Significance of Detecting the Levels of miR-29a, Survivin and Igras in Patients With Lung Cancer and Tuberculosis

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Abstract

Background:When lung cancer is combined with tuberculosis at the same time, it is easy to be misdiagnosed and missed. The purpose of this article is to provide reference for patients with lung cancer complicated by active pulmonary tuberculosis.

Methods:The concentration of survivin in diseased tissue and miR-29a and IGRAs in serum in 25 patients with non-small-cell lung carcinoma (NSCLC) complicated by active pulmonary tuberculosis (APT) and 32 patients with NSCLC and 30 patients with APT were measured.

Results:The expressions of miR-29a in serum of the APT group were higher than those of the NSCLC complicated by APT group and the NSCLC group ($p<0.05$). The expressions of survivin in diseased tissue of the NSCLC complicated by APT group and the NSCLC group were higher than those of the APT group ($p<0.05$).The expression level of IGRAs in serum of the NSCLC complicated by APT group and the APT group was higher than those of the NSCLC group ($p<0.05$), the expression level of IGRAs in serum of the NSCLC complicated by APT group was higher than the APT group.

Conclusion:We should be on guard against patients with APT complicated by lung cancer, when miR-29a was low expression in serum and survivin was high expression in diseased tissue of the patient who was pathologically diagnosed with APT. We should be on the alert for patients with lung cancer complicated by APT, when miR-29a was high expression in serum and survivin was low expression in diseased tissue and IGRAs was high level in serum of the patient with lung cancer.

Introduction

Lung cancer is a very common malignant tumor. Its morbidity and mortality are increasing year by year, posing a great threat to human health and life. Tuberculosis is also epidemic in China, causing a great economic burden. How to further improve the level of tuberculosis diagnosis and treatment has become an urgent problem to be solved [1]. The incidence of lung cancer in patients with tuberculosis is 10.9 times that of people without tuberculosis [2]. The incidence of both lung cancer and tuberculosis in China is relatively high. When lung cancer is combined with tuberculosis at the same time, it increases the difficulty of diagnosis and treatment, and is easy to be misdiagnosed and missed. When lung imaging indicates space occupying or exudative changes, the probability of diagnosis of lung cancer complicated with tuberculosis is not high. In clinical work, it is necessary to be vigilant. When some biomarkers are abnormal, it is often necessary to perform repeated lesion biopsy or lesion resection to confirm the diagnosis.

microRNA (miRNA) is a small molecule RNA that can be stably expressed in body fluids such as serum, plasma, saliva and can be sensitively detected. The blood circulating miRNA is mainly derived from a type of exosomes containing a variety of proteins and miRNAs that can be passed between cells and endocytic vesicles. miRNAs are stably expressed in exosomes and can be transmitted through exosomes [3]. Studies have found that miRNA can affect a variety of biological effects, such as DNA damage repair, cell cycle arrest, cell hypoxia, proliferation and apoptosis, etc. This makes miRNA a biological factor predicting NSCLC patients [4]. Some scholars have found that after Mycobacterium tuberculosis invades macrophages, miRNA participates in the body's anti-tuberculosis infection process [5]. In Das et al. [6] study, after THP-1 macrophages were infected with H37Rv and H37Ra, the expression of miR-29a in THP-1 cells increased

Survivin is the strongest inhibitor of apoptosis found so far. It is a new member of the Inhibitor of Apoptosis (IAP) family. It has complex functions and can inhibit cell apoptosis, promote cell transformation, participate in cell mitosis, 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. The survivin gene 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 hydroxyl terminal ring finger structure, in which the BIR molecule exerts an anti-apoptotic effect, while survivin only contains a single BIR functional region, the hydroxyl terminal does not contain a ring finger structure, but an interwoven spiral structure, which is different from other IAP family members.

Interferon-gamma release assay (IGRAs) is used as an auxiliary diagnostic test for tuberculosis infection [9]. Latent tuberculosis infection is very common in adults. Recently, IGRAs test is used to diagnose adult active tuberculosis [10–12].

