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Original Article

Expression of IRAK1 in lung cancer tissues and its clinicopathological significance: a microarray study

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Abstract: The interleukin-1 receptor associated kinases 1 (IRAK1) is a down stream effector molecule of the toll like receptor (TLR) signaling pathway, which is involved in inflammation, autoimmunity and cancer. However, the role of IRAK1 in lung cancer remains unclarified. Herein, we investigated the protein expression and the clinicopathological significance of IRAK1 in 3 formalin-fixed paraffin-embedded lung cancer tissue microarrays by using immunohistochemistry, which included 365 tumor and 30 normal lung tissues. We found that the expression of IRAK1 in lung cancer was significantly higher compared with that in normal lung tissues (P=0.002). Receiver operating characteristic (ROC) curves were generated to evaluate the power of IRAK1 to distinguish lung cancer from non-cancerous lung tissue. The area under curve (AUC) of ROC of IRAK1 was 0.643 (95% CI 0.550~0.735, P=0.009). Additionally, IRAK1 expression was related to clinical TNM stage (r=0.241, P < 0.001), lymph node metastasis (r=0.279, P < 0.001) and tumor size (r=0.299, P < 0.001) in lung cancer. In the subgroup of non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), the positive rates of IRAK1 were both higher than that in the normal lung tissues (P=0.003, P=0.002, respectively). Further spearman analysis showed that IRAK1 protein in NSCLC was positive correlated with clinical TNM stage (r=0.222, P < 0.001), lymph node metastasis (r=0.277, P < 0.001), tumor size (r=0.292, P < 0.001) and distal metastasis (r=0.110, P=0.043). In conclusion, the expression of IRAK1 protein might be valuable in identifying patients with increased risks of lung cancer and might act as a target for diagnosis and gene therapy for lung cancer.

Keywords: Lung cancer, IRAK1, immunohistochemistry, tissue microarray

Introduction

Lung cancer is the leading cause of cancer-related death worldwide [1-3]. In USA, lung cancer still ranks the first place in all tumor mortality. According to the statistics, 224, 210 new cases will be diagnosed with lung cancer and 159, 260 will die in year 2014 in the USA [4]. Consistently, lung cancer is also the leading cause of all cancer deaths in China [5]. Lung cancer is often diagnosed at an advanced stage when curative treatment is no longer possible [6]. The overall 5-year survival rate is relatively low due to the advanced disease [6-8]. Even in early-stage patients treated by surgery, the recurrence rate remains high [9]. Over 75-85% of newly diagnosed lung cancers are non-small cell lung cancer (NSCLC) [10-12]. Individualized lung cancer therapy based on genetics has progressed in the past 10 years, especially in NSCLC [13, 14]. However, the significant improvement in the five-year mortality rate is far from satisfaction [13, 15, 16]. Thus, there is an urgent need to discover new molecular diagnostic and therapeutic targets for lung cancer patients [3, 17].

There are 4 members in the interleukin-1 receptor associated kinases (IRAK) family: IRAK1, IRAK2, IRAKM and IRAK4. The IRAK1 gene is composed of 14 exons and locates on the X chromosome [18]. IRAK1 is the first member of this kinase family that is explicated as a key component of the interleukin-1 receptor (IL-1R) signaling pathway. Furthermore, IRAK1 is down stream effector molecule of the toll like recep-

Table 1. IRAK1 protein expression in lung cancer and normal lung tissue

Cancer VS non-cancerous		n	IRAK1 negative (n, %)	IRAK1 positive (n, %)	Z	*P*
Normal lung tissue		30	25 (83.3)	5 (16.7)		
Cancer		365	200 (54.8)	165 (45.2)	-3.031	0.002
SCLC		26	11 (42.3)	15 (57.7)	-3.167	0.002
NSCLC		339	189 (55.8)	150 (44.2)	-2.930	0.003
Adenocarcinoma		127	69 (54.3)	58 (45.7)	-2.906	0.004
	Acinar adenocarcinoma	83	47 (56.6)	36 (43.4)	-2.596	0.009
	Papillary adenocarcinoma	19	10 (52.6)	9 (47.4)	-2.294	0.022
	Broncholoalveolar cell carcinoma	18	6 (33.3)	12 (66.7)	-3.470	0.001
	Mucinous carcinoma	7	6 (85.7)	1 (14.3)	-0.152	0.879
Squamous cell carcinoma		175	100 (57.1)	75 (42.9)	-2.710	0.007
Adenosquamous carcinoma		28	15 (53.6)	13 (46.4)	-2.427	0.015
Undifferentiated carcinoma		8	4 (50)	4 (50)	-1.944	0.052
Large cell carcinoma		1	1 (100)	0	-0.439	0.661

NSCLC vs., SCLC *P*=0.185.

tor (TLR) signaling pathway [19]. Aberrant activation of TLR signaling has a significant impact on the onset of cancer, allergy, sepsis and autoimmunity [19].

