Expression of Cripto-1 gene protein and Activin-A in human lung adenocarcinoma tissue

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Abstract: To research the expression in human lung adenocarcinoma tissue of Cripto-1 (teratocarcinoma derived growth factor-1) gene protein and Activin-A gene protein, and explore the relationship and clinical significance between the two gene protein and clinical pathological characteristic of lung adenocarcinoma. This study had applied the immunohistochemical method to detect the 188 cases of lung adenocarcinoma and expression of Cripto-1 protein and Activin-A protein in 100 cases of normal lung tissue. Then, analysis the relationship between these two-gene protein and clinical lung adenocarcinoma histopathological features, and inherent correlation between these two genes. The positive expression rate of Cripto-1 protein in lung adenocarcinoma tissue was significantly higher in normal lung tissue, while, the positive expression rate of Activin-A protein in lung adenocarcinoma tissue was significantly lower than in normal lung tissue. The high expression of Cripto-1 and low expression of Activin-A was closely related (each $P<0.05$) to the TNM staging of lung adenocarcinoma, lymph node metastasis and the main pathological tissue staging of lung adenocarcinoma. And the correlation analysis showed that it was negative correlation for the expression of Activin-A protein and Cripto-1 protein in lung adenocarcinoma. The over expression of Cripto-1 and the expression lack of Activin-A were correlated with the occurrence, development, metastasis and malignant degree of lung adenocarcinoma.

Keywords: Lung adenocarcinoma; Cripto-1; Activin-A

INTRODUCTION

Lung adenocarcinoma was the world's common respiratory tract malignant tumor (William *et al*., 2011). In recent years, there was an upward trend in the incidence of lung adenocarcinoma. What had attracted more and more attention for the medical field was that the lung adenocarcinoma was with features of early local invasion and early blood vessels and lymphatic metastasis, high malignant degree. Lung adenocarcinoma belonged to the non-small cell lung cancer, according to the lung adenocarcinoma new international multidisciplinary classification scheme 1 in 2011, the lung adenocarcinoma were divided into In situ adenocarcinoma, micro invading adenocarcinoma and invading adenocarcinoma, and invading adenocarcinoma was divided into sidewall type, acini type, nipple type and micro nipple type and entity type, etc. Although scholars at home and abroad had extensive research on lung adenocarcinoma, it was still unclear for the molecular markers to regulate the cancerous process initiation and its progression and biological events. So it was important clinical significance to search for new molecular markers for diagnosis and prognosis of lung adenocarcinoma cancer patients.

Cripto-1 gene was also named teratocarcinoma-derived growth factor-1, belonging to the gene family of epidermal growth factor Cripto-FRL-Cripic (EGF-CFC). A number of research results showed that its abnormal expression may play an important role in the process of the occurence of tumor (Tadahiro *et al*., 2013). Activin-A gene belonged to TGF family. Many studies had proved that Activin-A had low expression in most tumor cells, high expression in normal tissue cells, and had inhibitory effect on a wide variety of tumor cells.

This study had applied immunohistochemical method to detect different expression of Cripto-1 and Activin-A gene protein in normal lung tissue and lung adenocarcinoma tissue. Then, analysis the relationship between these two-gene protein and clinical lung adenocarcinoma histopathological features and inherent correlation between these two genes. And to discuss the instruction significance of these two gene protein on tumor treatment.

MATERIALS AND METHODS

Materials

This study had chosen 188 cases of paraffin embedding specimens patients with lung adenocarcinoma in the first affiliated hospital of Zhengzhou university from January 2011 to April 2014. In these 188 cases of patients, there were 116 cases of male, with an average age of 49.8 years old, 76 cases of patients with no Lymph-Y node metastasis, and 112 cases of patients with lymph node metastasis. According to the lung cancer TNM staging criteria, the 188 cases of lung adenocarcinoma had been divided into I-II stage with 108 cases and III-IV stage with 80 cases. Combined with the international lung adenocarcinoma new classification scheme in 2011, 188 cases of lung adenocarcinoma was with invasion feature,

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and could be divided into: 80 cases with nipple type, 32 cases with micro nipple type, 20 cases of entity type and 56 cases of gland bubble type. In control group, this study had chosen 100 cases of the same period of normal lung tissue paraffin embedding specimens from thoracic surgery resection in the first affiliated hospital of Zhengzhou university, including 44 cases of male, 56 cases of female, the average age of 46.9 years old.

