Significance of detecting the levels of miR-29a, survivin and interferon gamma release assay in patients with lung cancer and tuberculosis

Lijuan Sun[A,B,D–F], Hongyun Li[A,B,D–F], Qun Fu[B–D,F], Shuangmin Hu[B–D,F], Wenfei Zhao[C,D,F]

Department of Respiratory and Critical Care, The Fifth Affiliated Hospital of Zhengzhou University, China

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online) *Adv Clin Exp Med*. 2022;31(10):1073–1080

Address for correspondence
Lijuan Sun
E-mail: sunlijuan_2020@126.com

Funding sources
None declared

Conflict of interest
None declared

Received on November 2, 2021
Reviewed on December 6, 2021
Accepted on May 20, 2022

Published online on September 12, 2022

Abstract

Background. When lung cancer is combined with concurrent tuberculosis (TB), it increases the difficulty of diagnosis and treatment, leading to missed and/or misdiagnosed cases.

Objectives. To provide reference markers for the clinical diagnosis of patients with lung cancer complicated by active pulmonary TB (APT).

Materials and methods. The concentration of survivin in diseased tissue, and miR-29a and IGRAs interferon gamma (IFN-γ) in serum were evaluated in 25 patients with non-small cell lung carcinoma (NSCLC) complicated by APT, 32 patients with NSCLC and 30 patients with APT.

Results. The expression of miR-29a in serum of patients with APT was higher than in patients with NSCLC complicated by APT (least significant difference (LSD)-t = 4.724, $p < 0.001$), and the NSCLC group (LSD-t = 6.619, $p < 0.001$). Furthermore, patients with NSCLC complicated by APT had higher miR-29a concentration than the NSCLC group. The rate of positive survivin expression in NSCLC ($\chi^2 = 23.418$, $p < 0.001$) and NSCLC combined with APT group ($\chi^2 = 17.160$, $p < 0.001$) was significantly higher than in patients with APT. The concentration of IFN-γ in serum of the NSCLC complicated by APT group (LSD-t = 2.912, $p = 0.004$) and the APT group (LSD-t = 4.452, $p < 0.001$) was higher than in the NSCLC group. The level of IFN-γ in serum of the NSCLC complicated by APT group were higher than in the APT group, but there was no statistical difference.

Conclusions. The levels of MiR-29a, Survivin and IFN-γ was helpful for differential diagnosis of lung cancer and tuberculosis.

Key words: NSCLC, miR-29a, survivin, APT, IGRAs

Cite as
Sun L, Li H, Fu Q, Hu S, Zhao W. Significance of detecting the levels of miR-29a, survivin and interferon gamma release assay in patients with lung cancer and tuberculosis. *Adv Clin Exp Med*. 2022;31(10):1073–1080. doi:10.17219/acem/150306

DOI
10.17219/acem/150306

Background

Lung cancer is a very common malignant tumor, posing a significant threat to human health and life. Its morbidity and mortality are increasing year by year. Tuberculosis (TB) is endemic in China, causing a great economic burden. Improving TB diagnosis and treatment has become an urgent public health issue.[1] The incidence rate of TB in China is 58/100,000, while the incidence of lung cancer in these patients is 10.9 times that of patients without TB (26.3 compared to 2.41 per 10,000 person-years).[2] Therefore, the incidence of both lung cancer and TB in China is relatively high. When lung cancer is combined with concurrent TB infection, it increases the difficulty of diagnosis and treatment, and leads to missed or misdiagnosed cases of both diseases. In such situations, bronchoscopy, computed tomography (CT) and transthoracic lung biopsy should be performed. If diagnosis is unclear after all diagnostic tests are performed, a biopsy is necessary. When lung imaging indicates space-occupying or exudative changes, the probability of lung cancer diagnosis complicated with TB is not high. In clinical evaluations, it is essential to be vigilant. When some biomarkers are abnormal, it is often necessary to perform lesion biopsy or lesion resection once again to confirm the diagnosis.

MicroRNAs (miRNAs/miRs) are small RNA molecules that can be stably expressed in body fluids such as serum, plasma or saliva, and can be detected with high sensitivity. MicroRNAs are also stably expressed in exosomes and can be transmitted through this mechanism. Studies have found that miRNAs can affect a variety of biological processes, such as DNA damage repair, cell cycle arrest, cell hypoxia, proliferation and apoptosis, etc. This enables miRNAs to act as a biological factor predicting non-small cell lung cancer (NSCLC) in patients.[4] Some scholars have found that after *Mycobacterium tuberculosis* spp. invades macrophages, miRNAs participate in the anti-TB infection process of the body.[5] In a study by Das et al.,[6] after THP-1 macrophages were infected with H37Rv and H37Ra, the expression of miR-29a in THP-1 cells increased.

Survivin is a new member of the inhibitor of apoptosis (IAP) family and it is the strongest IAP found so far. It has complex functions and can inhibit cell apoptosis, promote cell transformation, participate in cell mitosis and angiogenesis, and cause tumor cells to develop drug resistance.[7,8] The *survivin* gene is 15 kb in length, located at 17q25, and has 4 exons and 3 introns. Its coding product consists of 142 amino acids and has a molecular weight of 16.2 kD. Other members of the IAP family generally contain baculovirus IAP repeat (BIR) molecules composed of 2–3 tandem cysteine/histidine consensus sequences of 70 amino acids, and terminal hydroxyl RING finger structure, in which the BIR molecule exerts an anti-apoptotic effect. However, survivin contains only a single BIR functional region, while the terminal hydroxyl does not contain a ring finger structure, but an interwoven spiral structure, in contrast to other IAP family members.

