Expression and significance of DDX43 in lung adenocarcinoma

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Abstract: This paper aims to determine the expression and clinical significance of DDX43 in lung adenocarcinoma. Expression of DDX43 gene and protein of lung adenocarcinoma tissue and para-carcinoma tissues was observed in 27 cases by RT-PCR and immunohistochemistry. These patients were diagnosed as lung adenocarcinoma in the Huaihe Hospital of Henan University from February 2015 to December 2015. The relative ratio of DDX43 mRNA expression in lung adenocarcinoma and para-carcinoma tissues was 0.87±0.62 versus 0.21±0.77 and the difference between the two groups was statistically significant ($P<0.01$). The expression of DDX43 in normal lung tissues and lung adenocarcinoma tissues was different. The positive rate of DDX43 expression in lung adenocarcinoma tissues was significantly higher than that in normal lung tissues, and the difference was statistically significant ($P<0.05$). The analysis of clinical pathological characteristics showed that the increase of protein expression was related to the stage and metastasis of lung adenocarcinoma. DDX43 is highly expressed in lung adenocarcinoma, and the expression level is related to the stage and metastasis of lung adenocarcinoma, suggesting that DDX43 is closely related to the occurrence and development of lung adenocarcinoma, and may be a molecular marker for early diagnosis of lung adenocarcinoma.

Keywords: DDX43, lung adenocarcinoma, RT-PCR, immunohistochemistry, biomarker.

INTRODUCTION

Lung adenocarcinoma is one of the most lethal tumors in the world. Currently, cancerous metastasis and five-year survival rate have become research focuses. The pathological classification of tumor plays a key role in the therapeutic effect, and the complexity and heterogeneity of histological classification affect the use of conventional treatment methods (Sakashita *et al*., 2014). Lung adenocarcinoma, the most common pathological type of lung adenocarcinoma, belongs to the non-small cell lung adenocarcinoma, which is also the main cause of cancer-related death (Siegel *et al*., 2013). In Europe, the five-year survival rate of patients with lung adenocarcinoma is only 11.5% (Hauer *et al*., 2015). Despite advances in treatment technology, there has been no significant reduction in the number of patients who died of lung adenocarcinoma in the past few years, which is mainly due to metastasis and recurrence (Bodendorf *et al*., 2009).

Related research shows that the sooner the treatment of lung adenocarcinoma, the higher the five-year survival rate. The five year survival rate of stage IA and IB non-small cell lung adenocarcinoma was significantly higher than that of IIIA and IIIB, and patients who have been treated in the late stage are usually treated with chemotherapy (Goldstraw *et al*., 2007). Therefore, the early diagnosis and treatment of lung adenocarcinoma is very important. At present, the key and difficult point in the research of lung adenocarcinoma is the early diagnosis and metastasis. With the development of lung adenocarcinoma, many scholars have devoted themselves to the study of biomarkers of lung adenocarcinoma, but only a few can be used in clinical. Lung adenocarcinoma is a highly heterogeneous polygenic disease. Mutations in EGFR, ALK, KRAS, ROS1 and other genes can lead to the occurrence of cancer (Wang, 2014). In which DEAD (Aso-Glu-Ala-Asp) box polypeptide43 (DDX43) is a tumor-specific gene originally identified in LB23-SAR in human striated muscle tumor cell lines (Chen *et al*., 2015). Some literature had proved that DDX43 is connected with the invasion, metastasis and angiogenesis of tumor cells, and is able to activate a series of proto oncogene (Liu *et al*., 2007). Scanlan et al. have reported that the expression rate of DDX43 in lung cancer is 32%, which indicate that the high expression of this protein plays an important role in tumorigenesis and development (Scanlan *et al.*, 2004). DDX43 is one of the subunits of human ribonucleotide reductase, which plays an important role in tumorigenesis and development. In this study, the expression of DDX43 gene and protein in lung adenocarcinoma tissues were detected by RT- PCR and immunohistochemistry, and the correlation between the expression and clinicopathological features was analyzed to explore the significance of DDX43 in the early diagnosis of lung adenocarcinoma.