Immunohistochemical staining

Dyeing method was processed based on immunohistochemical streptomycin resistance biotin peroxidase (SP) method. Main reagents: rabbit anti human Cripto-1 polyclonal antibody (from abcam company in British, Lot No: Ab19917), goat anti human Activin-A polyclonal antibody (R&D company in the US, LotNo: CCK0312011), standard rabbit serum (Zhengzhou Yikang Bio-engineering Co.,Ltd, Lot No: 201901005), Biotinylated Rabbit anti goat IgG (from Beijing Kangwei Reagent Company, Lot No: 01912C), biotin labeled Goat anti rabbit two resistance (Beijing Kangwei Reagent Company, Lot No:1412E), closed with normal sheep serum (Beijing Kangwei Reagent Company, Lot No: 0212K), Immunohistochemical Color Kit (Beijing Kangwei Reagent Company, Lot No: 3012K).

Results

The positive expression product of Cripto-1 protein were mainly located in the cytoplasm and cell membrane, while, the positive expression product of Activin-A protein were mainly located in cytoplasm. In both parts, the positive cells were tan particles in the cytoplasm and / or cell membrane. Below was the immunohistochemical results criteria: positive cells quantity scoring: Each section been randomly selected at high magnification microscope vision 10, dyeing results determined the semi-quantitative method points, positive cells less than 5% was for 0 core, 5%-20% for 1 score, 21%-50% for 2 score, 51%-75% for 3 score, more than 75% for 4 score; cell staining intensity scoring: light yellow color for 1 score, tan yellow color for 2 score, tan color for 3 score, non color for 0 score. This study had added the score in and score in and got the results, more than or equal to 3 points was positive, less than 3 points was negative.

Statistical processing

This study had applied SPSS13.0 statistical software for data analysis, and used $\chi 2$ test for immunohistochemical results and clinical pathological parameters, applied Spearman test for the correlation analysis of Cripto-1 and Activin-A, inspection level was $\alpha=0.05$.

RESULTS

Expression of Cripto-1 protein in lung adenocarcinoma tissue and normal lung tissue (fig. 1)

The positive expression rate of Cripto-1 was 72.3% (136/188) in the 199 cases of lung adenocarcinoma tissues and 12.0% (12/100) in the 100 cases of normal lung tissues. The expression rate of Cripto-1 in lung adenocarcinoma tissue was significantly higher than in normal lung tissue. Comparative differences between groups was with statistical significance ($\chi 2=95.143$, $P<0.05$).

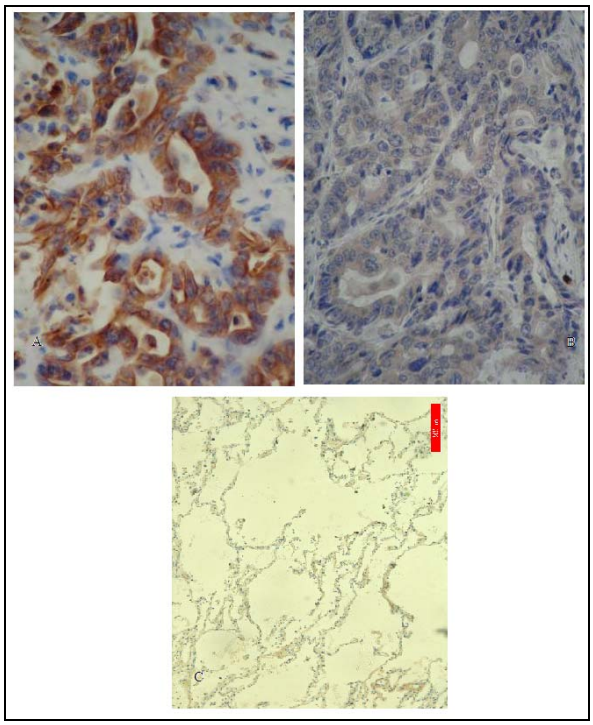

Fig. 1: Cripto-1 protein expression in lung adenocarcinoma tissues (A was for high expression, B was for lower expression) and normal lung tissues (negative) SP 200×

