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Expression and significance of myeloid differentiation factor 88 in non-small cell lung carcinoma and normal paracancerous tissues

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ABSTRACT. We studied the expression level of myeloid differentiation factor 88 (MyD88) in non-small cell lung carcinoma (NSCLC) and normal paracancerous tissues, to determine its relationship with clinical pathological characteristics and prognosis. In total, 82 NSCLC patients who had received surgical treatment in our hospital between September 2008 and December 2013 were selected for this study. Another 82 normal paracancerous lung tissue samples were used as controls. All patients had complete clinical records, and they were followed-up for 5 years. The expression level of MyD88 protein was detected by immunohistochemical assay. The positive expression rate of MyD88 in NSCLC tissues (62.2%) was markedly higher than that in normal tissues (10.9%), and was independent of patient characteristics such as age, gender, pathological pattern, history of smoking, and tumor size ($P > 0.05$). However, MyD88 expression was significantly correlated with degree of differentiation, clinical staging, and lymphatic metastasis ($P < 0.05$), and was negatively correlated with prognosis. The 5-year survival rate of patients with positive MyD88 expression was significantly lower than that of patients without

positive expression (P < 0.05). MyD88 was expressed at a higher level in NSCLC tissues and was closely associated with poor prognosis. MyD88 may be a novel eligible target for treating NSCLC.

Key words: Non-small cell lung carcinoma; Myeloid differentiation factor 88; MyD88

INTRODUCTION

As one of the most dangerous malignant tumors, lung cancer has become increasingly prevalent owing to population aging and industrial development. Non-small-cell lung carcinoma (NSCLC), which is the most common pathological type of lung cancer, may be either squamous cell carcinoma or adenocarcinoma. Since there are no typical clinical symptoms, most patients are in the middle or advanced stage upon diagnosis and can, therefore, no longer be treated by surgery. Instead, their survival can only be prolonged by chemotherapy, which has limited effects. Moreover, cytotoxic chemotherapeutic agents severely affect quality of life by causing gastrointestinal symptoms such as nausea and vomiting. Therefore, it is of great importance to study the onset and progression of NSCLC to discover novel targets and to develop efficient, low-toxicity drugs (Reck et al., 2013). Myeloid differentiation factor 88 (MyD88) is a key adaptor protein for many signaling transduction pathways in the human body, and predominantly controls the onset and progression of tumors. In this study, the relationships between MyD88 expression in NSCLC tissues and clinical pathological characteristics and prognosis were analyzed, with the aim of verifying the use of MyD88 as a therapy target.

MATERIAL AND METHODS

General information

In total, 82 NSCLC patients who had received surgical treatment in our hospital between September 2008 and December 2013 were selected for this study and were diagnosed by three experienced pathologists, following surgery. They received no chemotherapy, radiotherapy, or biotherapy before surgery, and complete clinical data were obtained. The patients' general clinical data are summarized in Table 1. This study was approved by the ethics committee of our hospital, and written consent was obtained from all patients.

Immunohistochemical and hematoxylin and eosin (HE) staining

Tissue samples were immersed in neutral formalin solution, embedded in paraffin, cut into 4-μm-thick sections, and subjected to HE staining. For immunohistochemical staining, paraffin sections were baked in an oven at 70°C for 30 min and at 56°C overnight. The sections were then deparaffinized in dimethylbenzene twice (5 min each time), dehydrated in 100, 95, 85, and 75% ethanol successively, washed with tap water, and finally washed twice with phosphate-buffered saline (PBS) while shaking (10 min each time). After antigen retrieval, the sections were blocked with goat serum at 25°C for 1 h, and then the blocking agent was spin-dried. Afterwards, the sections were incubated with diluted MyD88 antibody solution in a 37°C incubator for 2 h, washed thoroughly with PBS, incubated with secondary antibody for another 1 h, washed again with PBS, and reacted with

horseradish peroxidase-labeled streptavidin for 15 min. After another washing with PBS, the sections were stained by 3,3'-dinitrobenzidine, and the cell nuclei were counterstained with hematoxylin for 1 min. Subsequently, the sections were dehydrated in ethanol at gradient concentrations, and sealed with neutral resin after treatment with dimethylbenzene. Finally, the sections were investigated under a light microscope. The primary antibody was replaced by PBS as a negative control.

Immunohistochemical staining results were determined and graded according to the previous literature (Kim et al., 2012; Fu et al., 2013): brownish yellow precipitates in the cytoplasm were interpreted as positive MyD88 expression by two experienced pathologists. Fifteen fields were selected from each section at 400X magnification by using the double-blind method, and the number of cells in which MyD88 was positively expressed out of 100 cells was set as the positive expression rate. Positive immunohistochemical staining results (brownish yellow particles in the cytoplasm or cell nucleus) were determined according to a previous study (Tang and Ren, 2012). Percentage of positive cells: < 1%, 0 point; 1-10%, 1 point; 10-30%, 2 points; 30-60%, 3 points; > 60%, 4 points. Staining intensity of positive cells: negative, 0 points; pale yellow, 1 point; yellow, 2 points; brownish yellow, 3 points. Determination of the final results: 0-2 points, negative; 3-7 points, positive.