In this study, the levels of miR-29a, survivin and IGRAs in patients with non-small cell lung cancer combined with active tuberculosis were detected to provide references for clinical diagnosis.

Materials And Methods

1.1 General information: 25 patients with non-small cell lung cancer complicated with active tuberculosis, 32 patients with non-small cell lung cancer and 30 patients with active tuberculosis diagnosed from March 2017 to September 2019 in the Fifth Affiliated Hospital of Zhengzhou University were selected as the research objects. Inclusion criteria: The diagnosis of non-small cell lung cancer was confirmed by lung biopsy, bronchoscopy, surgical pathological tissue, lymph node biopsy, etc. The TNM staging standards refer to the NCCN Non-Small Cell Lung Cancer Clinical Practice Guidelines [13]. The diagnosis of active tuberculosis conforms to the 2018 version of the diagnostic criteria for tuberculosis [14]. The pathological diagnosis of non-small cell lung cancer with active tuberculosis can be diagnosed with active tuberculosis at the same time, before or after the diagnosis of lung cancer. Among them, 3 cases were diagnosed at the same time, 9 cases were diagnosed as active tuberculosis before the diagnosis of non-small cell lung cancer, 13 cases were diagnosed as non-small cell lung cancer first and then active tuberculosis. Exclusion criteria: Retreated tuberculosis; Have a history of malignant tumor; Have a history of anti-tuberculosis or tumor treatment before enrollment; Obvious complications such as infection. In the non-small cell lung cancer group, there were 22 males and 10 females, aged 46 to 65 years old, with an average of (52.92±4.63) years old. There were 15 cases of lung squamous cell carcinoma and 17 cases of lung adenocarcinoma. TNM stage was - , including 13 cases of stage - a and 19 cases of stage b - . There were 21 males and 9 females in the active tuberculosis group, aged 42-68 years, with an average of (50.62±4.88) years old. Non-small cell lung cancer combined with active tuberculosis group: 18 males and 7 females; aged 49-71 years, with an average of (55.06±5.17) years old, 13 cases of lung squamous cell carcinoma, 12 cases of lung adenocarcinoma, TNM staging is stage I-IV. Among them, 12 cases were stage - a and 13 cases were stage b- . There was no statistically significant difference in gender and age of the three groups (p>0.05), and they were comparable.

1.2 Detection method

1.2.1 Survivin detection method: Use immunohistochemistry to detect survivin protein expression. After the tissue wax section is dewaxed, debenzene, and gradient ethanol hydrated, the endogenous peroxidase activity is blocked by 3% hydrogen peroxide, and the antigen is repaired by high pressure and high temperature. After washing, block for 10 minutes, add survivin antibody dropwise, and incubate at 4°C for 12 hours. Wash after incubation, add dropwise biotin-labeled secondary antibody and horseradish enzyme-labeled streptavidin, and incubate overnight at 4 ℃. Wash with PBS, add DAB dropwise to develop color for 5 minutes, wash with water, counter-stain with hematoxylin, dehydrate and dry, and finally seal the slice and observe under a microscope. Result judgment: 5 high-power fields are randomly selected for each slice, and scored according to the percentage of positive cells. The percentage of positive cells> 75% is counted as 4 points, ≥50% 75% is 3 points, ≥25% 50% 2 points, ≥5% 25% is 1 point, <5% is 0 point. According to color development intensity, brown is 3 points, brown yellow is 2 points, light yellow is 1 point, and no color is 0 points. The score is calculated by multiplying the two items, and the score is divided from high to low. A score of 9 or more is high expression, 5 to 8 is medium expression, 2 to 4 is low expression, and 0 to 1 is negative expression. In this study, high, medium, and low expressions were defined as positive expressions.