Interferon gamma (IFN-γ) release assays (IGRAs) are used as an auxiliary diagnostic test for TB infection. They evaluate the ability of T cells to release IFN-γ upon stimulation with TB-specific antigens.[9] Latent TB infection is very common in adults. Recently, IGRAs have been utilized clinically to diagnose active adult TB.[10–12]

Objectives

Survivin is tumor-specific, being expressed only in tumor and embryonic tissues, while miR-29a and IFN-γ are mostly used in the differential diagnosis of TB. The aim of this study was to establish the clinical diagnosis value of miR-29a, survivin and IFN-γ in patients with NSCLC combined with active pulmonary TB (APT).

Materials and methods

Research objects

Patients with NSCLC combined with APT (n = 25), those with NSCLC (n = 32) and patients with APT diagnosed from March 2017 to September 2019 (n = 30) in our center were selected to participate in this prospective study. The diagnosis of NSCLC was confirmed by lung biopsy, bronchoscopy, surgical pathological tissue, lymph node biopsy, and imaging examination. The TNM staging standards refer to the National Comprehensive Cancer Network (NCCN) NSCLC Clinical Practice Guidelines.[13] The diagnosis of APT conforms to the 2018 version of the diagnostic criteria issued by National Health and Family Planning Commission of the People's Republic of China for TB.[14] The detailed diagnostic process was shown in Fig. 1. We performed the lung biopsy only for TB patients with difficult diagnoses, and included them in the study. Immunohistochemistry was used to diagnose and classify lung disease markers including TTF-1, Napsin A, CK7, p63/p40, CK5/6, DSG3, CgA, Syn, CD56, Ki67, and Bacillus Calmette-Guerin (BCG). Patients diagnosed with NSCLC combined with APT may be diagnosed with both diseases at the same time, or one of them may be diagnosed successively. Among patients with NSCLC combined with APT, in 3 cases NSCLC and APT were diagnosed at the same time, 9 cases were diagnosed as APT before the diagnosis of NSCLC, and 13 cases were diagnosed as NSCLC before the diagnosis of APT. Exclusion criteria included retreated TB, a history of a malignant tumor, a history of anti-TB or tumor treatment before enrollment, or other obvious complications, such as infection. Every patient signed an informed consent form before participating in the study. The study was conducted

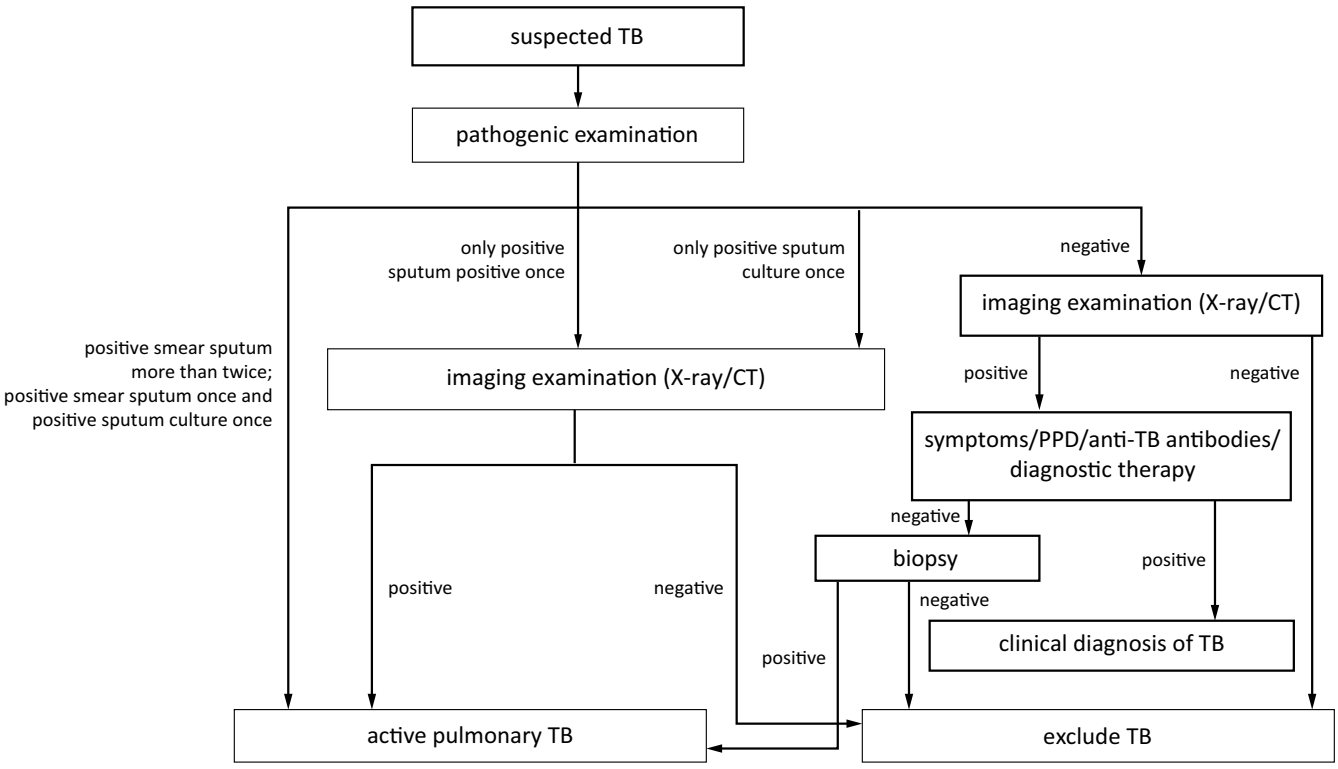

Fig. 1. Flowchart of active tuberculosis (TB) diagnosis

CT – computed tomography; PPD – purified protein derivative.

in accordance with the Declaration of Helsinki and approved by the Ethics Committee of The Fifth Affiliated Hospital of Zhengzhou University, Zhengzhou, China (approval No. 2017003).

