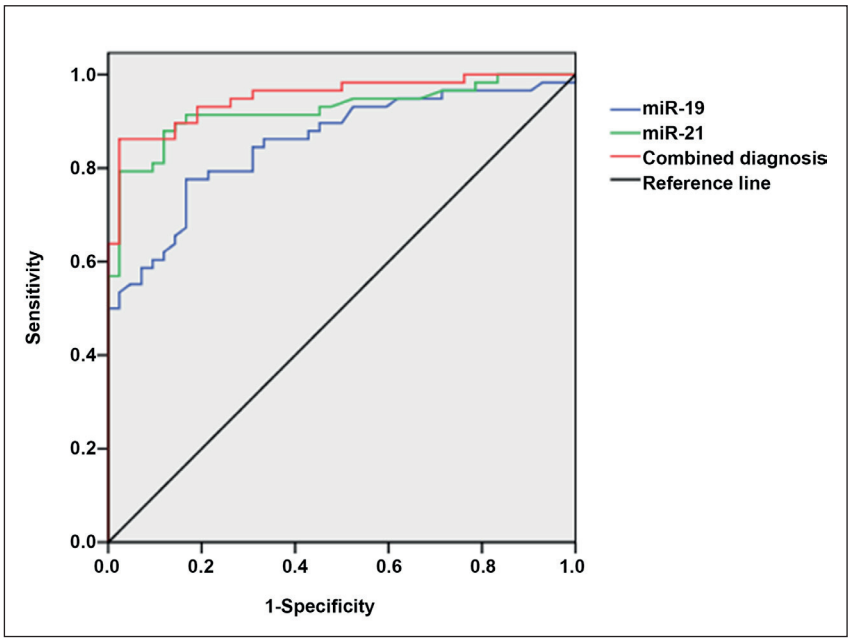

Figure 5. ROC curve analysis. The combined diagnosis was superior to the two single diagnosis, and the difference was statistically significant (p<0.05).

found that the expression level in the experimental group was significantly higher than that in control group, which suggested that the incidence and development of undifferentiated lung cancer was related to the abnormal expression of miR-19 and miR-21. Researches[19] have shown that tumor angiogenesis must be associated with the occurrence, development, and invasion, which also leads to the abnormal expression of various factors in the body. The degree of abnormal expression is closely related to the incidence and development of the tumor. In addition, there was no significant difference in the expression of miR-19 and miR-21 between large cell lung cancer and small cell lung cancer ($p>0.05$). The results of our work showed that the AUC of miR-19 in diagnosis of undifferentiated lung cancer was 0.854; the sensitivity was 98.30%; the specificity was 54.29% and the cut off was 3.54. The AUC of miR-21 in the diagnosis of undifferentiated lung cancer was 0.923; the sensitivity was 86.20%; the specificity was 76.19% and the cut off was 3.89. The AUC of combined detection in the diagnosis of undifferentiated lung cancer was 0.952; the sensitivity was 86.60%; the specificity was 97.62% and the cut off was 0.68. The specificity of combined detection was higher than that of miR-19 and miR-21, and the difference was statistically significant ($p<0.05$). The above data indicated that the diagnosis of undifferentiated lung carcinoma had certain accuracy, and miR-19 and miR-21 had certain diagnostic value. Therefore, detecting the expression level of miR-19 and miR-21 in undifferentiated lung cancer might increase the diagnostic rate of the tumor. MiR-19 combined with miR-21 has high sensitivity and specificity in the diagnosis of undifferentiated lung cancer. However, there are few reports on the sensitivity and specificity of miR-19, miR-21 and lung cancer biomarkers, so more studies are needed to verify that miR-19 and miR-21 can be used as biomarkers in the clinic. Foss et al[20,21] reported that miR-19 and miR-21 in serum could be used as diagnostic markers in patients with early undifferentiated lung carcinoma, and compared with common clinical tumor markers it has a strong ability of early differential diagnosis.

There are some shortcomings in this investigation. Whether the limitations of conditions and regional differences will affect this study is not known, and we will further supplement it in future studies.

Conclusions

We showed that miR-19 and miR-21 are highly expressed in peripheral blood of patients with undifferentiated lung cancer. MiR-19 and miR-21 are expected to be used as diagnostic markers for undifferentiated lung cancer.

Conflict of Interests:

The authors declare that they have no conflict of interest.

References

1) Ross JS, Wang K, Elkadi OR, Tarasen A, Foulke L, Sheehan CE, Otto GA, Palmer G, Yelensky R, Lipson D, Chmielecki J, Ali SM, Elvin J, Morosini D, Miller VA, Stephens PJ. Next-generation sequencing reveals frequent consistent genomic alterations in small cell undifferentiated lung cancer. J Clin Pathol 2014; 67: 772-776.
2) Rizvi NA, Mazieres J, Planchard D, Stinchcombe TE, Dy GK, Antonia SJ, Horn L, Lena H, Minenza E, Mennecier B, Otterson GA, Campos LT, Gandara DR, Levy BP, Nair SG, Zalcman G, Wolf J, Souquet PJ, Baldini E, Cappuzzo F, Chouaid C, Dowlati A, Sanborn R, Lopez-Chavez A, Grohe C, Huber RM, Harbison CT, Baudelet C, Lestini BJ, Ramalingam SS. Activity and safety of nivolumab, an anti-PD-1 immune checkpoint inhibitor, for patients with advanced, refractory squamous non-small-cell lung cancer (CheckMate 063): a phase 2, single-arm trial. Lancet Oncol 2015; 16: 257-265.
3) D'Incecco A, Andreozzi M, Ludovini V, Rossi E, Capodanno A, Landi L, Tibaldi C, Minuti G, Salvini J, Coppi E, Chella A, Fontanini G, Filice ME, Tornillo L, Incensati RM, Sani S, Crinò L, Terracciano L, Cappuzzo F. PD-1 and PD-L1 expression in molecularly selected non-small-cell lung cancer patients. Br J Cancer 2015; 112: 95-102.
4) Reck M, Popat S, Reinmuth N, De Ruysscher D, Kerr KM, Peters S. Metastatic non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2014; 25 Suppl 3: iii27-39.
5) Hu J, Qian GS, Bai CX. Chinese consensus on early diagnosis of primary lung cancer (2014 version). Cancer 2105; Suppl 17: 3157-3164.
6) Li P, Zhang J, Yu S, Guo Y, Yang X. Radiological Modalities for Diagnosis. In: Radiology of Parasitic Diseases: a practical approach. Hongjun Li, Ed. Springer, Dordrecht 2017; pp. 15-24.
7) Rimm DL, Han G, Taube JM, Yi ES, Bridge JA, Flieder DB, Homer R, West WW, Wu H, Roden AC, Fujimoto J, Yu H, Anders R, Kowalewski A, Rivard C, Rehman J, Batenchuk C, Burns V, Hirsch FR, Wistuba II. A prospective, multi-institutional, pathologist-based assessment of 4 immunohistochemistry assays for PD-L1 expression in non-small cell lung cancer. JAMA Oncol 2017; 3: 1051-1058.