Table 1. Relationships between myeloid differentiation factor 88 (MyD88) and the clinical pathological characteristics of non-small-cell lung carcinoma (NSCLC).

Characteristic	Group	Total number of cases	MyD88 expression		χ^2	P
			Positive	Negative		
Gender	Male	60	36	24	0.46	0.49
	Female	22	15	7		
Age (years)	<60	44	25	19	1.17	0.28
	≥60	38	26	12		
Pathological type	Squamous-cell carcinoma	45	32	13	3.37	0.06
	Adenocarcinoma	37	19	18		
History of smoking	Yes	51	29	22	1.63	0.20
	No	31	22	9		
Differentiation degree	Low	21	18	3	18.98	<0.001
	Medium	34	25	9		
	High	27	8	19		
Clinical stage	I, II	52	27	25	4.2	0.14
	III, IV	30	18	12		
Lymphatic metastasis	Yes	49	37	12	9.18	0.002
	No	33	14	19		
Tumor size (cm)	<4	50	30	20	0.26	0.61
	≥4	32	21	11		

Statistical analysis

All data were analyzed by SPSS 17.0. The numerical data were compared by χ^2 test, and survival rates were analyzed by the Kaplan-Meier method. $P < 0.05$ was considered statistically significant.

RESULTS

Expression levels of MyD88 protein in NSCLC and normal tissue

MyD88 was mainly located in the cytoplasm of NSCLC cells. Appearing brownish yellow, MyD88 had a positive expression rate of 62.2% in NSCLC tissues, which was significantly higher than in normal cancerous tissues (10.9%) ($P < 0.05$, Figures 1 and 2, Table 2).

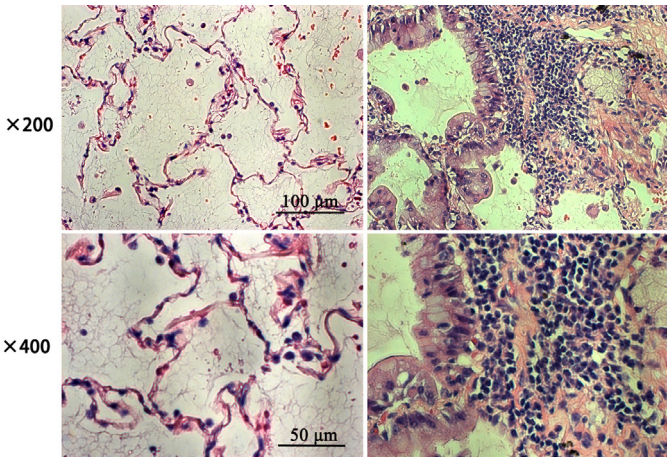

Figure 1. Hematoxylin and eosin (H_E) staining of non-small-cell lung carcinoma (NSCLC) (right) and normal tissues (left).

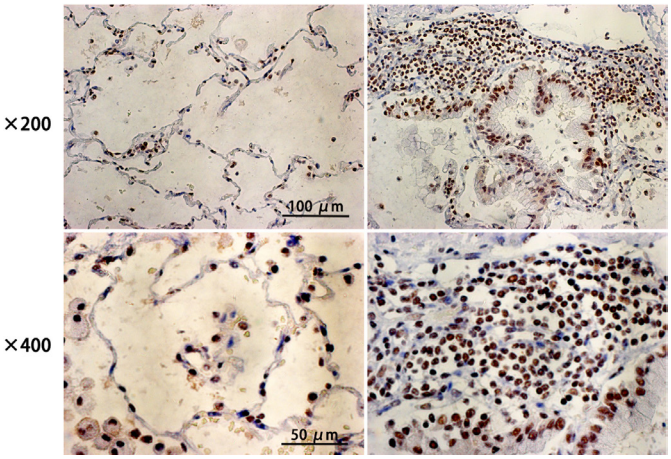

Figure 2. Immunohistochemical staining of non-small-cell lung carcinoma (NSCLC) (right) and normal tissues (left).

Table 2. Expression levels of myeloid differentiation factor 88 (MyD88) in non-small-cell lung carcinoma (NSCLC) and normal tissue samples.

Group	Total number of cases	MyD88 expression		χ^2	P
		Positive cases (%)	Negative cases (%)		
NSCLC tissue	82	51 (62.2)	31 (37.8)	46.36	<0.001
Normal tissue	82	9 (10.9)	73 (89.1)		

Relationships between MyD88 and clinical pathological characteristics of NSCLC

The positive expression rate of MyD88 was not related to age, gender, differentiation degree, tumor size, or clinical stage in the NSCLC patients ($P > 0.05$). MyD88 expression was significantly associated with clinical stage and lymphatic metastasis ($P < 0.05$). Higher MyD88 expression was associated with lymphatic metastasis and increasing stage (Table 1).

Correlation between MyD88 expression and prognosis

The 5-year survival rate of patients with positive MyD88 expression levels was significantly lower than that of patients with negative expression levels ($P < 0.05$, Figure 3).