Reports on the relationship between IRAK1 and tumors, such as melanoma, Burkitt lymphoma, lymphoma and gastric cancer, have popped up frequently in recent years [20-23]. Toll-like receptor pathway molecules (TLR2, TLR4, TRAF6 and IRAK1) active in peripheral blood immunocompetent cells can determine tumor invasiveness of laryngeal cancer infiltration [24]. Using bioinformatic methods, Pilarsky et al found that IRAK1 was one of commonly overexpressed genes in solid tumors, including lung cancer [25]. However, to date, the exact role of IRAK1 in lung cancer remains unclarified. In the present study, we studied the expression pattern of IRAK1 protein in lung cancer and explored the association between IRAK1 expression and clinicopathological features in lung cancer patients, especially in NSCLC.

Materials and methods

Study design

This retrospective study included 395 cases containing 365 cancers (age ranged from 11 to 84 years; mean 57.67 years) and 30 normal lung tissues (age ranged from 19 to 73 years; mean 54.03 years). Histologic examination revealed the lung cancer specimens mainly consisted of 339 cases of NSCLCs, including 127 cases of adenocarcinomas, 175 cases of squamous cell carcinomas, 28 cases of adenosquamous carcinomas, 8 cases of undifferentiated carcinomas and 1 case of large cell carcinoma. The 127 adenocarcinomas were split into 83 cases of acinar adenocarcinoma, 19 cases of papillary adenocarcinomas, 18 cases of broncholoalveolar cell carcinomas and 7 cases of mucinous carcinomas. Other clinicopathological information provided from medical records was summarized in **Table 2**, including pathological grading, TNM staging, lymph node metastasis, tumor size and distal metastasis. All cases were primary pneumonectomies without treatment and were randomly selected from pneumonectomies performed in the First Affiliated Hospital of Guangxi Medical University, P. R. China from January 2010 to December 2012. The study protocol was approved by the Ethical Committee of the First Affiliated Hospital of Guangxi Medical University. Written informed consent was achieved from the patients and clinicians for the usage of the samples for research. All samples were reviewed and the diagnoses were confirmed by two independent pathologists.

Immunohistochemistry

IRAK1 antibody was purchased from Santa Cruz Biotechnology, Inc. (Heidelberg, Germany) (F-4, sc-5288, 1:50 dilution). The total IRAK1 immunostaining score was calculated as the sum of the positivity rate of stained tumor cells and staining intensity. The mean percentage of positive cells were scored as 0 (0%), 1 (1-25%), 2 (26-50%), 3 (51-75%), and 4 (76-100%). The staining intensity was scored on a four-tier scale: 0, no staining; 1, mild staining; 2, moder-