Detection method

Survivin detection method

We used immunohistochemistry to detect survivin protein expression. After the tissue wax section was dewaxed, debenzened and hydrated with gradient ethanol, the endogenous peroxidase activity was blocked using 3% hydrogen peroxide, with the antigen being repaired by high pressure and high temperature. After washing with phosphate-buffered saline (PBS) and blocking with normal goat serum (supplied in the kit) for 10 min, the survivin antibody (cat. No. ZN2428; 1:2000 dilution; Beijing Baiaolaibo Technology Co., Ltd., Beijing, China) was added and incubated at 4°C for 12 h. Next, the slides were washed and the biotin-labeled secondary antibody was added dropwise with labeled streptavidin and incubated overnight at 4°C. Slides were washed following incubation, and then the secondary antibody and horseradish enzyme-labeled streptavidin (Beijing Baiaolaibo Technology Co., Ltd.) were added dropwise and incubated overnight at 4°C. Samples were then washed with PBS and 3,3'-diaminobenzidine (DAB) (Beijing Baiaolaibo Technology Co., Ltd.) added dropwise, and incubated at room temperature for 5 min to develop color. Then, the samples were washed with water, counterstained with hematoxylin (Beijing Baiaolaibo Technology Co., Ltd.), dehydrated and dried, and finally coverslipped for observation under a microscope (model CX31-LV320; Olympus Corp., Tokyo, Japan). Survivin protein expression was identified according to the following criteria: 5 high-power fields were randomly selected for each slice and scored according to the percentage of positive cells. The percentage of positive cells >75% was counted as 4 points, ≥50–75% as 3 points, ≥25–50% as 2 points, ≥5–25% as 1 point, and <5% scored 0 points. According to the intensity of color development, brown meant 3 points, brown yellow 2 points, light yellow 1 point, and no color 0 points. The score was calculated by multiplying the 2 items, and it was segregated from high to low. A score of 9 or more meant high expression, 5–8 medium expression, 2–4 low expression, and 0–1 negative expression. In this study, high, medium and low results were defined as positive expressions (representative images of immunohistochemical staining are shown in Fig. 2).

Detection method of miR-29a

Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to detect the levels of miR-29a in peripheral blood. Fasting venous blood was collected, centrifuged at 2000 rpm/min for 20 min at 4°C, and the supernatant was collected into a centrifuge tube and stored at –80°C for testing. The TRIzol (Thermo Fisher Scientific, Waltham,