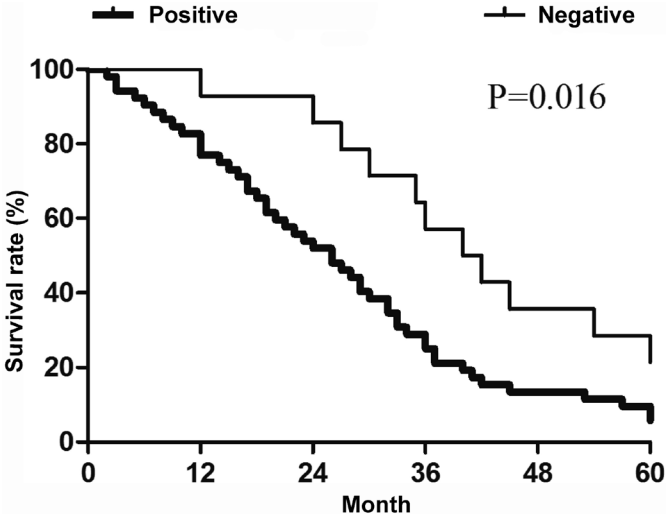

Figure 3. Correlation between myeloid differentiation factor 88 (MyD88) expression and prognosis.

DISCUSSION

Toll-like receptors (TLRs), which are an important type of non-specific immune protein, bridge non-specific and specific immunity. They are mainly divided into two types, i.e., TLR1, 2, 4, 5, 6, and 11 (distributed on the cell surface with the microbial membrane as the ligand, and activated by small molecules or antibodies), and TLR3, 7, 8, and 9 (mainly distributed in intracellular vesicles, and recognizing microbial nucleic acids). Recently, TLRs have been closely associated with gastric cancer and intestinal cancer, and play crucial roles in lung cancer tissues (TLR4 and TLR9 in particular) (Samara et al., 2012). With significantly increased expression in NSCLC tissues, TLR4 is closely associated with prognosis (Fu et al., 2013). Moreover, activation of the TLR4 signaling pathway in tumor tissues can cause the release of considerable quantities of inflammatory factors and produce immune tolerance, thereby allowing tumor cells to escape the immune system. He et al. (2006) reported that TLR expression is significantly elevated in NSCLC tissues, without being associated with the differentiation degree of the tumor. Belmont et al. (2014) found that the NSCLC cells of mice in which the *Tlr9* gene had been knocked out grew significantly more slowly than did the cells of wild-type mice, suggesting that TLR9 was a potential target for NSCLC therapy. Although blocking any of the TLR signaling pathways may inhibit the proliferation and invasion of NSCLC, no drugs targeting TLRs have been confirmed as being useful for the treatment of this cancer, probably because a single target is not universally applicable. Therefore, the discovery of a drug target that facilitates the inhibition of several TLR signaling pathways simultaneously may be of great significance to NSCLC treatment. MyD88 may be such a target.

As a member of the Toll/IL-1R family and the death domain family, MyD88 is intrinsically a cytosolic soluble protein mainly comprising three functional domains. The N-terminal death domain,

which consists of 90 amino acids, mediates interactions between the proteins containing death sequences. Comprising 130 amino acids, the C-terminal Toll domain is similar to the cytosolic domain, which transmits signals by recruiting connexins (Janssens and Beyaert, 2002). After the death domain of IL-1R-associated kinase is bound by that of MyD88, this kinase is subjected to autophosphorylation and a series of activations. As a result, IκB is activated and NF-κB is activated and translocated, thus inducing the synthesis and release of inflammatory cytokines, and ultimately the inflammatory response. As an essential adaptor protein of the TLR4 pathway, MyD88 has shown evident effects on inflammation and autoimmune diseases, and its downregulation can mitigate inflammatory tissue injury (Naugler et al., 2007). MyD88 deletion is able to prevent DEN-induced liver cancer in mice (Rakoff-Nahoum and Medzhitov, 2007). In addition, the MyD88-mediated signaling pathway can regulate the formation of intestinal tumors, and deletion of the *Myd88* gene can suppress the formation of tumors in a mouse familial adenomatous polyposis model (Silasi et al., 2006). Furthermore, MyD88-mediated signaling transduction is able to promote tumor formation. In a study on Stage III ovarian cancer, patients with high MyD88 expression levels had significantly worse prognoses than did those with low expression levels (Warner and Núñez, 2013). In the present study, MyD88 was expressed at a significantly higher level in NSCLC tissues than in normal tissues and was closely associated with clinical stage and lymphatic metastasis. Moreover, the 5-year survival rate of patients with positive MyD88 expression was significantly lower than that of patients with negative expression, indicating that MyD88 dominantly controls the onset and progression of NSCLC as well as the prognosis.

In summary, MyD88 may play a critical role in the pathogenesis of NSCLC, and may inspire the development of a treatment for NSCLC. However, *in vitro* and *in vivo* experiments are still required.

Conflicts of interest

The authors declare no conflict of interest.

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