MATERIALS AND METHODS

Study subjects

27 patients with lung adenocarcinoma admitted to our hospital from February 2015 to December 2015 were

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collected. Out the total patients there were 12 males and 15 females, with aged between 52-75 years old, mean aged 61.83±8.59 years old, and 10 cases in clinical stage I-II, 17 cases in stage III - IV. All the included patients were diagnosed as lung adenocarcinoma through pathological examination and had not received any therapies before undergoing surgery, with complete clinical data. An informed consent was signed from each patient and the collected tissue samples were kept in the liquid nitrogen.

Detection of DDX43 mRNA expression by RT-PCR method

Tissue total RNA was extracted according to the TRIzol® Reagent specification, and the concentration and purity of RNA samples were determined. The integrity of RNA was determined by agarose gel electrophoresis. The RNA was reverse transcribed into cDNA under the condition of 42 ˚C for 60 min or 95˚C for 5 min (Cao *et al.*, 2015), and then put them on the ice for follow-up test or frozen storage. PCR was amplified with cDNA as template, and the gene primer sequence of DDX43 was as follows: F-TGAACTGAAGATGTGCCCTTAC, R-TTACGGACAA TTCATGGTGTG (Grossi *et al.*, 2015). The ABI 7500 Real Time PCR system was applied under the condition of 40 cycles (95˚C for 30 s; 60˚C for 30 s; 72˚C for 30 s) (Song *et al*., 2012). GAPDH was used as the control of this study, the relative expression of the target gene was detected by 2 Ct method.

Paraffin embedded tissues were cut into 4-5 pm slices on the slicing machine, then put them on the slide glass for tissue section, and each section was divided into lung tumor group and normal control group. After the slice was baked on the 70^{o} constant temperature oven for 10-20 min, xylene and ethanol were used for dewaxing and sodium citrate buffer (pH=6) was used for high-pressure antigen retrieval, and 3% H2O2 (with 80% methanol) was used to quench endogenous peroxidase, while the nonspecific background was sealed by the serum of normal goat. Next, DDX43 primary antibody with a certain concentration was added and incubated overnight at 4□ refrigerator. And then the secondary anti-working fluid was added and redyed for 40 s with hematoxylin. Finally, the slice was sealed for microscopic examination after alcohol differentiation (WangN *et al*., 2014). The results were analyzed by semi-quantitative method. Under the microscope of 200 times, the results of staining were determined by two people, and presence of brown granules in cells was the target positive protein. The criteria included the number of positive staining cells and the staining intensity: (1) The percentage of positive staining cells: 0 point for <10%, 1 point for 10%-50%, and 2 point for >50%; (2) Staining intensity score: 1 point for light yellow, 2 point for tan, and 3 point for sepia. The expression intensity of these two proteins was divided into three grades: 0 point: "-"; 1 point: "1+2", "2"; 4-6 point (Hakim and Raboh, 2015).

STATISTICAL ANALYSIS

SPSS 19 statistical software was used to analyze the data, t test and variance analysis were used to compare the difference of the measured data and the rank test was used to compare the data between the groups. The difference was statistically significant of $P<0.05$.

RESULTS

Expression of DDX43 in lung adenocarcinoma and normal lung tissue

The expression of DDX43 protein in lung adenocarcinoma tissues and normal lung tissues was detected by immunohistochemistry. According to the depth of dyeing, the expression intensity of "negative" and the three grades of "1 +", "+ +", "+ +", the results are shown in table 1. The positive expression rate of DDX43 in lung adenocarcinoma was 100%, while the positive rate of DDX43 in normal lung tissue was 77.8%, which is lower than that of lung adenocarcinoma, and the difference between them was statistically significant ($P< 0.01$), as shown in fig. 1.

