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Expression and clinical significance of MMP-9 and P53 in lung cancer

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Abstract. – OBJECTIVE: This study aimed to investigate the expression of MMP-9 (matrix metalloproteinases 9) and P53 (tumor suppressor proteins, tumor protein P53) in serum of patients with lung cancer. The relation between the two factors and their clinical pathological features were also explored.

PATIENTS AND METHODS: 150 patients were selected as the experimental group. They were diagnosed with lung cancer in Shandong Province Chest Hospital from January 2014 to January 2017. Meanwhile, 90 healthy subjects were selected as the control group. The expression levels of MMP-9 and P53 in serum were detected by fluorescence quantitative Real-Time Polymerase Chain Reaction (RT-PCR). The diagnostic value of MMP-9 and P53 in patients with lung cancer was analyzed by receiver operating characteristic curve (ROC). Pearson test was used to analyze the correlation between MMP-9 and P53 in the experimental group. The patients were divided into high expression and low expression groups according to the median of MMP-9 and P53 expressions.

RESULTS: The expressions of MMP-9 and P53 in patients with lung cancer were higher than the normal level ($p<0.05$). MMP-9 and P53 in serum of the experimental group were closely related to patients' TNM-stage, degree of differentiation, lymph node metastases, smoking history and pattern of organization ($p<0.05$). The expressions of MMP-9 and P53 were positively correlated ($p<0.001$).

CONCLUSIONS: The expressions of MMP-9 and P53 played important roles in lung cancer and were closely related to clinicopathology and three-year survival rate. They could provide help for the diagnosis and treatment of clinical lung cancer.

Key Words:
MMP-9, P53, Lung cancer, Clinicopathologic features, Diagnostic value, Three-year survival rate.

Introduction

Lung cancer is a common malignant tumor, which is mainly divided into non-small cell lung cancer and small cell lung cancer. Most of the patients are smokers. In recent years, the incidence and death rate of lung cancer are increasing rapidly, becoming one of the malignant tumors with the worst threat to people's health and life[1,2]. About 1.6 million new patients with lung cancer are diagnosed every year in the world, and its fatality rate ranks first among deaths caused by malignant tumors[3]. With the improvement of medical level in recent years, the diagnosis and treatment of lung cancer has made some progress, but there are still many problems in the prevention, early diagnosis and prognosis[4-6]. The chance of recovery is relatively greater in the early period of lung cancer. In the late period, the survival rate of lung cancer would be lower. Therefore, it is important to improve the prognosis of patients and the survival rate to reduce the morbidity and mortality of lung cancer. The study of medical personnel is essential[7,8].

MMP-9 and P53 play crucial roles in some cancer processes[9-12]. MMP-9 gene is located in chromosome 20q11.1-13.1, 26-27 kbp, with 13 and 9 genes. They are closely related to tumor invasion and metastasis. MMP-9 can degrade extracellular matrix, and its overexpression can enhance the ability of tumor cells to break through basement membrane, promoting tumor invasion and metastasis[13,14].

P53 gene is named for encoding a molecular weight of 53KD protein, which is an important tumor suppressor protein gene. It is located on

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chromosome 17 of human and contains 11 exons. P53 has the highest correlation with human tumor[15]. Mutations in the P53 gene are the main cause of P53 inactivation. Due to the absence of functional domains, they may exert "dominant negative effects" or induce more aggressive cancers through "functional acquisition"[16,17]. The relationship between the expression of MMP-9 and P53 in lung cancer and the clinicopathologic features are still unclear. Therefore, we conducted this study to provide more effective prognostic references for the clinical treatment of lung cancer and to improve the clinical treatment level of lung cancer.

Patients and Methods

Sample Collection

A total of 150 patients were selected as the experimental group. They were diagnosed with lung cancer in Shandong Province Chest Hospital from January 2014 to January 2017, with 98 male cases, 52 female cases and an average age 44-75 (61.5 ±10.4) years old. Meanwhile, a total of 90 healthy subjects were selected as the control group, with 56 male cases, 34 female cases and an average age 45-78 (61.8±10.6) years old. This study has been approved by the Medical Ethics Committee of Shandong Province Chest Hospital, and all patients signed the informed consent.