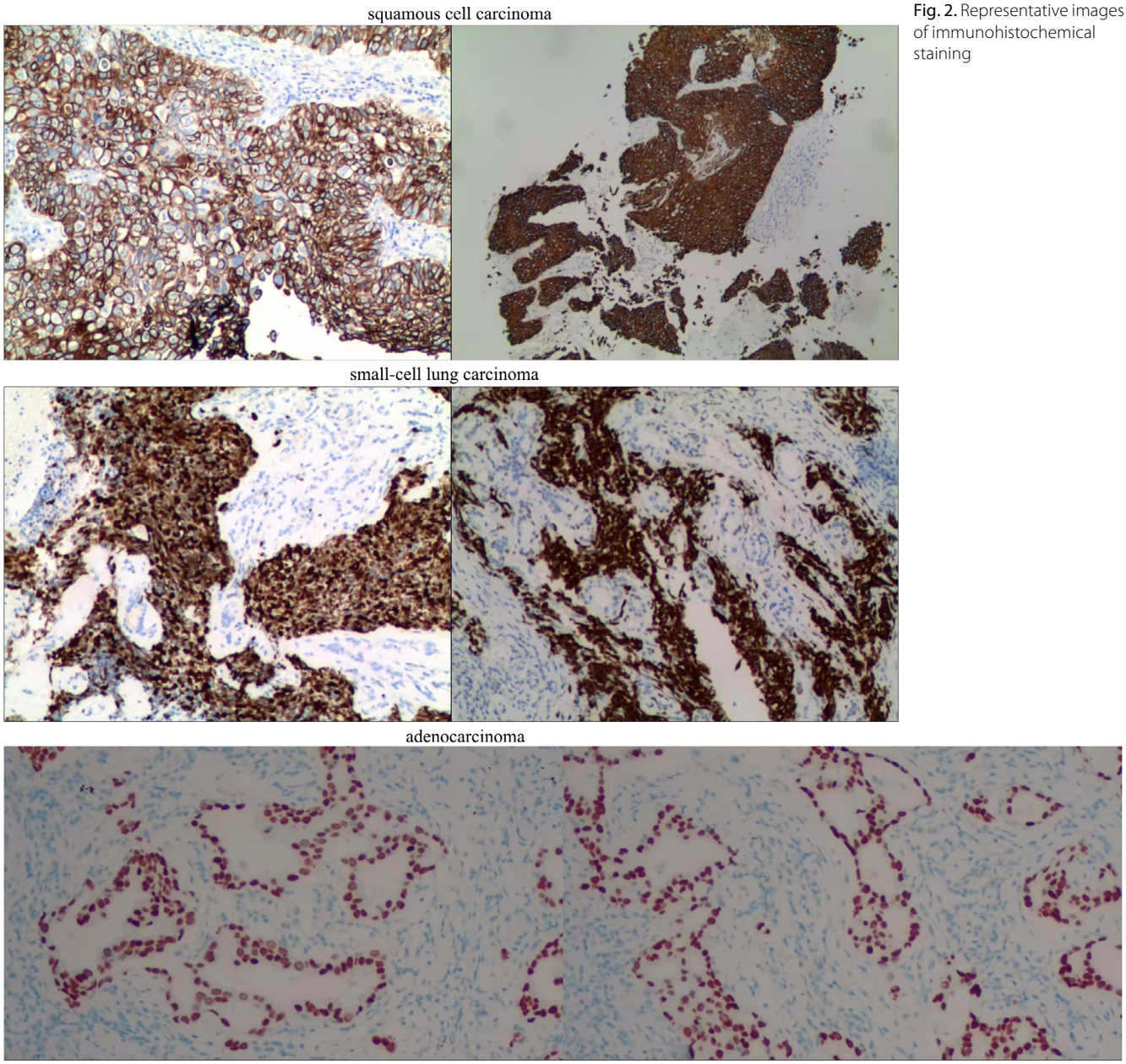

Fig. 2. Representative images of immunohistochemical staining

USA) was used to extract total RNA from the collected cellular material, and after detection of nucleic acid concentration, reverse transcription of miRNA was performed to generate cDNA. Using cDNA as a template and U6 as an internal reference gene, SYBR Premix EX TaqTM II (TaKaRa Bio Inc., Kusatsu, Japan) was used to detect the expression of miR-29a, using the following primer sequences: upstream primer: 5'-GGGTAGCACCATCTGAAA-3', downstream primer: 5'-CAGTGCGTGTCCTGGAGT-3', U6 primer: 5'-GACTTATGTTAGGAGACGA-3'. The reaction system consisted of 20 μL, including cDNA (2 μL), miR-29a primer (1 μL), SYBR Premix EX TaqTM II (10 μL), and double distilled water (ddH_2O) (7 μL). A total of 40 cycles were performed, and the relative expression of miR-29a was calculated using the $2^{-\Delta\Delta Ct}$ method. The experiment was repeated 3 times to ensure validity.

IFN-γ detection method

Peripheral venous blood was collected, divided (1 mL per tube) into N (negative control), T (test culture) and P (positive control) tubes, and mixed gently for 2 h. Tubes were incubated at 37°C for 24 h, then centrifuged at 3000 rpm for 10 min, and the upper layer of plasma was used for testing. A human IFN-γ enzyme-linked immunosorbent assay (ELISA) kit (cat. No. 1605023) was purchased from Shanghai Jianglai Biotech Co., Ltd. (Shanghai, China) and the absorbance (A) was measured using a microplate reader at 450 nm. A standard curve was prepared according to the calibrator to calculate the IFN-γ level. When the value of IFN-γ is 0–14 pg/mL, it indicates a negative result. If it exceeds 14.0 pg/mL, it indicates a positive result, and the patient may have TB infection.[15] A positive test result (IFN-γ (+)) was interpreted

as latent *M. tuberculosis* spp. infection, whereas a negative IGRA (IFN-γ (–)) result meant no infection with M.tb.

Statistical analyses

All data were analyzed using IBM SPSS v. 20.0 software (IBM Corp., Armonk, USA). The Shapiro–Wilk test was used to test normality and the Levene's test was used to test homogeneity of variance. The measurement data were expressed as mean ± standard deviation (M ±SD). The comparison between groups was performed using t-test or one-way analysis of variance (ANOVA) followed by least significant difference (LSD) test. The count data were expressed as a percentage, and the comparison between groups was performed using Fisher's exact test or χ^2 test. The value of $p < 0.05$ indicated a statistically significant difference.

Results

General information

The average age in the NSCLC group was 52.92 ±4.63 years. Among 15 cases of lung squamous cell carcinoma, 14 cases were p63+, 10 cases were syn+ and 15 cases were CK 5/6+. Among 17 cases of lung adenocarcinoma, 16 cases were TTF-1+, 13 cases were Napsin A and 17 cases were CK 7+. The TNM stage ranged between I and IV. In the APT group, the average age was 50.62 ±4.88 years. The NSCLC combined with APT group had an average age of 55.06 ±5.17 years. Of these patients, 13 cases were diagnosed as lung squamous cell carcinoma, and 12 cases were diagnosed as lung adenocarcinoma. The TNM stage ranged between I and IV (Table 1). There was no statistically significant difference in gender or age of the 3 groups.

Table 1. Clinical and demographic characteristics of enrolled patients

Variable	NSCLC	APT	NSCLC+APT
Gender, n (%)			
male	22 (68.75)	21 (70.00)	18 (72.00)
female	10 (21.25)	9 (30.00)	7 (28.00)
Age, range [years]	46–65	42–68	49–71
Cytological typing, n (%)			
Squamous cell carcinoma, n (%)	15 (46.88)	–	13 (52.00)
p63+	14 (93.33)	–	–
syn+	10 (66.67)	–	–
CK 5/6+	15 (100.00)	–	–
Adenocarcinoma, n (%)	17 (53.12)	–	12 (48.00)
TTF-1+	16 (94.12)	–	–
Napsin A	13 (76.47)	–	–
CK 7+	17 (100.00)	–	–
TNM stage, n (%)			
I–IIIa	13 (40.63)	–	12 (48.00)
IIIb–IV	19 (59.37)	–	13 (52.00)

NSCLC – non-small cell lung carcinoma patients; APT – patients with pulmonary tuberculosis; NSCLC+APT – patients with NSCLC combined with APT.