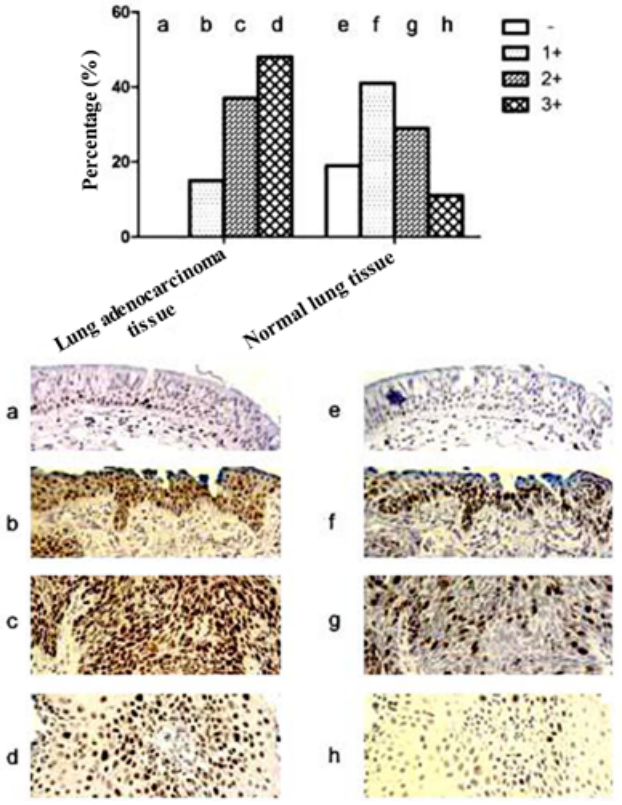

Fig. 1: Expression of DDX43 in lung adenocarcinoma and normal lung tissue

Expression of DDX43 protein and its clinicopathological features

Expression of DDX43 protein and its clinicopathological features were analyzed, including gender, age, tumor size, clinical stage, lung adenocarcinoma metastasis, as shown in table 2. And the results showed that the increase of

protein expression was correlated with the clinical stage and metastasis of lung adenocarcinoma. With the increase of clinical stage, the expression level of DDX43 protein was increased (P=0.019), and the expression of DDX43 protein was significantly increased after mediastinal lymph node metastasis (P=0.017).

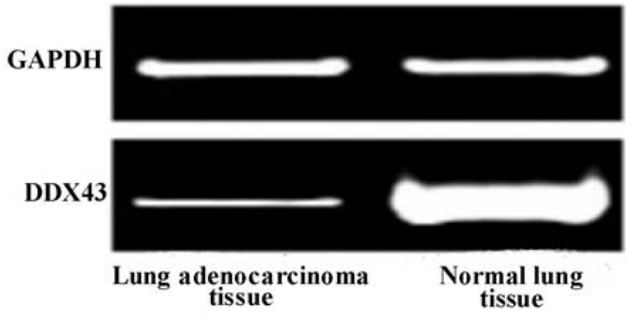

Fig. 2 DDX43 mRNA expression in the normal lung tissue and lung adenocarcinoma

Detection of DDX43 mRNA expression in lung adenocarcinoma tissues and normal lung tissues by RT-PCR

DDX43 mRNA expression in lung adenocarcinoma tissues and normal lung tissues were detected by RT-PCR, as shown in fig. 2 and the mRNA expression level was analyzed by $2^{-\Delta\Delta ct}$ method shown in table 3. The relative ratio of DDX43 mRNA expression in lung adenocarcinoma was 0.87+/-0.62, and the relative ratio of DDX43 expression in normal lung tissue was mRNA + / - 0.77, and the difference between the two groups was statistically significant (P<0.01).

Expression of DDX43 mRNA and its clinicopathological features

Expression of DDX43 mRNA and its clinicopathological features were analyzed, including gender, age, tumor size, clinical stages, lung adenocarcinoma metastasis, as shown in table 4. And the results showed that the increased expression of mRNA was correlated with the clinical stage and metastasis of lung adenocarcinoma. With the increase of clinical stage, the expression level of DDX43 mRNA was increased (P<0.01), and the expression of DDX43 mRNA was significantly increased after mediastinal lymph node metastasis (P=0.011).