Inclusion criteria: patients pathologically diagnosed with lung cancer according to the criteria in American Joint Committee on Cancer (AJCC), 2010; patients without preoperative chemoradiotherapy; patients whose predicted survival time was greater than 3 months; patients who can be followed up by telephone with complete clinical data.

Exclusion criteria: patients with severe liver and kidney dysfunction; patients with other malignant tumors; pregnant or lactating women; patients with severe cardiovascular and cerebrovascular diseases; patients with cognitive and communication disorders; patients who did not cooperate with the experiment.

Reagents and Instruments

PCR instrument (ABI Company, Tampa, FL, USA; 7500), total RNA extraction Kit EasyPure miRNA Kit (Beijing TransGen Biotech Company, ER601-01, Beijing, China), reverse transcription + PCR Kit TransScript miRNA First-Strand cDNA Synthesis SuperMix (Beijing TransGen Biotech Company, Beijing, China, AT351-01), TransScript Green Two-Step qRT-PCR SuperMix (TransGen Biotech, Beijing, China, AQ201-01). All primers were designed and synthesized by Sangon Biotech Co., Ltd (Shanghai, China). More details were shown in Table I.

qRT-PCR Detection Methods

The collected serum was extracted by EasyPure miRNA Kit. The total RNA was detected by UV spectrophotometer and agarose gel electrophoresis for the purity, concentration and integrity of the total RNA. TransScript® miRNA RT Enzyme Mix and 2×TS miRNA Reaction Mix were used for reverse transcription of the total RNA, and the procedures were strictly in accordance with the manufacturer's kit. Then, the PCR amplification experiment was carried out. PCR reaction system: cDNA 1 μL, sense primers and reverse primers 0.4 μL, 2×TransTaq® Tip Green qPCR SuperMix 10 μL, Passive Reference Dye (50×) 0.4 μL, and ddH_2O was added to reach 20 μL in the end. PCR reaction conditions: pre-denaturation at 94°C for 30 s, denaturation at 94°C for 5 s, annealing extension at 60°C for 30 s, a total of 40 cycles were performed. There were 3 repeat holes set for each sample, and the experiment was carried out for 3 times. GAPDH was used as the internal reference in this study, and $2^{-\Delta ct}$ was used to analyze the data.

Follow-Up Survey

A total of 150 patients and their family members were followed up by telephone and interview for 3 years. The deadline of follow-up was

Table I. Primer sequence list.

Groups	Sense primer	Reverse primer
MMP-9	5'-TGTGTCTTCCCCTTCGTCTTCC-3'	5'-GCCCCACTTCTTGTCGCTGT-3'
P53	5'-ATGAAGCTCCCAGAATGC-3'	5'-GGGCCGCCGGTGTAG-3'
GAPDH	5'-TGGCAGAAGAAGGAGACAATAA-3'	5'-ACGCAGGAAGGCTTGAATAT-3'

January 2020. The overall survival was from the first day after surgery to the last follow-up or death.

Observational Indexes

Main observational indexes: the expression of MMP-9 and P53 between the experimental group and the control group was compared, and the survival conditions of the patients in 3 years was calculated. Pearson test was used to analyze the correlation between MMP-9 and P53 in lung cancer.

Secondary observational indexes: The diagnostic value of MMP-9 and P53 in lung cancer was analyzed by receiver operating characteristic curve (ROC). The patients were divided into high expression and low expression groups according to the median of MMP-9 and P53 expressions. and the Kaplan-Meyer survival curve was plotted.

Statistical Analysis

SPSS 20.0 (SPSS IBM, Armonk, NY, USA) medical statistical analysis software was used for the statistical analysis of the collected data. GraphPad Prism 7 (GraphPad software Co., Ltd., San Diego, CA, USA) was used for image rendering of the collected data. Data utilization (%) was counted by chi-square test, expressed by χ^2, and the measurement data was expressed by mean±-standard deviation (Mean±SD). All measurement data were in accordance with normal distribution. Independent sample *t*-test was used for comparison between the two groups. The 3-year survival of patients was analyzed by Kaplan-Meyer survival analysis. The log-rank test was used for analysis. ROC was used to evaluate the ability of MMP-9 and P53 in the diagnosis of lung cancer. Pearson was used to examine the relationship between MMP-9 and P53 in lung cancer. $p<0.05$ was considered statistically significant.