The expression and comparison of survivin, miR-29a and IFN-γ in the 3 groups

Results for survivin, miR-29a and IFN-γ in the 3 groups are shown in Table 2, including normality and homogeneity of variance. The positive rate of survivin in NSCLC (NSCLC compared to APT, 78.12% compared to 16.67%, $\chi^2 = 23.418$, $p < 0.001$) and NSCLC combined with APT group (NSCLC combined with APT compared to APT, 72.00% compared to 16.67%, $\chi^2 = 17.160$, $p < 0.001$) was significantly higher than in the APT group. However, there was no statistical difference between the NSCLC group and the NSCLC combined with APT group (NSCLC compared to NSCLC combined with APT, 78.12% compared to 72%, $\chi^2 = 0.284$, $p = 0.758$). The survivin expression had no significant relationship with the age, sex, pathological type, or clinical stage of NSCLC combined with APT patients (Table 3).

One-way ANOVA followed by the LSD-t-test was used to analyze the differences between 3 groups. The expression of miR-29a in the APT group was significantly higher than in the NSCLC combined with APT group (APT compared to NSCLC combined with APT, 4.43 ±1.91 compared to 2.27 ±1.98, post hoc LSD-t-test, LSD-t = 4.724, $p < 0.001$) and the NSCLC group (APT compared to NSCLC, 4.43 ±1.91 compared to 1.59 ±1.53, post hoc LSD-t-test, LSD-t = 6.619, $p < 0.001$). However, there was no statistical difference between the NSCLC combined with APT group and the NSCLC group (NSCLC combined with APT compared to NSCLC, 2.27 ±1.98 compared to 1.59 ±1.53, post hoc LSD-t-test, LSD-t = 1.509, $p = 0.151$) (Table 2 and Fig. 3A). The expression of miR-29a had no significant relationship with the age, gender and pathological type of NSCLC combined with APT patients, but was related to the clinical stage. Moreover, the difference in TNM stage between the 2 groups was also significant (2.87 ±1.65 compared to 1.33 ±0.92, t-test, t = 2.861, $p = 0.011$) (Table 3).

The concentration of IFN-γ in the APT group (APT compared to NSCLC, 132.43 ±122.28 compared to 36.72 ±50.66, post hoc LSD-t-test, LSD-t = 4.452, $p < 0.001$) and the NSCLC combined with APT (NSCLC combined with APT compared to NSCLC, 102.48 ±60.55 compared to 36.72 ±50.66, post hoc LSD-t-test, LSD-t = 2.912, $p = 0.004$) was higher than that in the NSCLC group. Furthermore, the IFN-γ level in the NSCLC combined with APT group was lower than that in the APT group, although there was no statistical difference between them (102.48 ±60.55 compared to 132.43 ±122.28, post hoc LSD-t-test, LSD-t = 1.307, $p = 0.195$) (Table 2 and Fig. 3B).

Discussion

Survivin is the most powerful inhibitor of apoptosis within the IAP family, and it is only expressed in embryonic and developing fetal tissues. It is not expressed

Table 2. Expression of survivin, miR-29a and IFN-γ in 3 groups of patients

Group	Value	NSCLC (n = 32)	APT (n = 30)	NSCLC+APT (n = 25)
Survivin, n (%)	positive	25 (78.12)	5 (16.67)	18 (72.00)
	negative	7 (21.88)	25 (83.33)	7 (28.00)
	χ^{2*}	23.418	17.160	0.284
	p-value	<0.001[a]	<0.001[b]	0.758[c]
miR-29a (M ±SD)	–	1.59 ±1.53	4.43 ±1.91	2.27±1.98
Shapiro–Wilk test	Sig.	0.093	0.114	0.337
Homogeneity of variances test	p-value		0.24	
	t-value**	6.619	4.724	4.724
	p-value	<0.001[a]	<0.001[b]	0.151[c]
IFN-γ (M ±SD)	–	36.72 ±50.66	132.43 ±122.28	102.48 ±60.55
Shapiro–Wilk test	Sig.	0.236	0.522	0.417
Homogeneity of variances test	p-value		0.09	
	t-value**	4.452	1.307	2.912
	p-value	<0.001[a]	0.195[b]	0.004[c]

IFN-γ – interferon gamma; M – mean; SD – standard deviation; NSCLC – non-small cell lung cancer patients; APT – patients with pulmonary tuberculosis; NSCLC+APT – patients with NSCLC combined with APT; * significance testing performed using χ^2 test; ** significance testing was done using one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) t-test; [a] NSCLC compared to APT; [b] APT compared to NSCLC+APT; [c] NSCLC compared to NSCLC+APT.

Table 3. Expression of survivin and miR-29a in patients with NSCLC+APT

Clinicopathological features	n	Survivin			miR-29a		
		positive	negative	Fisher's exact p-value*	M ±SD	t-value	p-value**
Age				0.601		0.570	0.577
≤55 years	10	7	3		2.29 ±1.73		
>55 years	15	11	4		1.92 ±1.39		
Sex				0.607		−0.652	0.532
male	18	12	6		1.92 ±1.35		
female	7	5	2		2.45 ±1.94		
Cytological typing				0.560		−1.349	0.193
squamous cell carcinoma	13	8	5		1.68 ±1.19		
adenocarcinoma	12	8	4		2.50 ±1.76		
TNM stages				0.440		2.861	0.011
I–IIIa	12	7	5		2.87 ±1.65		
IIIa–IV	13	9	4		1.33 ±0.92		

NSCLC+APT – patients with non-small cell lung carcinoma (NSCLC) combined with APT; * significance testing was performed using Fisher's exact test; ** significance testing was done using t-test; M – mean; SD – standard deviation.