DISCUSSION

Non-small cell lung adenocarcinoma is the main histological type of lung adenocarcinoma, and the subtype of lung adenocarcinoma accounts for >40% of lung adenocarcinoma (Ferlay *et al*., 2010). Early treatment of lung adenocarcinoma is usually surgical treatment. However, most of the patients have been found to be advanced cancer, and cancer cells have been transferred, and only about 30-40% patients without transfer in the diagnosis (Grossi *et al*., 2015). The majority of patients will relapse within two years, the patient's five year survival rate is very low. Therefore, in recent years, the early diagnosis of lung adenocarcinoma has attracted much attention, and it is important to find a new molecular marker to improve the clinical diagnosis and prognosis of lung adenocarcinoma. So far, the potential markers associated with it are being discovered. Zhao J et al have found that the high expression of cytidine and uridine guanylate binding protein 1 (CUGBP1) in the lung adenocarcinoma patients can increase the recurrence rate of cancer, and to make the prognosis of patients poorer (Zhao *et al*., 2015), CUGBP1 may be used to predict prognosis in patients with stage IB adenocarcinoma of the lung. In addition, the epidermal growth factor receptor (EGFR), excision repair cross complementing 1 (ERCC1), ribonucleotide reductase M1 (RRM1), and tubulin-β3 (TUBB-3) et al. were also found heterogeneity distribution in non-small cell lung adenocarcinoma (Jakobsen *et al*., 2013). In order to improve the diagnosis of lung adenocarcinoma, more biomarkers are needed to be explored. In this study, RT-PCR and immunohistochemistry were used to detect the expression of DDX43 gene and protein in lung adenocarcinoma, and to analyze the correlation between the expression of PCR gene and clinicopathological features.

The main role of the nucleotide reductase in the organism is to catalyze the reduction of four ribonucleotides to produce the corresponding deoxyribonucleotides. Studies have shown that the activity of nucleotide reductase is closely related to the division and differentiation of cancer cells. As a candidate oncogene, DDX43 can be expressed in a variety of solid tumors and hematological systems, and is associated with the prognosis of lung adenocarcinoma. DDX43 is involved in the composition of ribonucleotide reductase, which is a key enzyme in the replication of DNA in cell cycle, activating tumorigenesis and playing a vital role in the development of tumor (Furuta *et al*., 2010). Studies have found that DDX43 is highly expressed in the tumor, including ovarian cancer, gastric cancer, and its high expression is correlated with tumor grade and survival time (Ferrandina *et al*., 2010; Kang *et al*., 2014). Angiogenesis plays a key role in the process of tumor progression, and plays an important role in tumor growth and metastasis. Research of Zhang K (ZhangK *et al*., 2009) have shown that DDX43 can accelerate the progression of cancer by regulating Thrombospondin-1 (TSP-1) and vascular endothelial growth factor (VEGF) to promote tumor angiogenesis. In addition, other studies have shown that DDX43 can be used as a biomarker for tumor diagnosis and prognosis. In addition, Su YF et al have found that DDX43 is involved in the development and progression of cervical cancer, and can be used to predict the survival period of patients, and maybe a potential targeted target by cell biology experiments and tissue micro array technique (Su *et al*., 2014). Morikawa T, Liu X et al have found that DDX43 is associated with the tumor invasion and metastasis and the

Table 1: Expression of DDX43 in lung adenocarcinoma and normal lung tissue

Groups	Cases	DDX43 protein expression				U/χ^2	P
		-	1+	2+	3+		
Observation group	27	0	4	10	13	-3.809	<0.01
Control group	27	6	10	8	3		