Results

General Clinical Data of the Experimental Group and the Control Group

There were no differences between the experimental group and the control group in age, gender, BMI, alcoholism, residence, disease course, triglyceride, low-density lipoprotein, serum cholesterol and other general clinical baseline data, without statistical significance ($p>0.05$). There were statistically significant differences in the baseline data of smoking history ($p<0.05$). More details were shown in Table II.

Expression of MMP-9 and P53 in Lung Cancer

RT-PCR was used to detect the expression levels of MMP-9 and P53 in the serum of patients of the two groups. The results showed that the expression level of MMP-9 in the experimental group were (0.673±0.132), significantly higher than that of the control group (0.472±0.098) (*p*

Table II. General clinical data of the experimental group and the control group [n(%)].

Groups	Experimental group (n = 150)	Control group (n = 90)	*t* or χ^2 value	*p*-value
Age	61.5 ± 10.4	61.8 ± 10.6	0.215	0.830
Gender			0.237	0.627
Male	98 (65.33)	56 (62.22)		
Female	52 (34.67)	34 (37.78)		
Smoking history			14.000	0.001
With	84 (56.00)	28 (31.11)		
Without	66 (44.00)	62 (68.89)		
Alcoholism			0.401	0.527
With	72 (48.00)	47 (52.22)		
Without	78 (52.00)	43 (47.78)		
BMI (kg/m^2)	21.152 ± 3.183	22.087 ± 3.474	2.128	0.034
Residence			0.056	0.813
Cities	89 (59.33)	52 (57.78)		
Countryside	61 (40.67)	38 (42.22)		
Disease course (years)	2.52 ± 1.64	2.21 ± 1.34	1.515	0.131
Triglyceride (mmol/L)	1.36 ± 0.23	1.35 ± 0.34	0.271	0.786
Low-density lipoprotein (mmol/L)	6.42 ± 1.25	6.25 ± 1.43	0.966	0.335
Serum cholesterol (mmol/L)	4.48 ± 1.04	4.47 ± 1.09	0.071	0.944

Table III. Expression of MMP-9 and P53 in the experimental group and the control group.

Groups	Experimental group	Control group	*t*-value	*p*-value
MMP-9	0.673 ± 0.132	0.472 ± 0.098	12.520	< 0.001
P53	2.137 ± 0.304	1.865 ± 0.281	7.039	< 0.001

Table IV. The diagnostic value of MMP-9 and P53 in lung cancer.

Indexes	AUC	95% CI	Specificity	Sensitivity	Youden index	Cut-off
MMP-9	0.866	0.822-0.911	72.67%	90.00%	62.67%	< 0.584
P53	0.787	0.727-0.846	74.67%	73.33%	50.52%	< 1.976

< 0.001). The expression level of P53 in the experimental group was (2.137±0.304), which was significantly higher than that of the control group (1.865±0.281) (p < 0.001). More details were shown in Table III.

Diagnostic Value of MMP-9 and P53 in Patients with Lung Cancer Analyzed by ROC Curve

ROC curve was plotted according to the expression of MMP-9 and P53 to analyze the diagnostic value of the two indexes in lung cancer. It was found that the area under the MMP-9 curve was 0.866, the specificity was 72.67%, the sensitivity was 90.00%, and the cut-off value was 0.584. The area under the P53 curve was 0.787, the specificity was 74.67%, the sensitivity was 73.33%, and the cut-off value was 1.976. More details were shown in Table IV and Figure 1.

The Relationship Between MMP-9 and P53 and Clinicopathological Features in Patients with Lung Cancer

According to MMP-9 and P53 of patients, we found that there were significant differences between the expression of mmp-9 and TNM stage, differentiation degree, lymph node metastasis, smoking history and pattern of organization of patients (p<0.05). There were significant differences between the expression of P53 and TNM stage, differentiation degree, lymph node metastasis, smoking history and tissue type of patients (p<0.05). More details were shown in Table V.