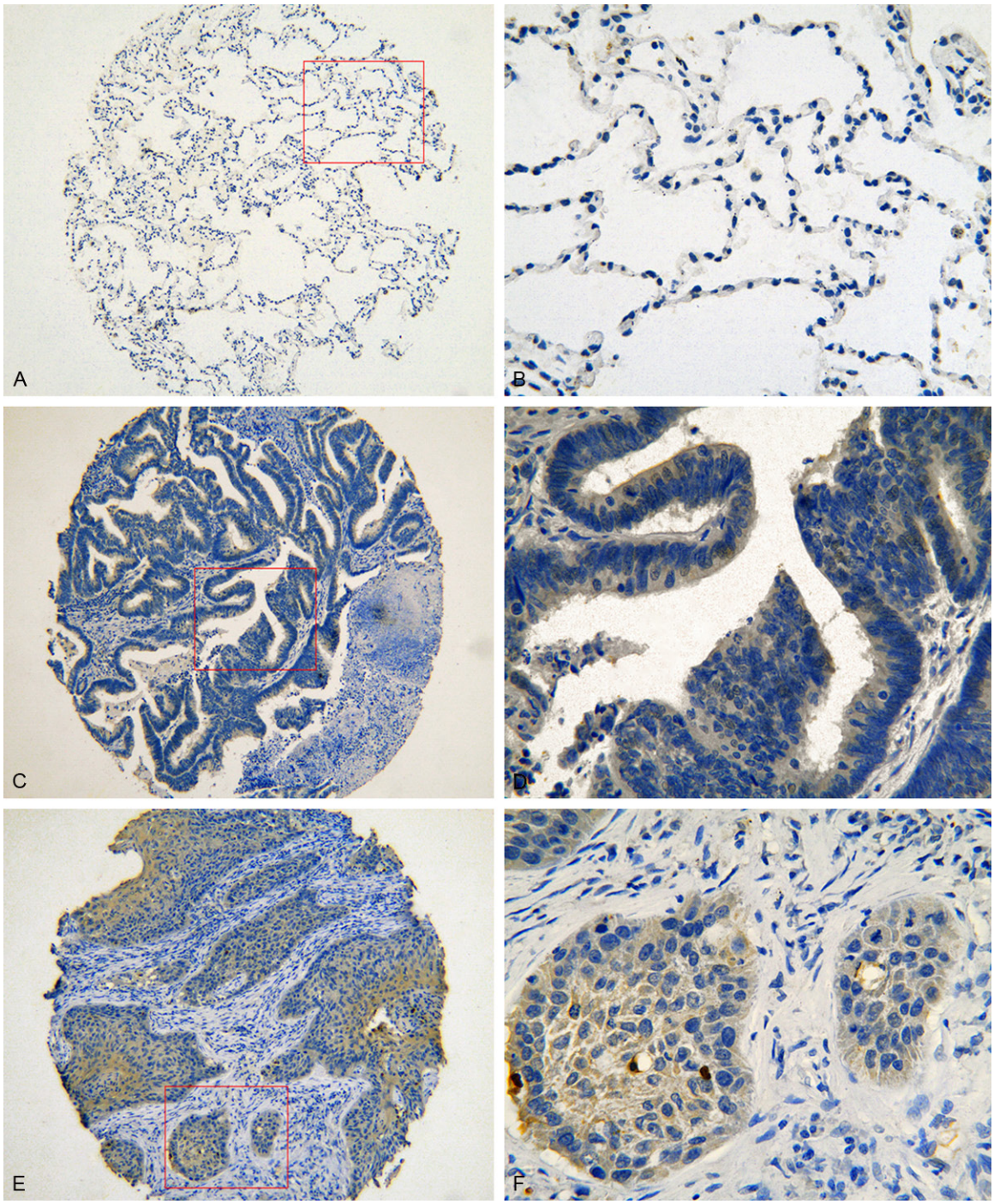

Figure 1. Representative images of IRAK1 expression in lung cancers. Negative staining for IRAK1 in normal lung tissues (A, x100; B, x400). Strong cytoplasmic staining for IRAK1 in adenocarcinoma (C, x100; D, x400) and squamous cell carcinoma (E, x100; F, x400). Immunohistochemisty.

ate staining; 3, strong staining. Final pathological scores over 2 were regarded as positive. All stained microarray sections were evaluated and scored individually by two pathologists with no prior information of the clinicopathological consequences of the patients.

Statistical analysis

All the statistical analysis was carried out using the Statistical Package for the Social Science (SPSS), version 20.0. For comparisons between the two groups, Mann-Whitney *U* tests were

Table 2. Differential expression of IRAK1 protein with clinicopathological factors in overall lung cancer patients