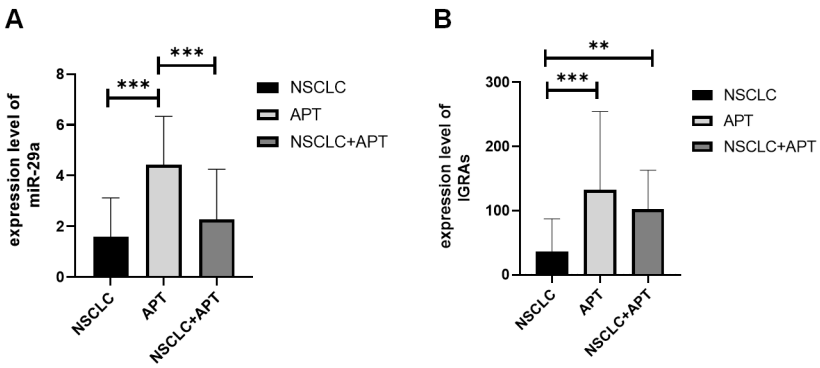

Fig. 3. The expression level of indicated genes, miR-29a (A) and interferon gamma (IFN-γ) (B)

IGRAs – interferon gamma release assays; NSCLC – non-small cell lung cancer patients; APT – patients with pulmonary tuberculosis; NSCLC+APT – patients with NSCLC combined with APT.

in most of the normal, mature adult tissues, but has abnormal expression in many tumors.[16–18] Survivin promotes the occurrence and development of NSCLC by inhibiting the apoptosis of cancer cells, thereby allowing them to escape from monitoring remain undetected. Tamm et al. detected the expression of survivin in 60 human tumor cell lines, of which the expression was the highest in lung and breast cancer.[19] Our results demonstrated

that survivin was highly expressed in the NSCLC and the NSCLC combined with APT groups, and its expression was significantly higher than the positive rate of benign lung lesions and consistent with related reports.[20] In addition, the present study found that positive survivin expression is not related to clinical tumor stage ($p > 0.05$). Interestingly, this result is consistent with those of Zhao and Zheng,[21] but Niu et al.[22] found that the positive rate of survivin was related to TNM staging. The later the TNM staging was performed, the higher the positive rate of survivin. We consider this discrepancy to be due to variation in cases included in each report.

MicroRNAs are a type of non-coding RNAs with 19–22 nucleotides. Because they can directly target a variety of proteins, they have multiple functions. The miR-29a is a part of the small RNA family which, in addition to the 3'untranslated region (3'UTR), can interact with miRNA regulatory elements (MRE) in the non-3'UTR region. For example, there are a total of 14 miR-29a-binding sites in the 3'UTR region and coding region of elastin. It was confirmed using luciferase reporter gene analysis that miR-29a could simultaneously bind to the MRE in the coding region and 3'UTR region to inhibit elastin expression.[23] Studies found that miR-29a could directly inhibit the expression of a variety of collagen molecules, with target genes being mainly extracellular matrix and migration protein.[24,25] The miR-29a target gene-related proteins participated in multiple signaling pathways, and could inhibit extracellular matrix remodeling by combining with the downstream of the transforming growth factor (TGF-β)/Smad-3 signaling pathway.[26] Furthermore, the expression of miR-29a in normal lung tissues gradually increases as the lung matures.[27] Moreover, miRNAs participate in the proliferation and apoptosis of various tumors by regulating the expression of oncogenes. The expression of miR-29a was downregulated in NSCLC tissues, which was significantly correlated to tumor staging and metastasis, and had certain value for NSCLC diagnosis and the evaluation of the disease.[28] Studies found that miR-29a also played an important role in the body's immune response, and had certain clinical value in the diagnosis of APT. Fu et al. used a microarray-based expression profiling to screen the serum of patients with active TB and found that the expression of miR-29a was significantly upregulated, which was consistent with the upregulation of miR-29a in the sputum of patients with APT.[29] Sharbati et al. also confirmed that M.tb could upregulate the expression of miR-29a after infecting human macrophages.[30]

The incidence of TB is relatively high, and infection is most common in the lungs. Tumor patients are susceptible to TB due to low immunity. At this stage, the most commonly used screening method for TB infection in China is the tuberculin skin test (TST), but its specificity is reduced because the pure protein derivative of the antigen is similar to the antigen of the BCG vaccine. When the body's immune function declines, the sensitivity of TST diagnosis also decreases,[31] and it is prone to cross-reaction and false positive results.[32,33] The bacteriological examination is a common method for diagnosing active TB, but it takes a long time to culture tubercle bacillus. Therefore, finding a fast and accurate detection method is particularly important for the prevention and diagnosis of TB. Interferon gamma is a specific cytokine released by T lymphocytes sensitized by TB. In recent years, IGRAs have become a new type of immunological diagnosis method, which is gradually applied in the clinical diagnosis of TB.[34] After an individual is infected with M.tb, 2 specific antigens, namely CFP-10 and ESAT-6, can be produced. These antigens stimulate T lymphocytes to produce IFN-γ. Therefore, by detecting the level of IFN-γ in the peripheral blood of patients, it is possible to determine whether there is M.tb infection, and to distinguish true TB infection, eliminating the interference of vaccination and nontuberculous infection.[35] Research by Huang and Chen found that malignant tumors and purulent infections can also cause IFN-γ to increase.[36] Furthermore, other results have confirmed that IGRAs show high sensitivity and specificity in diagnosis, and is more useful than TST in auxiliary diagnosis of APT.[37]