Correlation Analysis of MMP-9 and P53

According to Pearson correlation analysis of the relationship between MMP-9 and P53 in the experimental group, we found that the expression of MMP-9 and P53 in the experimental group was positively correlated. It meant that the expression of P53 increased with the increase of MMP-9 expression, with differences (r=0.577, p<0.001). More details were shown in Figure 2.

The Relationship Between the Expression of MMP-9 and P53 and the Three-Year Survival of Patients

We made a statistic on 3-year survival of patients. A total of 150 cases of patients and their families were followed up, with 0 patient lost in the follow-up period (Figure 3A). According to the median of MMP-9 and P53 expressions, the patients were divided into high and low expression groups. By drawing the Kaplan-Meyer survival curve, it was found that the survival rate of MMP-9 with high expression was 12.67% and that with low expression was 29.33%. The survival of patients with low expression of MMP-9

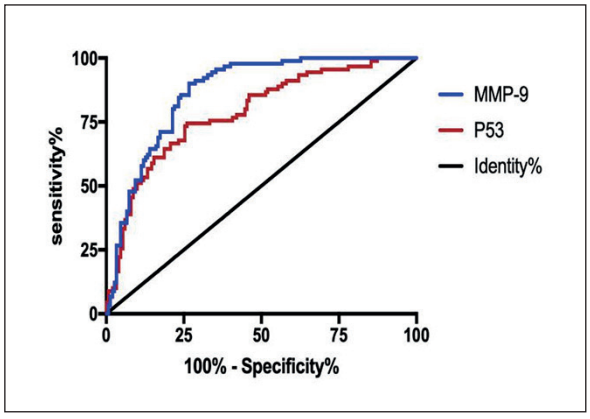

Figure 1. MMP-9 and P53 on prediction of the ROC curve of lung cancer efficacy. The optimal cut-off point 0.584 of MMP-9 could be obtained when the sensitivity was 90.00% and the specificity was 90.00%, the optimal cut-off point 1.976 of P53 could be obtained when the sensitivity was 73.33% and the specificity was 74.67%.

Table V. The relationship between MMP-9 and P53 and clinicopathological characteristics in patients with lung cancer.

Clinicopathologic features	N	MMP-9 (n = 150)	*t*-value	*p*-value	P53 (n = 150)	*t*-value	*p*-value
Age			1.846	0.066		1.917	0.056
≤ 50	78	0.659 ± 0.121			2.098 ± 0.274		
> 50	72	0.685 ± 0.123			2.158 ± 0.268		
TNM stage			9.485	< 0.001		20.350	< 0.001
Grade I+II	65	0.613 ± 0.118			1.932 ± 0.286		
Grade III+IV	85	0.745 ± 0.123			2.616 ± 0.296		
Grade of Differentiation			6.416	< 0.001		27.140	< 0.001
Undifferentiation	58	0.718 ± 0.115			2.725 ± 0.298		
Low differentiation	92	0.635 ± 0.109			1.828 ± 0.274		
Lymph node metastasis			9.246	< 0.001		18.040	< 0.001
with	67	0.746 ± 0.113			2.541 ± 0.294		
without	83	0.628 ± 0.108			1.947 ± 0.276		
Smoking history			2.711	0.007		25.360	< 0.001
Yes	90	0.693 ± 0.117			2.623 ± 0.273		
No	60	0.657 ± 0.113			1.838 ± 0.263		
Alcoholism			1.858	0.064		1.706	0.089
With	76	0.683 ± 0.118			2.147 ± 0.271		
Without	74	0.658 ± 0.115			2.094 ± 0.267		
Pattern of organization			4.648	< 0.001		19.500	< 0.001
Squamous carcinoma	79	0.697 ± 0.114			2.487 ± 0.270		
Adenocarcinoma	71	0.635 ± 0.117			1.915 ± 0.237		

was significantly higher than that of patients with high expression, and there were significant differences of 3-year survival between the two groups of patients (p=0.001) (Figure 3B). The survival rate of patients with high expression of P53 was 14.00%, while the survival rate of patients with low expression was 28.00%. The survival of patients in the low-expression group was significantly higher than that in the high-expression group, and there were significant differences between the two groups in 3-year survival (p=0.001) (Figure 3C).