The relationship between Cripto-1 protein expression and lung adenocarcinoma clinical pathological features

In the 188 cases of patients, the lung adenocarcinoma of clinical pathology data statistics results showed that the high expression of Cripto-1 protein was closely correlation ($P<0.05$) with the TNM staging, lymph node metastasis and main histopathologic stage of lung adenocarcinoma (see table 1). Cripto-1 protein positive expression in III-IV stage of lung adenocarcinoma tissues had increased significantly than I-II stage. And the positive the positive expression rate in tissue with lymph node metastasis group was much higher than that in tissue without lymph node metastasis. The positive expression rate of Cripto-1 was statistically significant differences in different pathological tissue stage specimens of lung adenocarcinoma, besides that the positive expression rate of Cripto-1 in entity type and the micro-nipple type than that in nipple type and acini type.

The express of activin-A protein in the lung adenocarcinoma tissue and normal lung tissue (fig. 2)

The positive expression rate of Activin-A in lung adenocarcinoma tissue of 188 cases of patients was 59.6%

(112/188) and the positive expression rate in normal lung tissues of 100 cases was 92.0% (92/100). Expression rate of Activin-A in the lung adenocarcinoma tissue was far lower than that in normal lung tissue and the difference between groups was with statistical significance ($\chi 2$=71.440, P<0.05).

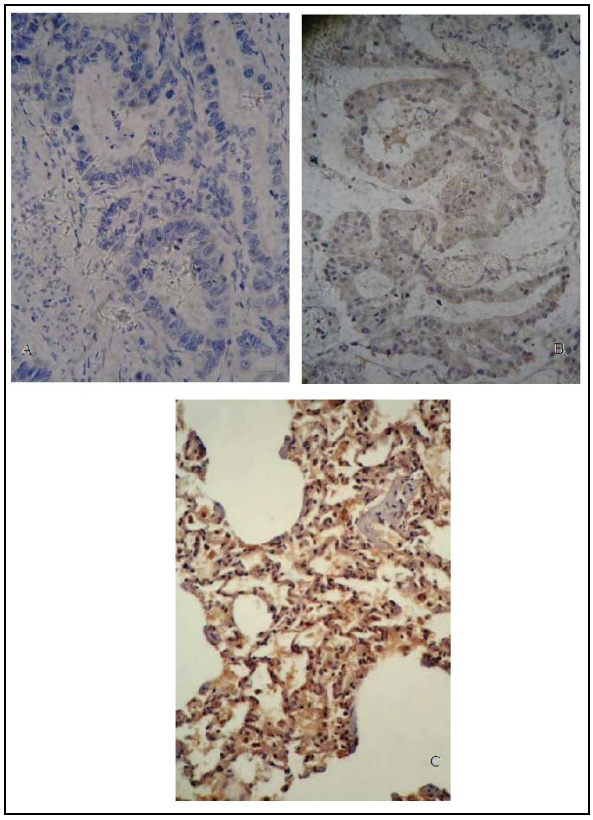

Fig. 2: Activin-A protein in the lung adenocarcinoma tissue (A for negative, B for lower expression) and normal lung tissue (high expression) expression. SP 200 x

Relationship between the activin-A protein expression and every clinical pathological features of lung adenocarcinoma

The statistically results of clinical pathology data for 188 patients with lung adenocarcinoma showed that low expression of Activin-A protein was closed related (P<0.05) with TNM staging, lymph node metastasis and Lung adenocarcinoma of lung adenocarcinoma patients. The positive expression rate of Activin-A protein in-group with lymph node metastasis group of lung adenocarcinoma was significantly lower than that in groups without lymph node metastasis. The positive expression rate in cancerous tissue of III-IV stage was obviously lower than that in I-II period, and the positive expression rate in entity type and micro-nipple type was significantly lower than that in nipple type and acini type.