Table 2: Expression of DDX43 protein and its clinicopathological features

Clinical manifestation	Cases	DDX43protein expression			U/χ^2	P
		1+	2+	3+		
Gender						
Male	12	2	4	6	-0.053	>0.05
Female	15	2	6	7		
Age						
< 60	10	1	4	5	-0.302	>0.05
≥ 60	17	3	6	8		
Max diameter of tumor						
T1 d ≤ 3 cm	11	2	5	4	0.893	>0.05
T2 3 cm < d ≤ 7 cm	9	1	3	5		
T3 d > 7 cm	7	1	2	4		
Clinical stages						
I	5	3	1	1	7.895	0.019
II	9	4	4	1		
III	13	1	4	8		
Mediastinal lymph node						
+	18	0	7	11	-2.56	0.017
-	9	4	3	2		

Table 3: DDX43 mRNA expression in the normal lung tissue and lung adenocarcinoma

Samples	$2^{-\triangle\triangle Ct}$	P value
Lung adenocarcinoma tissue	0.87±0.62	< 0.01
Normal lung tissue	0.21±0.77	

Table 4: Analysis of DDX43mRNA expression level and clinicopathologic features

Clinical manifestation	Cases	DDX43 mRNA expression level $2^{-\triangle\triangle Ct}$	F	P
Gender				
Male	12	0.84±0.36	0.031	> 0.05
Female	15	0.89±0.27		
Age				
< 60	10	0.83±0.49	0.936	> 0.05
≥ 60	17	0.88±0.62		
Max diameter of the tumor				
T1 d ≤ 3 cm	11	0.81±0.39	0.816	> 0.05
T2 3 cm < d ≤ 7 cm	9	0.85±0.71		
T3 d > 7 cm	7	0.88±0.38		
Clinical stages				
I	5	0.61±0.73	10.013	< 0.01
II	9	0.79±0.39		
III	13	0.97±0.54		
Mediastinal lymph node metastasis				
+	18	0.96±0.81	8.035	0.011
-	9	0.73±0.94		

silencing of DDX43 in tumor cells can obviously inhabit the cell growth and weaken its invasion ability (Morikawa *et al*., 2010; LiuX *et al*., 2013).

The study of DDX43 as a tumor biomarker is mainly focused on other cancers. In the study of lung adenocarcinoma, Grossi F et al have found that DDX43 is obviously correlated with the overall survival of patients, and the high expression of DDX43 could be used to predict the poor prognosis of patients (Grossi *et al*., 2015). Whether it can be used as a molecular marker for early diagnosis of lung adenocarcinoma remains to be further explored. In this study, RT-PCR and immunohistochemistry were used to detect the expression of DDX43 gene and protein in lung adenocarcinoma tissues, and to analyze the correlation between expression and clinicopathological features, and the results showed that the positive expression rate of DDX43 in lung adenocarcinoma was 100%, and its protein expression level and mRNA expression level in the tissues were significantly higher than those in normal lung tissues, and the increase of DDX43 protein expression in lung adenocarcinoma tissue was correlated with the clinical stage of disease and the metastasis of lung adenocarcinoma ($P<0.05$). Combined with its molecular biological function, it can be predicted that if the expression level of DDX43 in lung adenocarcinoma patients is not high, the function of DNA repair is decreased, which may be more conducive to the control of the disease. In addition, the results of this study showed that the expression level of DDX43 was related to the metastasis of cancer, which was consistent with the study of Zhang K et al that DDX43 could promote tumor angiogenesis and accelerate cancer progression (ZhangK *et al*., 2009). It is concluded that DDX43 can be used as a target for the treatment of lung adenocarcinoma.

CONCLUSION

All in all, DNA repair function plays an important role in the occurrence and progress of cancer. This study have found that DDX43 protein and mRNA levels in lung adenocarcinoma are highly expressed, indicating that DDX43 is closely related to the occurrence and development of lung adenocarcinoma, and its expression level may lead to the deterioration of patients. DDX43 is expected to become the molecular markers for early diagnosis of lung cancer. This study laid the experimental foundation for exploring the molecular marker of early diagnosis of lung adenocarcinoma. While the exact mechanism of DDX43 in lung adenocarcinoma remains to be further studied.

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