In clinical practice, doctors need to treat patients with high suspicion of tumor or TB with caution during diagnosis and treatment; the possibility of lung cancer complicated with TB should be fully considered. A correct diagnosis in patients with the coexistence of TB and lung cancer is difficult. In such cases, bronchoscopy, CT and transthoracic lung biopsy should be performed. If diagnosis is unclear after these diagnostic tests, surgery becomes necessary. The 3 biomarkers (survivin, IFN-γ and miR-29) proposed in this study can provide other avenues for differential diagnoses. However, we should be vigilant to patients with APT complicated by lung cancer, especially those with low serum expression of miR-29a, and high expression of survivin in diseased tissues who have been pathologically diagnosed with APT. According to the clinical situation, a reasonable diagnosis and treatment plan can be developed to avoid missed or misdiagnosed cases, which is of great significance for patients with lung cancer complicated with TB.

Limitations

The present study has several limitations. First, although abovementioned biomarkers (survivin, IFN-γ and miR-29) could be seen to help diagnose lung cancer in patients with TB, we did not take cost into account. Second, this study did not include the prognostic information of each group, and the significance of those biomarkers for the prognosis of lung cancer combined with TB could not be evaluated. However, it will be evaluated in future studies. Finally, this study is limited to the conditions of local population, and the discussed issue requires larger, multi-center, multi-field future studies.

Conclusions

In the present study, our results demonstrated that detecting the levels of miR-29a, survivin and IFN-γ was helpful for differential diagnosis of lung cancer and TB.

ORCID iDs

Lijuan Sun https://orcid.org/0000-0002-4105-1678
Hongyun Li https://orcid.org/0000-0001-8205-1036
Qun Fu https://orcid.org/0000-0002-4819-0697
Shuangmin Hu https://orcid.org/0000-0001-5679-7934
Wenfei Zhao https://orcid.org/0000-0002-2494-4925