The relationship between the activin-A protein and Cripto-1 protein in lung adenocarcinoma

In 112 cases of Activin-A positive expression in lung adenocarcinoma tissues, Cripto-1 positive expression was 12 cases and negative expression was 100 cases. In the negative expression of 76 cases of lung adenocarcinoma tissues, Cripto-1 positive expression was 36 cases, negative expression was 40 cases. Statistical results showed that the Activin-A protein and Cripto-1 protein expression in lung adenocarcinoma were negative correlation, P<0.05 (γ=-0.381, P=0.001).

DISCUSSION

Lung adenocarcinoma was a kind of respiratory tract malignant tumor with high malignant degree. And prognosis was significantly different between verity types of lung adenocarcinoma. Which had a better prognosis was situ adenocarcinoma and micro invading adenocarcinoma. The situ adenocarcinoma and micro invading adenocarcinoma had a good prognosis. In the invading adenocarcinoma, the stick-wall type, nipple type and acini type had a relatively good prognosis, while, entity type and micro nipple were with poor prognosis (Yoshizawa *et al*., 2011; Russell *et al*., 2011). Research showed that the occurrence of malignant tumor and cell cycle disorder were closely related to the disorder of differentiation and proliferation. In the early stages, tumor were mainly excessive proliferated, and then cell and hyperplasia regulating mechanism was disordered, eventually leaded to cancer. At present, with the discovery and in-depth study of cancer genes, people understood the oncogene and tumor suppressor genes regulating function imbalance was an important factor in tumor occurrence and development. Search for cancer gene, the early detection of cancer genes, and the related molecular targeted therapy of cancer gene were becoming more and more significant in the treatment of patients with tumor, and many cancer gene targeting molecules had been identified in a variety of tumor (Jianxin *et al*., 2012; Jun *et al*., 2012), cancer gene targeting molecules had become the focus of medical profession research in recent years.

Many studies had confirmed that Cripto-1 gene protein had cancer gene characteristics, and it had been reported the expression positive rate of Cripto-1 were ranging from around 40%-80% in most malignant tumor in, for example: Skin melanoma, breast cancer, gastric cancer, oral squamous carcinoma and cervical cancer, while, the expression of lung adenocarcinoma were rarely reported. This study had applied immunohistochemical technique to research the expression situation of Cripto-1 in normal lung tissues and lung adenocarcinoma tissues, the results showed that the high expression of Cripto-1 in lung adenocarcinoma tissues was closely related to the occurrence of lung adenocarcinoma. The clinical pathological data results of the statistical analysis of lung adenocarcinoma patients showed the high expression of Cripto-1 protein was significantly correlated with the TNM staging, lymph node metastasis of lung adenocarcinoma and main histopathologic type and

Table 1: Cripto-1 protein expression and clinical pathological features of lung adenocarcinoma

Pathological Features	N	Cripto-1 Protein Express				
		Positive Case	Negative Case	Positive Expression Rate (%)	$\chi 2$	P
Lymph node metastasis						
No	76	40	36	52.6	24.764	0.001
Yes	112	96	16	85.7		
TNM Installment						
I, II	108	64	44	59.3	21.704	0.001
III, IV	80	72	8	90.0		
Main Histology Stage						
Nipple Type	80	44	9	55.0	31.928	0.000
Micro-nipple type	32	32	0	100		
Entity Type	20	20	0	100		
Acini Type	56	40	4	71.4		

Table 2: The relationship between Activin1A protein expression and clinical pathological features of lung adenocarcinoma

Pathological Features	N	Activin-A Protein				
		Positive Case	Negative Case	Positive Expression Rate (%)	$\chi 2$	P
Lymph node metastasis						
No	76	44	32	57.9	16.164	0.001
Yes	112	32	80	28.6		
TNM Staging						
I, II	108	60	48	55.6	24.124	0.000
III, IV	80	16	64	20.0		
Main Histology Stage						
Nipple Type	80	39	41	48.8	33.476	0.001
Micro-nipple Type	32	1	28	12.5		
Entity Type	20	0	20	0.0		
Acini Type	56	24	32	42.9		

proved the Cripto-1 protein had a very important role in the development, invasion and metastasis of lung adenocarcinoma. And the high expression of Cripto-1 protein indicated the high malignant degree and poor prognosis of lung adenocarcinoma.