Lung cancer	n.	IRAK1 negative (n, %)	IRAK1 positive (n, %)	Z	*p*
Gender				-0.808	0.383
Male	299	174 (58.2)	125 (41.8)		
Female	96	51 (53.1)	45 (46.9)		
Age (years)				-0.548	0.435
< 60	218	128 (58.7)	90 (41.3)		
≥ 60	177	97 (54.8)	80 (45.2)		
Pathological grading				1.881[a]	0.409
I	39	24 (61.5)	15 (38.5)		
II	92	56 (60.9)	36 (39.1)		
III	131	69 (52.7)	62 (47.3)		
TNM				-4.577	< 0.001
I-II	299	180 (60.2)	119 (39.8)		
III-IV	63	18 (28.6)	45 (71.4)		
LNM				-5.295	< 0.001
Yes	128	46 (35.9)	82 (64.1)		
No	234	152 (65)	82 (35)		
Tumor diameter (cm)				-5.675	< 0.001
≤ 7	314	190 (60.5)	124 (39.5)		
> 7	48	8 (16.7)	40 (83.3)		
Distal metastasis				-1.924	0.054
Absent	346	193 (55.8)	153 (44.2)		
Present	16	5 (31.2)	11 (68.8)		

[a]Kruskal-Wallis H test was performed.

used. While for more than two groups, such as the comparisons of IRAK1 status among different histological subtypes, pathological classifications and grading, Kruskal-Wallis H test was used. Spearman correlation was applied to study the relationship between IRAK1 expression and clinicopathological parameters. *P* values less than 0.05 was considered statistically significant (two-tailed).

Results

IRAK1 expression in lung cancer

Cytoplastic staining was showed for the immunostaining of IRAK1 protein. The expression level of IRAK1 was significantly higher in the lung cancer, compared with normal lung tissues (P=0.002, **Table 1**). Receiver operating characteristic (ROC) curves were generated to evaluate the power of IRAK1 to distinguish lung cancer from non-cancerous lung tissue. The area under curve (AUC) of ROC of IRAK1 was 0.643 (95%CI 0.550~0.735, p=0.009). Typical immunohistochemical staining of the IRAK1 protein was shown in **Figure 1**.

The alteration of IRAK1 expression in lung cancer in different groups with the clinicopathological characteristics was shown in **Table 2**. With regard to clinical TNM stages, the positive ratio of IRAK1 protein expression was remarkably higher in advanced stages (III and IV) than that in early stages (I and II) (P < 0.001). Higher levels of IRAK1 protein were found in lung cancer patients with lymph node metastasis (P < 0.001) and tumor larger than 7 cm (P < 0.001), compared with the corresponding traits. Moreover, analyzed by Spearman coefficient of correlation, IRAK1 expression level showed close correlations with TNM stage (r=0.241, P < 0.001), lymph node metastasis (r=0.279, P < 0.001) and tumor size (r=0.299, P < 0.001). We also detected a marginal correlation between IRAK1 and distal metastasis (r=0.101, P= 0.054). Statistical analysis showed that there was no significant correlation between IRAK1 upregulation and other clinicopathological fea-

Table 3. The relationship of IRAK1 with other clinical pathological parameters in NSCLC

NSCLC	n	IRAK1 negative (n, %)	IRAK1 positive (n, %)	Z	*P*
Gender				-0.350	0.726
Male	254	143 (56.3)	111 (43.7)		
Female	85	46 (54.1)	39 (45.9)		
Age (years)				-0.676	0.499
< 60	181	104 (57.5)	77 (42.5)		
≥ 60	158	85 (53.8)	73 (46.2)		
TNM				-4.073	< 0.001
I-II	286	173 (60.5)	113 (39.5)		
III-IV	53	16 (30.2)	37 (69.8)		
LNM				-5.100	< 0.001
Yes	115	42 (36.5)	73 (63.5)		
No	224	147 (65.6)	77 (34.4)		
Tumor diameter (cm)				-5.371	< 0.001
≤ 7	295	181 (61.4)	114 (38.6)		
> 7	44	8 (18.2)	36 (81.8)		
Distal metastasis				-2.019	0.044
Yes	16	5 (31.2)	11 (68.8)		
No	323	184 (57)	139 (43)		
Pathological grading				1.7[a]	0.434
I	39	24 (61.5)	15 (38.5)		
II	92	56 (60.9)	36 (39.1)		
III	130	69 (53.1)	61 (46.9)		
Histology				1.193[a]	0.970
Adenocarcinoma	127	69 (54.3)	58 (45.7)		
Squamous cell carcinoma	175	100 (57.1)	75 (42.9)		
Adenosquamous carcinoma	28	15 (53.6)	13 (46.4)		
Undifferentiated carcinoma	8	4 (50)	4 (50)		
Large cell carcinoma	1	1 (100)	0		
Adenocarcinoma classification				6.127[a]	0.105
Acinar adenocarcinoma	83	47 (56.6)	36 (43.4)		
Papillary adenocarcinoma	19	10 (52.6)	9 (47.4)		
Broncholoalveolar cell carcinoma	18	6 (33.3)	12 (66.7)		
Mucinous carcinoma	7	6 (85.7)	1 (14.3)		