1.2.2 Detection method of miR-29a

QRT-PCR method is used to detect the level of miR-29a in peripheral blood. 3ml of fasting venous blood is collected early in the morning, centrifuged at 2000r/min for 20 minutes under low temperature conditions, and the supernatant is collected into a centrifuge tube and stored at -80℃ for testing. TRIZOL is used to extract total RNA from whole blood, and after nucleic acid concentration detection, reverse transcription of miRNA is performed to generate cDNA. Using cDNA as template and U6 as internal reference gene, SYBR Premix EX TaqTM is used to detect the expression level of miR-29a. Primer sequence miR29a-Primer upstream primer 5'-GGGTAGCACCATCTGAAA-3', downstream primer 5'-CAGTGCGTGTCCTGGAGT-3', U6-Primer 5'-GACTTATGTTAGGAGACGA-3'. The reaction system is 20μl, including cDNA 2μl, miR-29a-Primer 1μl, SYBR Premix EX TaqTM 10μl, and dd H20 7μl. A total of 40 cycles are amplified, and the relative expression of miR-29a is calculated using the $2^{-\triangle\triangle Ct}$ method. The experiment is repeated 3 times.

1.2.3 IGRAs detection method

Take 4ml of peripheral venous blood, and divide the whole blood into N (background control culture tube), T (test culture tube), and P (positive control culture tube) within 2 hours. 1ml for each tube. Mix gently. Place and incubate in a constant temperature incubator at 37°C for 24 hours, then centrifuge at 3000 rpm for 10 minutes, and take the upper layer of plasma. The ELISA method is used to detect the level of interferon-γ (IFN-γ) in the supernatant, the absorbance A is measured by a microplate reader at a wavelength of 450nm, and a standard curve is prepared according to the calibrator to calculate the IFN-γ level. When the value of IFN-γ is 0-14 pg/ml, it indicates that the result is negative. If it exceeds 14.0pg/ml, it indicates that the result is positive, and it is judged that the patient may have tuberculosis infection [15].

1.3 Statistical analysis All data are analyzed using SPSS 20.0 software. Among them, the measurement data are expressed as mean ± standard deviation, the comparison between groups is by t test; the count data is expressed by percentage, and the comparison between groups is by $\chi 2$ test. $P < 0.05$ the difference is statistically significant.

Results

2.1 The expressions of survivin, miR-29a, and IGRAs in the three groups are shown in Table 1.

The positive rate of survivin in lung cancer and lung cancer combined with tuberculosis group was significantly higher than that of tuberculosis, the difference was significant ($p<0.05$), there was no statistical difference between the non-small cell lung cancer group and the non-small cell lung cancer combined with active tuberculosis group ($p >0.05$) (Table 1), survivin expression had no significant relationship with the age, sex, pathological type, and clinical stage of NSCLC patients ($p>0.05$) (Table 2).

The expression of miR-29a in the active tuberculosis group was higher than that of the non-small cell lung cancer combined with active tuberculosis group and the non-small cell lung cancer group ($p<0.05$), and the non-small cell lung cancer combined with active tuberculosis group was higher than the non-small cell lung cancer group. However, there was no statistical difference ($p>0.05$). The expression of miR-29a had no significant relationship with the age, gender, and pathological type of NSCLC patients, but was only related to the clinical stage. The difference between the two was significant ($p<0.05$) (Table 3).

The level of IGRAs in the active tuberculosis group and the non-small cell lung cancer combined with active tuberculosis group was higher than that in the non-small cell lung cancer group ($p<0.05$), and the IGRAs level in the non-small cell lung cancer combined with active tuberculosis group was higher than that in the active tuberculosis group ($p >0.05$), but there is no statistical difference (Table 1).

