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Expression and significance of MMP-9 and MDM2 in the oncogenesis of lung cancer in rats

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ABSTRACT

Objective: To observe the expression of matrix metalloproteinase-9 (MMP-9) and mouse double minute 2 homolog (MDM2) in the oncogenesis of lung cancer in rats and to explore their clinical value. **Methods:** A total of 140 rats were selected, of which 20 were selected randomly as the control group; and the remaining 120 as the observation group. The observation group was injected with benzopyrene to establish diseases model such as tissue proliferation, abnormal proliferation and lung cancer. Detected the MMP-9 levels of lung tissue by enzyme-linked assay, detected the MDM2 levels of lung tissue by immunochcmistry assay. **Results:** The MMP-9 and MDM2 expression of the lung cancer group and the abnormal proliferation group were significantly higher than that in the tissue proliferation group and the control group, the difference was significant ($P<0.05$). And the MDM2 expression of the tissue proliferation group was significantly higher than that in the control group, the difference was significant ($P<0.05$). There was no significant difference in the MMP-9 expression between the tissue proliferation group and the control group ($P>0.05$). The MDM2 and MMP-9 expression were increased in turn in the small cell carcinoma, squamous cell carcinoma and adenocarcinoma, the difference was statistically significant ($P<0.05$). The MMP-9 and MDM2 expressions of stage Ⅲ and stage Ⅳ lung cancer tissue in rats were significant higher than that during stage Ⅰ and stage Ⅱ, the difference was significant ($P<0.05$). There was no significantly different in the MMP-9 and MDM2 expressions between stage Ⅲ and stage Ⅳ ($P>0.05$), and there is no significant difference of the MMP-9 and MDM2 expressions between stage Ⅰ and stage Ⅱ ($P>0.05$). **Conclusions:** The expression of MMP-9 and MDM2 in lung tissue was associated with lung disease and lung cancer, both of them may be involved in the development and metastasis of lung cancer. Combined detection can be used as therapy and prognostic indicators for lung cancer.

1. Introduction

Lung cancer is one of the most common malignant tumor in the world today, its incidence and mortality rank the highest among all malignant tumors. Deaths from lung cancer accounts for more than 17% of all cancer[1]. Results of existing research showed that the invasion and metastasis is the main cause of death in patients with advanced lung cancer, and metalloproteinase (MMP) may promote cancer cell invasion to surrounding tissue through the degradation of extracellular matrix (ECM)[2]. Matrix metalloproteinase-9 (MMP-9) is the enzyme with the biggest relative molecular weight of the MMPs family, which degraded N-type and V-type collagen and gelatin. Its expression increased in malignant cell lines[3], and correlated with the angiogenesis, invasiveness and metastasis of tumor[4]. There were other studies showed that mouse double minute 2 homolog (MDM2) amplification was closely correlated with tumor growth and metastasis[5,6]. In this study, we use enzyme-linked immunosorbent assay (ELISA) and immunohistochemical staining (SP) to measure the expression MMP-9 and MDM2 of lung tissue and explore their correlation with lung cancer growth, differentiation, invasion and metastasis, provide indicators to determine the clinical treatment and prognosis of lung cancer.

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2. Materials and methods

2.1. Animals

A total of 140 rats were selected and were randomly into the control group (n=20) and observation group (n=120). Benzopyrene were injected into rats in observation group, specific methods[7,8] was applied to established disease models such as the tissue proliferation group, abnormal proliferation group, and lung cancer group *etc*. Pathological changes were observed including tissue proliferation (6 cases), abnormal proliferation (11 cases), lung cancer (103 cases). Pathological types included small cell carcinoma (36 cases), squamous cell carcinoma (57 cases), and adenocarcinoma (37 cases).

2.2. Experimental methods

MMP-9 expression was detected by ELISA. The lesions from deep anesthesia rats was taken, cut half and made into 10% homogenate with saline. MMP-2 expression levels were detected by ELISA. MMP-9 ELISA kit was purchased from Beijing Jingmei Co., strictly in accordance with instruction manual[9].