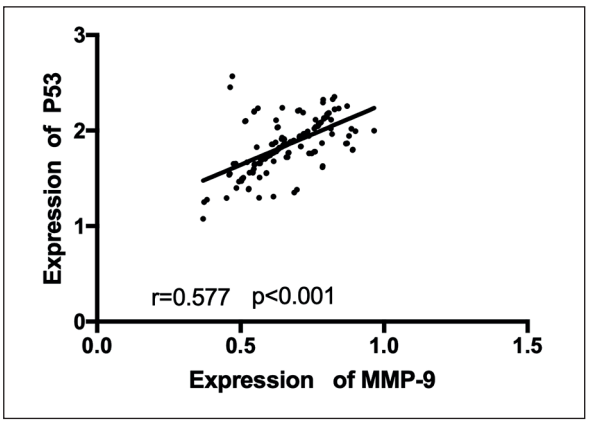

Figure 2. Correlation analysis of MMP-9 and P53. The expression of MMP-9 and P53 was positively correlated (r=0.577, p<0.001).

Discussion

With the acceleration of China's industrialization process, the incidence and death rate of lung cancer have become the first among malignant tumors[18,19], and the incidence of lung cancer is still gradually increasing. Invasion and metastasis are the main biological characteristics of malignant tumors, and also the main cause of failure in clinical treatment of lung cancer and death of patients[20]. There have been new methods of treatment for lung cancer in recent years. However, the specialty of early lung cancer has no evident symptoms, so it is hard to find lung cancer in the early days, leading to poor prognosis of lung cancer[21]. Hence, in order to improve the prognosis of lung cancer and reduce the incidence of lung cancer, more and more diagnostic methods need to be studied, so as to find out patients with lung cancer in the early stage, and effectively improve the effect of treatment.

In this study, it was found that the expressions of P53 protein and MMP-9 in cancer patients increased compared with those in normal people. It is suggested that P53 protein and MMP-9 may play certain roles in lung cancer. Firstly, we explored the relationship between the expression of MMP-9 in lung cancer patients and the clinical pathological characteristics of patients. We found that MMP-9 was significantly related to patients'