References

1. Yu YH, Liao CC, Hsu WH, et al. Increased lung cancer risk among patients with pulmonary tuberculosis: A population cohort study. *J Thorac Oncol*. 2011;6(1):32–37. doi:10.1097/JTO.0b013e3181fb4fcc
2. Wallis RS, Kim P, Cole S, et al. Tuberculosis biomarkers discovery: Developments, needs, and challenges. *Lancet Infect Dis*. 2013;13(4):362–372. doi:10.1016/S1473-3099(13)70034-3
3. Tomasetti M, Lee W, Santarelli L, Neuzil J. Exosome-derived microRNAs in cancer metabolism: Possible implications in cancer diagnostics and therapy. *Exp Mol Med*. 2017;49(1):e285–e285. doi:10.1038/emm.2016.153
4. Chen X, Xu Y, Liao X, et al. Plasma miRNAs in predicting radiosensitivity in non-small cell lung cancer. *Tumor Biol*. 2016;37(9):11927–11936. doi:10.1007/s13277-016-5052-8
5. Bao M, Fu YR, Yi ZJ. Research progress of microRNA in anti-tuberculosis immunity and tuberculosis diagnosis [in Chinese]. *Zhonghua Jie He He Hu Xi Za Zhi*. 2015;38(12):918–921.
6. Das K, Saikolappan S, Dhandayuthapani S. Differential expression of miRNAs by macrophages infected with virulent and avirulent *Mycobacterium tuberculosis*. *Tuberculosis*. 2013;93:S47–S50. doi:10.1016/S1472-9792(13)70010-6
7. Münscher A, Prochnow S, Gulati A, et al. Survivin expression in head and neck squamous cell carcinomas is frequent and correlates with clinical parameters and treatment outcomes. *Clin Oral Invest*. 2019;23(1):361–367. doi:10.1007/s00784-018-2444-8
8. Zhang S, Xiao JY, Xie CN, Liu Y, Wang CL, Tian YQ. Effect of survivin gene silencing on malignant phenotype of nasopharyngeal carcinoma cell line CNE-2 [in Chinese]. *Ji Nan Da Xue Xue Bao*. 2011;32(6):607–610.
9. Pai M, Dheda K, Cunningham J, Scano F, O'Brien R. T-cell assays for the diagnosis of latent tuberculosis infection: Moving the research agenda forward. *Lancet Infect Dis*. 2007;7(6):428–438. doi:10.1016/S1473-3099(07)70086-5
10. Jiang W, Shao L, Zhang Y, et al. High-sensitive and rapid detection of *Mycobacterium tuberculosis* infection by IFN-γ release assay among HIV-infected individuals in BCG-vaccinated area. *BMC Immunol*. 2009;10(1):31. doi:10.1186/1471-2172-10-31
11. Goletti D, Raja A, Ahamed Kabeer BS, et al. IFN-γ, but not IP-10, MCP-2 or IL-2 response to RD1 selected peptides associates to active tuberculosis. *J Infect*. 2010;61(2):133–143. doi:10.1016/j.jinf.2010.05.002
12. Abdel-Samea SA, Ismail YM, Fayed SMA, Mohammad AA. Comparative study between using QuantiFERON and tuberculin skin test in diagnosis of *Mycobacterium tuberculosis* infection. *Egypt J Chest Dis Tuberc*. 2013;62(1):137–143. doi:10.1016/j.ejcdt.2013.02.003
13. Ettinger DS, Wood DE, Akerley W, et al; National Comprehensive Cancer Network. Non-small cell lung cancer, version 6.2015. *J Natl Compr Canc Netw*. 2015;13(5):515–524. doi:10.6004/jnccn.2015.0071
14. National Health and Family Planning Commission of the People's Republic of China. Diagnostic criteria for tuberculosis (WS 288-2017) [in Chinese]. *Electron J Emerg Infect Dis*. 2018;3(1):59–61.
15. Tao YH, Yang XL, Jin FX, Lv QQ, Dong HQ. Application of T lymphocyte interferon gamma release test in the diagnosis of tuberculosis [in Chinese]. *Zhong Hua Yi Yuan Gan Ran Xue Za Zhi*. 2017;27(18):4081–4084.
16. Altieri DC. Survivin, cancer networks and pathway-directed drug discovery. *Nat Rev Cancer*. 2008;8(1):61–70. doi:10.1038/nrc2293
17. Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. *Nat Med*. 1997;3(8):917–921. doi:10.1038/nm0897-917
18. Roshdy N, Mostafa T. Seminal plasma survivin in fertile and infertile males. *J Urol*. 2009;181(3):1269–1272. doi:10.1016/j.juro.2008.10.158
19. Tamm I, Wang Y, Sausville E, et al. IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. *Cancer Res*. 1998;58(23):5315–5320. PMID:9850056.
20. Cao Y, Dilimulati T, Yang HG. Clinical significance of survivin and p53 protein expression in non-small cell lung cancer [in Chinese]. *Chin J Cancer Prev Treat*. 2011;18(10):773–775.
21. Zhao SC, Zheng DR. Expression and significance of PPTG, survivin and bFGF mRNA in non-small cell lung cancer [in Chinese]. *J Clin Pulm Med*. 2017;22(3):542–545.
22. Niu YQ, Deng LY, Wang YF. Expression and clinicopathological significance of Sox2 and Survivin in non-small cell lung cancer [in Chinese]. *J Diagn Pathol*. 2015;22(10):594–597.
23. Liu SJ, Zhang F, Wei W, et al. Expression of miR-29a-3p and miR-365a-3p in peripheral blood of patients with active pulmonary tuberculosis and latent tuberculosis infection [in Chinese]. *Clin Study*. 2013;10(22):36–38.
24. Gebeshuber CA, Zatloukal K, Martinez J. miR-29a suppresses tristetraprolin, which is a regulator of epithelial polarity and metastasis. *EMBO Rep*. 2009;10(4):400–405. doi:10.1038/embor.2009.9
25. Fort A, Borel C, Migliavacca E, Antonarakis SE, Fish RJ, Neerman-Arbez M. Regulation of fibrinogen production by microRNAs. *Blood*. 2010;116(14):2608–2615. doi:10.1182/blood-2010-02-268011
26. Wang Y, Liu J, Chen J, Feng T, Guo Q. MiR-29 mediates TGFβ 1-induced extracellular matrix synthesis through activation of Wnt/β-catenin pathway in human pulmonary fibroblasts. *Technol Health Care*. 2015;23(s1):S119–S125. doi:10.3233/thc-150943
27. Cushing L, Kuang PP, Qian J, et al. miR-29 is a major regulator of genes associated with pulmonary fibrosis. *Am J Respir Cell Mol Biol*. 2011;45(2):287–294. doi:10.1165/rcmb.2010-0323OC
28. Xiao J, Meng XM, Huang XR, et al. miR-29 inhibits bleomycin-induced pulmonary fibrosis in mice. *Mol Ther*. 2012;20(6):1251–1260. doi:10.1038/mt.2012.36
29. Fu Y, Yi Z, Wu X, Li J, Xu F. Circulating microRNAs in patients with active pulmonary tuberculosis. *J Clin Microbiol*. 2011;49(12):4246–4251. doi:10.1128/JCM.05459-11
30. Sharbati J, Lewin A, Kutz-Lohroff B, Kamal E, Einspanier R, Sharbati S. Integrated micro-RNA-mRNA-analysis of human monocyte derived macrophages upon *Mycobacterium avium* subsp. *hominissuis* infection. *PLoS ONE*. 2011;6(5):e20258. doi:10.1371/journal.pone.0020258
31. Li TX, He Y, Zhou G, et al. The value of whole blood interferon gamma release test in the diagnosis of tuberculosis [in Chinese]. *Chin J Tuberculosis*. 2016;38(8):623–629.
32. Huebner RE, Schein MF, Bass JB. The tuberculin skin test. *Clin Infect Dis*. 1993;17(6):968–975. doi:10.1093/clinids/17.6.968
33. Black GF, Weir RE, Floyd S, et al. BCG-induced increase in interferon-gamma response to mycobacterial antigens and efficacy of BCG vaccination in Malawi and the UK: Two randomised controlled studies. *Lancet*. 2002;359(9315):1393–1401. doi:10.1016/S0140-6736(02)08353-8
34. Wu Y, Li Q, Zhang ZD. The value of interferon gamma release test in the diagnosis of senile pulmonary tuberculosis [in Chinese]. *Chin J Tuberculosis*. 2016;38(2):122–128.
35. Zeng Y, Li TS, Song MM, Huang L. The value of interferon gamma release test in the diagnosis of active pulmonary tuberculosis [in Chinese]. *J Clin Lung*. 2017;22(5):777–780.
36. Huang X, Chen J. Laboratory diagnosis of tuberculous pleural effusion [in Chinese]. *J Mod Lab Med*. 2014;29(1):97–100.
37. Zhang SM, Zhou H, Fu YQ, Shen YH, Zhou JY. The clinical value of γ-interferon release test in the diagnosis of active tuberculosis [in Chinese]. *Chin J Tuberc Resp Dis*. 2014;37:372–373.