MDM2 expression in lung tissue was measured by immunohistochemical staining (SP). The other half of the lung lesions were washed with saline and fixed in 10% formaldehyde solution for 24 h. They were embedded with paraffin, and cut into sections. Expression of MDM2 was detected by immunohistochemical SP method. Operation was conducted as follows: antigen retrieval buffers was added in sections for repair, they were washed twice with PBS after cooling at room temperature for 30 min. After 10 min they were incubated at room temperature, added with 1% concentration of primary antibody (produced by Beijing Zhongshan Biotechnology Inc.), and then incubated in the greenhouse, wahsed with PBS. Secondary antibody (produced was added by Beijing Zhongshan Biotechnology Company), washed with PBS after incubation at 37 ℃. They were stained with DAB solution then washed, conterstained, dehydrated, cleared and finally mounted[10]. PBS solution was used instead of primary antibody as negative control[11].

2.3. Result determination

HV-D30P-S4 (manufactured by Hitachi, Japan) microscopic cameras was used to observe MDM2 protein, the positive cells cytoplasm showed brown or brown-yellow particles, the results were determined by semi-quantitative method. Five randomly vision was observed at high magnification, 200 cells was counted respectively. Slice positive cells <1% is 0 point, 1% -25% is 1 point, 25% -50% is 2 points, 50% -75% is 3 points, ⩾ 75% is 4 points. For dye depth: no positive cells is 0 point, yellow 1 point, brownish yellow 2 points, chocolate brown 3 points. The total score of the immune response was immune percentage of positive cells × dye depth[12,13].

2.4. Statistical analysis

The data were analyzed by SPSS 18.0 statistics software and the measurement data were expressed as mean±SD values, t- test was used and Pearson was applied in the correlation analysis, P<0.05 was considered as statistical significant difference.

3. Results

3.1. MMP-9 and MDM2 expression in normal lung tissue and lesions of lung tissue

The MMP-9 and MDM2 expression of the lung cancer group and the abnormal proliferation group were significantly higher than that in the tissue proliferation group and the control group (P<0.05). And the MDM2 expression of the tissue proliferation group was significantly higher than that in the control group (P<0.05). There was no significantly

Table 1
MMP-9 and MDM2 expression in normal lung tissue and lesions of lung tissue.

Groups	Number of cases (n)	MMP-9(μg/L)	MDM2 (min)
Control group	20	9.65±2.38	1.59±0.76
Tissue proliferation group	6	10.17±2.51[c]	2.18±0.83[b]
Abnormal proliferation group	11	27.45±4.83[a]	3.29±0.94[a]
Lung cancer group	103	39.84±5.67[a]	4.35±1.06[a]

Note: compared with the tissue proliferation group and the control group, [a]P<0.05, compared with the control group, [b]P<0.05; compared with the control group, [c]P>0.05.

Table 2
MMP-9 and MDM2 expression of different pathological types of lung tissue.

Type	The number of cases (n)	MMP-9 (μg/L)	MDM2 (min)
Small cell carcinoma	26	22.76±4.92	3.79±0.98
Squamous cell carcinoma	57	28.19±5.36[a]	4.29±0.94[a]
Adenocarcinoma	37	45.96±5.78[ab]	4.65±1.09[ab]

Note: compared with small cell carcinoma, [a]P<0.05, compared with squamous cell carcinoma, [b]P<0.05.

different in MMP-9 expression between the tissue proliferation group and the control group (P> 0.05) (Table 1).

3.2. MMP-9 and MDM2 expression of different pathological types of lung tissue

MDM2 and MMP-9 expression were increased in turn in the small cell carcinoma, squamous cell carcinoma and adenocarcinoma, the difference was statistically significant (P<0.05) (Table 2).

3.3. Expression of MMP-9 and MDM2 in different TNM staging lung cancer

MMP-9 and MDM2 expressions of stage Ⅲ and stage Ⅳ lung cancer tissue in rats were significantly higher than that during stage Ⅰ and stage Ⅱ (P<0.05). There was no significantly different in MMP-9 and MDM2 expressions between stage Ⅲ and stage Ⅳ (P>0.05), and there was also no significantly different in MMP-9 and MDM2 expressions between stage Ⅰ and stage Ⅱ (P>0.05) (Table 3).

3.4. Correlation between MMP-9 expression and MDM2 expression

Pearson correlation analysis showed that the MMP-9 expression was positively correlated with MDM2 expression in the lung tissue of rats with lung cancer (r= 0.795, P<0.01).

4. Discussion

Lung cancer is one of the world's leading deaths from tumors today, 5 year survival rate is only 15%.The mortality rate of lung cancer ranked first in the male cancer patients, the rate in women has more than breast cancer now[14], and it showed an increasing trend[15]. China is one of the regional cancer area, the incidence and mortality are increasing year by year. There are studies confirm that tumor invasion and metastasis is the main reason leading to low survival rate of patients[16]. Tumor invasion and metastasis first through the barrier of extracellular matrix (ECM) and vascular basement membrane (BM).