Discussion

Survivin is the most powerful member of the apoptosis inhibitor family. Survivin gene is only expressed in embryonic tissues and developing fetal tissues. It is not expressed in most mature normal adult tissues, but has abnormal expression in many tumor tissues. [16-18]. Survivin promotes the occurrence and development of NSCLC by inhibiting the apoptosis of cancer cells, allowing cancer cells to escape from monitoring. Tamm et al. [19] detected the expression of survivin in 60 human tumor cell lines, and the results were all positive, of which the expression was relatively highest in lung cancer and breast cancer. The experimental results of this group show that survivin is highly expressed in NSCLC and NSCLC combined with tuberculosis, which is significantly higher than the positive rate of benign lung lesions, which is consistent with related reports [20]. In addition, this study found that the positive expression of survivin has nothing to do with age, gender, tissue type, and clinical stage ($p>0.05$), which is consistent with the results of Zhao Shucan et al. [21], but Niu Yanqing et al. [22] found that the positive rate of survivin is related to TNM staging. The later the TNM staging is, the higher the positive rate of survivin will be. It is considered that there may be a certain difference in the included cases.

MicroRNAs (miRNAs) are a type of non-coding RNA with 19-22 nucleotides. Because they can directly target a variety of proteins, they have multiple functions. MicroRNA-29a (miR-29a) is a small RNA family. In addition to the 3'untranslated region (3'UTR), it can also interact with miRNA regulatory elements (MRE) in the non-3'UTR region. There are a total of 14 miR-29 binding sites in the 3'UTR region and coding region of elastin. It was confirmed by luciferase reporter gene analysis that miR-29 can simultaneously bind to the MRE in the coding region and 3'UTR region to inhibit elastin expression [23]. Studies have found that miR-29 can directly inhibit the expression of a variety of collagen, and the target genes are mainly extracellular matrix and migration protein [24-25]. miR-29a target gene-related proteins participate in multiple signaling pathways, and can inhibit extracellular matrix remodeling by combining with the downstream of the TGF-β/Smad3 signaling pathway [26]. The expression of miR-29 in normal lung tissues gradually increases as the lung matures [27]. miRNA participates in the proliferation and apoptosis of various tumors by regulating the expression of oncogenes. The expression level of miR-29a is down-regulated in non-small cell lung cancer tissues, which is significantly related to tumor staging and metastasis, and has certain value for NSCLC diagnosis and disease evaluation [28]. Studies have found that miR-29a also plays an important role in the body's immune response and has certain clinical value in the diagnosis of active tuberculosis. Fu et al. [29] used a chip to screen the serum of patients with active tuberculosis and found that the expression of miR-29a was significantly up-regulated, which was consistent with the up-regulation trend of sputum in patients with active tuberculosis. Jutta et al. [30] also confirmed that Mycobacterium tuberculosis can upregulate the expression of miR-29a after infecting human macrophages. This study found that the expression level of miR-29a in the serum of patients with active tuberculosis was significantly higher than that of patients with lung cancer.

The incidence of tuberculosis is relatively high. Tuberculosis infection is the most common in the lungs. Tumor patients are susceptible to tuberculosis due to low immunity. At this stage, the most commonly used screening method for tuberculosis infection in China is still the tuberculin test (TST), but the 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 positives [32-33]. Bacteriological examination is a common method for active tuberculosis, but the culture time of tuberculosis bacteria is longer. Therefore, finding a fast and accurate detection method is particularly important for the prevention and diagnosis of tuberculosis.

Interferon-γ (IFN-γ) is a specific cytokine released by T lymphocytes sensitized by tuberculosis. In recent years, Interferon-γ release test (IGRAs) has become a new type of immunological diagnosis method, which is gradually applied in the clinical diagnosis of tuberculosis [34]. After a human is infected with Mycobacterium tuberculosis, two specific antigens, CFP-10 and ESAT-6, can be produced. The antigen stimulates T lymphocytes to produce γ-interferon. Therefore, by detecting the level of γ-interferon in the peripheral blood of patients, it can be judged whether there is Mycobacterium tuberculosis infection, and can distinguish true tuberculosis infection, eliminating the interference of vaccination and non-tuberculous infection [35]. Research by Huang Xiang et al. [36] found that malignant tumors and purulent infections can also cause γ-interferon to increase. Research results have confirmed [37] that IGRAs show high sensitivity and specificity in diagnosis, and play a better role in auxiliary diagnosis of active tuberculosis.