Activating Activin was transforming growth factor-β(TGF-β) family, its signal were through Activin receptor (Alk4) and Activin II receptor (ActR II / II B) to delivery (Gray *et al*., 2003). Activin-A was a complex hormone that has a variety of biological functions. And it could be expressed in several of normal body tissue cells. Its functions included regulating cell growth, reproduction, differentiation, and participated in the body's inflammatory response, damage repairation and even participated in regulating tumor cell growth and apoptosis process (Elspeth *et al*., 2013). Therefore, it was regarded as a tumor suppressor gene. Activin signal transduction pathways mediated disorder that could make the cells to escape from this growth inhibition, resulting in the occurrence of tumor. This study had found in previous studies, Activin-A could inhibit the growth of varieties of tumor cell lines, while, there were rarely reported the correlation research between Activin-A and lung adenocarcinoma in home and abroad. The immunohistochemical staining results showed that the positive expression rate of Activin-A protein in lung adenocarcinoma tissues was significantly hower than that of normal lung tissue, which showed that the low expression of Activin-A in lung adenocarcinoma tissues was closely related with the occurrence of lung adenocarcinoma. High expression of Activin-A protein indicated the better prognosis of tumor. So, ActivinA played the role of tumor suppressor genes on the occurrence of tumor. The clinical pathological data of the statistical analysis results for lung adenocarcinoma patients also showed the low expression of Activin-A was significantly correlated with TNM staging, lymph node metastasis of lung adenocarcinoma and main histopathologic type of lung adenocarcinoma, also indicated that the low expression or lack expression of Activin-A protein played an important role in the development and transference of ung adenocarcinoma, also indicated the poor prognosis of lung adenocarcinoma. This study also showed Cripto-1 and Activin-A expression had significant negative correlation in lung adenocarcinoma, indicated both had the mutual inhibition function in the occurrence and development of lung

adenocarcinoma. Related studies showed that the promoting cancer gene function of Cripto-1 was realized through adjusting Actvin signal transduction pathways. The over expression of Cripto-1 protein could hinder the cell signal transduction within Activin-A, Cripto-1 could directly link with the receptor (Alk4) of Activin I, and inhibited the combination with Actvin-A and Alk4. In tumor cells, due to formation of the Cripto-1, Activin and ActiR II compound of Activin II, the blocked signal then blocked the function of Activin's function of inhibiting the proliferation of lung adenocarcinoma. In addition, the combination of Cripto-1 and Activin AB to block Actvin-A and inhibit the growth of tumor cells. The antibody inhibitor of Cripto-1 and Activin-A could obviously inhibit the growth of tumor cells and the inhibition rate could reach 70% (Xiufeng and Peixiang, 2005), this provided guidance for our further study on tumor cells targeted therapy. The most tumor biological target therapy about the Cripto-1 was: Cripto-1 antisense oligonucleotide research, RNA interference technique and tumor monoclonal antibody research, etc. In animal models, antisense oligonucleotides research not only could effectively inhibit the growth of a wide variety of tumor cell lines, but also reduce microvascular density in the tumor tissue (Caterina *et al*., 2005). RNA interference technology was more expected to be applied to clinical treatment rather than the antisense oligonucleotide technology in recent years. Activin-A and Cripto-1 signal transduction pathways of Cripto-1/ActR II Blocker was a new method for the treatment of potential cancer targeted therapy in the latest research. Alantolactone block the Cripto-1 signal conduction to perform the function of anti-tumor, through blocking the combination of Cripto-1 and Activin II receptor. Related data showed that the drug *in vitro* culture had specifically anti-tumor function of proliferation in human colonic cancer cells, and mostly don't have toxicity to normal cell (Ying *et al*., 2011). In addition, the direct application of Activin-A treatment in some tumors could help to promote the role of muscle atrophy, tumor in mice the Activin-A systemic therapy which applied on mice could promote tumor tissue muscle atrophy and apoptosis effect (Thissen and Loumaye, 2013). Although the study above could not be applied to study in the human body due to several of limitations temporarily, had pointed out the new direction for clinical tumor targeted biological treatment in the future.

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