In the process of tumor invasion,tumor cell direct secreted MMYs or induce host cells to produce MMPs after combined with the extracellular matrix surface receptors,partially degraded ECM. Tumor cells infiltrated by the missing extracellular matrix, resulting in tumor invasion and metastasis[11]. Current research is more on MMP, mainly studies MMP-9. MMP-9 is a proteolytic enzyme secreted by a variety of cells, which is the enzymes with maximum molecular weight in MMP family. It can degrade ECM and promote cancer cell invasion, then promote tumor proliferation and metastasis[17]. Recent studies have found that MMP-9 was significantly higher in oral cancer and gynecologic tumors and is associated with invasion and metastasis[18,19].

The study of Wang *et al*[20] showed that MMP-9 play an important role in the process of lung cancer development, invasion and metastasis, but may be not related to the pathological type. MDM2 is an oncogene which can be combined with the transcriptional activation domain of p53. It mediated p53 transported out of cell nucleus and inhibit activation of transcription, but also promote p53 protein degradation by ubiquitin-proteasome, reducing its level; MDM2 can affect the cell cycle and proliferation and differentiation by regulating the p53 protein activity and levels,thereby affecting tumor occurrence and development. The study of Aikawa *et al*[21] showed that MDM2 protein expression are closely related to the differentiation, stage and lymph node metastasis of lung cancer. Studies of Chen *et al*[22] also showed that MDM2 protein-positive rate in patients with advanced lung cancer is higher than patients with early stage lung cancer, and patients with lymph node metastasis was significantly higher than those without lymph node metastasis, poorly differentiated type was significantly higher than well-differentiated. Dworakowska D *et al*[23] also considered: MDM2 gene may be involved in the occurrence and development of non-small cell lung cancer.

However, the research results on the relationships of MMP-9 and MDM2 with lung cancer have different conclusions. For example, the study of Hu *et al*[24] showed that the differences of MMP 9-expression in different pathological grade and lymph node metastasis have no statistically significant. In this study, the MMP-9 and MDM2 expression of the lung cancer group and the abnormal proliferation group were significantly higher than that in the tissue proliferation group and the control group, the difference was statistically significant (P<0.05). This is close to the result of Fan *et al*[8], Song *et al*[9], Safranek *et al*[25], which indicating that MMP-9, MDM2 are closely related to lung cancer occurrence and development .

In this study, the MDM2 and MMP-9 expression were increased in turn in the small cell carcinoma, squamous cell carcinoma and adenocarcinoma, the difference was statistically significant (P<0.05). This is close to the result of

Table 3

The expression of MMP-9 and MDM2 in different TNM staging lung cancer($\bar{x}$±sd).

TNM staging	The number of cases (n)	MMP-9 (μg/L)	MDM2 (min)
Ⅰ	21	25.48±5.23	3.95±0.96
Ⅱ	37	26.34±5.41[c]	4.07±1.02[c]
Ⅲ	38	38.97±5.64[a]	4.46±1.08[a]
Ⅳ	24	39.65±5.67[ab]	4.50±1.09[ab]

Note: Compared with stage Ⅰ, Ⅱ, [a]P <0.05, compared with stage Ⅲ, [b]P> 0.05; compared with stage Ⅰ, [c]P> 0.05.

Luo *et al*[10], Hao *et al*[11], Zhang *et al*[13], which showed the MMP-9, MDM2 expression in lung tissue may be associated with the pathological type. In addition, in this study the MMP-9 and MDM2 expressions of stage Ⅲ and stage Ⅳ lung cancer tissue in rats were significantly higher than that during stage Ⅰ and stage Ⅱ, the difference was statistically significant($P<0.05$). The study of Zhang *et al*[15], Chen[22] *et al* study also shows that MMP-9 and MDM2 have a certain relationship between lung cancer staging. In this study, Pearson correlation analysis showed that MMP-9 and MDM2 expression in lung cancer tissue in rats were positively correlated, which indicating that both may participate development, transfer and other processes in the occurrence of lung cancer.

In summary, the expression of MMP-9 and MDM2 in lung tissue were associated with lung disease and lung cancer, both of them may be involved in the development and metastasis of lung cancer. Combined detection can be used as therapy and prognostic indicators for lung cancer.

Conflict of interest statement

We declare that we have no conflict of interest.

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