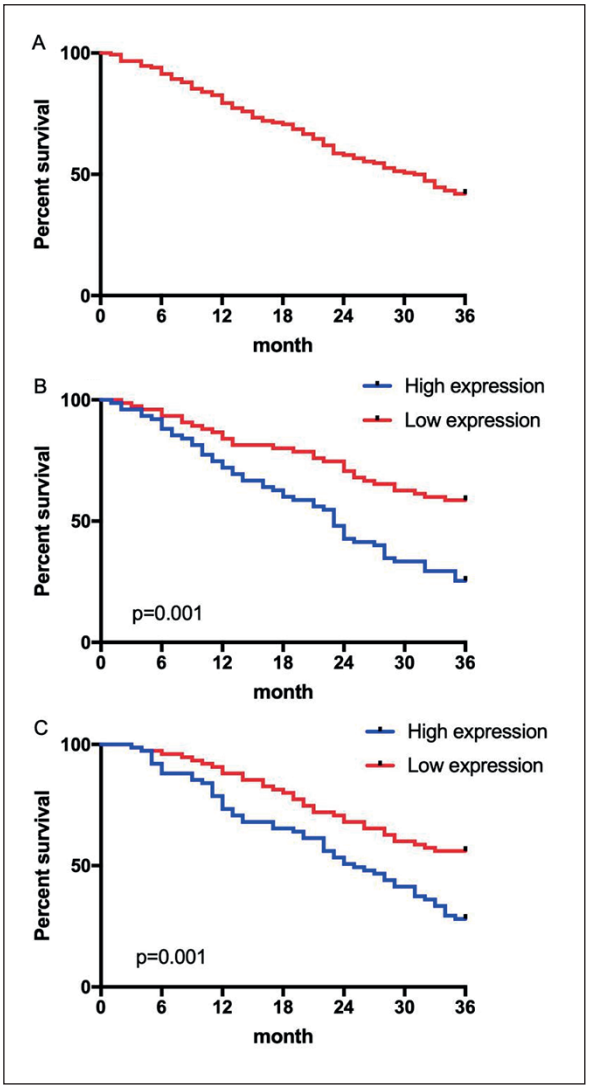

Figure 3. The relationship between the expression of MMP-9 and P53 and the three-year survival of patients. **A**, Patients were followed up for 3 years, with 42.00% overall survival rates of 3 years. **B**, survival in patients of MMP-9 low expression group was significantly higher than that in the high expression group. There were significant differences on 3-year survival of patients (p = 0.001). **C,** Survival in patients of P53 high expression group was significantly lower than that in the low expression group. There were significant differences on 3-year survival of patients (p = 0.001).

TNM stages, differentiation, lymph node metastases, smoking history, pattern of organization. Studies[22,23] have shown that MMP-9 shows high expression in breast cancer, gastric cancer and other tumor cells, and it is correlated with differentiation stage and prognosis. MMP-9 showed high expression in patients with lung cancer in the research of Zheng et al[24], which was similar to our research. MMP-9 is an important protease that can cut many extracellular matrix proteins to regulate extracellular matrix remodeling and cut many plasma surface proteins to release them from the cell surface. Zheng et al[24], as well as our study, suggest that MMP-9 may be an important target to promoting the occurrence and development of cancer. P53 is a tumor suppressor protein gene, which tends to be upregulated in this study. Avery-Kiejda et al[25] approved that the expression of P53 in breast cancer was also upregulated, which was similar to our results. Unlike Avery-Kiejda et al[25], we studied lung cancer in our study, and P53 was upregulated by genetic mutations. We speculated that in some cancers, the mutation of P53 gene may inactivate its mechanism and cause functional dysfunction, which may easily lead to cancer. Therefore, expression of P53 can be used as an important target for cancer discovery. Secondly, we also analyzed the diagnostic value of MMP-9 and P53 in lung cancer patients through ROC curve. We found that the optimal cut-off point 0.584 of MMP-9 could be obtained when the sensitivity was 90.00% and the specificity was 90.00%, the optimal cut-off point 1.976 of P53 could be obtained when the sensitivity was 73.33% and the specificity was 74.67%. It suggested that MMP-9 and P53 could be used as diagnostic indexes of lung cancer. We also analyzed the relationship between MMP-9 and P53 in lung cancer patients through Pearson correlation analysis, and found that the expression of MMP-9 and P53 was positively correlated. We speculated that the high expression of MMP-9 and P53 could be used as important indexes to predict lung cancer. Finally, we conducted a follow-up survey on the 3-year survival of the patients. According to the statistics, a total of 150 patients with lung cancer and their families were followed-up, with 87 patients died and 63 survived within 3 years, and the survival rate was 42.00%. Patients were divided into a high expression group and low expression group according to the median of MMP-9 and P53 expressions. By drawing the Kaplan-Meyer survival curve, it was found that the 3-year survival of patients in MMP-9 high expression group was significantly lower than that in the low expression group. The 3-year survival of patients in P53 high expression group was significantly lower than that in the low expression group. This indicated that the higher the expression of MMP-9 and P53, the higher the mortality rate of patients, which can be used as a prognostic indicator of lung cancer.

However, there are still some shortcomings in this work. First of all, although we found that the expression levels of MMP-9 and P53 were related to clinicopathological features, we did not conduct in-depth exploration. The specific regulatory mechanism was not clear, which needs to be further observed in follow-up experiments. Secondly, we are still unclear about the interaction and mechanism between MMP-9 and P53, which still needs to be further confirmed through follow-up experiments. Finally, it is not clear whether the expression of MMP-9 and P53 of cancer patients could be affected after a period of treatment, and we hope to observe it in the follow-up research.

Conclusions

In summary, the expression of MMP-9 and P53 in patients with lung cancer is higher than that of normal people. The expression of MMP-9 and P53 is related to the differentiation degree of lung cancer, TNM stage, lymph node metastasis, smoking history, and pattern of organization. The higher the expression of MMP-9 and P53, the worse the survival condition. The expression of MMP-9 and P53 is positively correlated.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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