8) Ma J, Lin Y, Zhan M, Mann DL, Stass SA, Jiang F. Differential miRNA expressions in peripheral blood mononuclear cells for diagnosis of lung cancer. Lab Invest 2015; 95: 1197-1206.
9) Dorsey JF, Kao GD, MacArthur KM, Ju M, Steinmetz D, Wileyto EP, Simone CB 2nd, Hahn SM. Tracking viable circulating tumor cells (CTCs) in the peripheral blood of non-small cell lung cancer (NSCLC) patients undergoing definitive radiation therapy: pilot study results. Cancer 2015; 121: 139-149.
10) Earl J, Garcia-Nieto S, Martinez-Avila JC, Montans J, Sanjuanbenito A, Rodríguez-Garrote M, Lisa E, Mendía E, Lobo E, Malats N, Carrato A, Guillen-Ponce C. Circulating tumor cells (CTC) and KRAS mutant circulating free DNA (cfDNA) detection in peripheral blood as biomarkers in patients diagnosed with exocrine pancreatic cancer. BMC Cancer 2015; 15: 797.
11) Zhu J, Wang S, Chen Y, Li X, Jiang Y, Yang X, Li Y, Wang X, Meng Y, Zhu M, Ma X, Huang C, Wu R, Xie C, Geng S, Wu J, Zhong C, Han H. miR-19 targeting of GSK3beta mediates sulforaphane suppression of lung cancer stem cells. J Nutr Biochem 2017; 44: 80-91.
12) Ma J, Lin Y, Zhan M, Mann DL, Stass SA, Jiang F. Differential miRNA expressions in peripheral blood mononuclear cells for diagnosis of lung cancer. Lab Invest 2015; 95: 1197-1206.
13) Li B, Ren S, Li X, Wang Y, Garfield D, Zhou S, Chen X, Su C, Chen M, Kuang P, Gao G, He Y, Fan L, Fei K, Zhou C, Schmit-Bindert G. MiR-21 overexpression is associated with acquired resistance of EGFR-TKI in non-small cell lung cancer. Lung Cancer 2014; 83: 146-153.
14) Sheng WZ, Chen YS, Tu CT, He J, Zhang B, Gao WD. MicroRNA-21 promotes phosphatase gene and protein kinase B/phosphatidylinositol 3-kinase expression in colorectal cancer. World J Gastroenterol 2016; 22: 5532-5539.
15) Fu W, Zhuo J, Hu L. Differential effects of recombinant human endostatin treatment on differentiated and undifferentiated blood vessels in Lewis lung cancer. Oncol Lett 2017; 13: 196-200.
16) Liao Y, Cheng S, Xiang J, Luo C. lncRNA CCHE1 increased proliferation, metastasis and invasion of non-small lung cancer cells and predicted poor survival in non-small lung cancer patients. Eur Rev Med Pharmacol Sci 2018; 22: 1686-1692.
17) Zhang L, Xiao H, Zhou H, Santiago S, Lee JM, Garon EB, Yang J, Brinkmann O, Yan X, Akin D, Chia D, Elashoff D, Park NH, Wong DTW. Development of transcriptomic biomarker signature in human saliva to detect lung cancer. Cell Mol Life Sci 2012; 69: 3341-3350.
18) Xu-Welliver M, Carbone DP. Blood-based biomarkers in lung cancer: prognosis and treatment decisions. Transl Lung Cancer Res 2017; 6: 708-712.
19) Tang H, Tian H, Yue W, Li L, Li S, Gao C, Si L, Qi L, Lu M. Overexpression of LAPTM4B is correlated with tumor angiogenesis and poor prognosis in non-small cell lung cancer. Med Oncol 2014; 31: 974.
20) Zaporozhchenko IA, Morozkin ES, Skvortsova TE, Ponomaryova AA, Rykova EY, Cherdyntseva NV, Polovnikov ES, Pashkovskaya OA, Pokushalov EA, Vlassov VV, Laktionov PP. Plasma miR-19b and miR-183 as potential biomarkers of lung cancer. PLoS One 2016; 11: e0165261.
21) Yang JS, Li BJ, Lu HW, Chen Y, Lu C, Zhu RX, Liu SH, Yi QT, Li J, Song CH. Serum miR-152, miR-148a, miR-148b, and miR-21 as novel biomarkers in non-small cell lung cancer screening. Tumour Biol 2015; 36: 3035-3042.