Conclusion

patients with high suspicion of tumor or tuberculosis in lung imaging should be particularly cautious in their diagnosis and treatment, fully consider the possibility of lung cancer complicated with tuberculosis, and develop a reasonable diagnosis and treatment plan based on actual conditions to avoid misdiagnosis or missed diagnosis.

Abbreviations

NSCLC: non-small-cell lung carcinoma

APT: active pulmonary tuberculosis

miRNA: microRNA

IAP: Inhibitor of Apoptosis

BIR: baculovirus IAP repeat

IGRAs: Interferon-gamma release assay

IFN-γ: interferon-γ

miR-29a: MicroRNA-29a

3'UTR: 3'untranslated region

MRE: miRNA regulatory elements

TST: tuberculin test

IFN-γ: Interferon-γ

IGRAs: Interferon-γ release test

Declarations

Ethics approval

This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of The Fifth Affiliated Hospital of Zhengzhou University.

Consent to participate

All patients informed and agreed to participate in this study.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflicts of interest

All of the authors had no any personal, financial, commercial, or academic conflicts of interest separately.

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Authors' contributions

Sun L J , Li H Y conceived of the study, and Fu Q and Hu S M participated in its design and coordination and Zhao W F helped to draft the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1 Expression of survivin, miR-29a and IGRAs in three groups of subjects

Group	n	Survivin				miR-29a			IGRAs		
		positive	negative	χ^2	p	`x±s	t	p	`x±s	t	p
①Lung cancer group	32	25	7			2.68±1.56			36.72±50.66		
②Tuberculosis group	30	5	25			6.04±2.09			132.43±122.28		
③Lung cancer with pulmonary tuberculosis group	25	18	7			3.14±1.47			102.48±60.55		
① Compared with ②				23.418	0.000		-11.50	0.000		-3.979	0.000
① Compared with ③				0.284	0.758		-1.442	0.153		-4.366	0.000
② Compared with ③				17.160	0.000		6.444	0.000		1.179	0.245

Note：p<0.05,There was statistical difference.

Table 2. Expression of Survivin and miR-29a in lung cancer

Clinicopathological features		n	Survivin				miR-29a		
			positive	negative	χ^2	p	`x±s	t	p
Age									
	≤55 years	12	8	4	0.696	0.454	1.53±1.68	0.575	0.573
	＞55 years	20	15	5			1.21±1.16		
Sex									
	Male	22	16	6	1.000	0.595	1.91±1.53	1.542	0.145
	Female	10	7	3			1.07±1.22		
Cytological typing									
	Squamous cell carcinoma	15	9	6	0.450	0.267	1.53±1.58	0.749	0.461
	Adenocarcinoma	17	13	4			1.16±1.15		
TNM by stages									
	Ⅰ-Ⅱastage	13	8	4	0.219	0.150	2.51±1.31	5.152	0.000
	Ⅲa-Ⅳ stage	19	16	3			0.52±0.58		

Note：p<0.05,There was statistical difference.

Table 3. Expression of Survivin and miR-29a in patients with lung cancer and pulmonary tuberculosis

Clinicopathological features		n	Survivin				miR-29a		
			positive	negative	χ^2	p	`x±s	t	p
Age									
	≤55 years	10	7	3	1.000	0.601	2.29±1.73	0.570	0.577
	＞55 years	15	11	4			1.92±1.39		
Sex									
	Male	18	12	6	1.000	0.607	1.92±1.35	-0.652	0.532
	Female	7	5	2			2.45±1.94		
Cytological typing									
	Squamous cell carcinoma	13	8	5	1.000	0.560	1.68±1.19	-1.349	0.193
	Adenocarcinoma	12	8	4			2.50±1.76		
TNM by stages									
	Ⅰ-Ⅱa stages	12	7	5	0.688	0.440	2.87±1.65	2.861	0.011
	Ⅱa-Ⅳ stages	13	9	4			1.33±0.92		

Note：p<0.05,There was statistical difference.