Pathological grading I vs. II Z=-0.072, *P*=0.943, I vs. III Z=-0.929, *P*=0.353, II vs. III Z=-1.151, *P*=0.250. Broncholoalveolar cell carcinoma vs. mucinous Z=-2.306, *P*=0.021. No difference of IRAK1 expression was found between other parameters. [a]Kruskal-Wallis H test was performed.

tures, such as, gender, age, histological differentiation grades.

IRAK1 expression in NSCLC

After lung cancer was divided into subgroups of NSCLC and SCLC, the ratio of IRAK1 positive expression was higher in NSCLC than that in the normal lung tissues (*P*=0.003, **Table 1**). The positive rate of IRAK1 expression was higher in adenocarcinoma (*P*=0.004), squamous cell carcinoma (*P*=0.007) and adenosqumous carcinoma (*P*=0.015), than that in the normal lung tissue (**Table 1**). In 339 NSCLCs, the expression of IRAK1 protein was significantly higher in advanced stages (III and IV) (*P* < 0.001), larger tumor (*P* < 0.001) and with lymph node metastasis (*P* < 0.001), compared with early stage (I and II), smaller tumor, without lymph node metastasis, respectively (**Table 3**). The positive IRAK1 expression was 68.8% (11/16) in the cases with distal metastasis, markedly higher than that of cases of non-distal metastasis (139/323, 43%, Z=-2.019,

Table 4. The relationship of IRAK1 with other clinical pathological parameters in SCLC

SCLC	n	IRAK1 negative (n, %)	IRAK1 positive (n, %)	Z	*P*
Gender				-2.089	0.037
Male	21	11 (52.4)	10 (47.6)		
Female	5	0	5 (100)		
Age (years)				-0.273	0.785
< 60	15	6 (40)	9 (60)		
≥ 60	11	5 (45.5)	6 (54.5)		
TNM				-1.613	0.107
I-II	13	7 (53.8)	6 (46.2)		
III-IV	10	2 (20)	8 (80)		
LNM				-0.916	0.360
Yes	13	4 (30.8)	9 (69.2)		
No	10	5 (50)	5 (50)		
Tumor diameter (cm)				-1.726	0.084
≤ 7	19	9 (47.4)	10 (52.6)		
> 7	4	0	4 (100)		

P=0.044, **Table 3**). Further spearman interrelated analysis concluded that the positive expression of IRAK1 in NSCLC were correlated with the following clinicopathological parameters: clinical TNM stages (r=0.222, $P < 0.001$), lymph node metastasis (r=0.277, $P < 0.001$), tumor size (r=0.292, $P < 0.001$) and distal metastasis (r=0.110, P=0.043).

IRAK1 expression in SCLC

Among the 26 SCLC studied, 11 cases (42.3%) were identified as negative IRAK1 expression, and 15 cases (57.7%) were as positive IRAK1 expression. The significant difference occurred between SCLC and normal lung tissues (P= 0.002, **Table 1**). For the relationship between IRAK1 expression and clinicopathological parameters in SCLC, the statistical difference in the level of IRAK1 expression has only been noticed in gender (P=0.037, **Table 4**).

Discussion

By using bioinformatic methods and hybridization of a gene-specific probe to a CPA II, Pilarsky et al found that IRAK1 was a upregulated gene in the different cancer entities, including lung cancer [25]. We also observed the similar results on ECgene (http://genome.ewha.ac.kr/ECgene/) database. To our knowledge, no studies on the potential role of IRAK1 protein in lung cancer patients have been reported so far. In the present study, we, for the first time, detected the expression of IRAK1 protein by using microarray and immunohistochemistry in lung cancer and normal lung tissues. The expression of IRAK1 was remarkably higher in lung cancer than in normal lung. When lung cancer was divided into NSCLC and SCLC, IRAK1 also showed the similar trend of being upregulated in both NSCLC and SCLC tissues, as compared to the normal lung. After subdividing NSCLC into different pathology types, we found the significantly higher levels of IRAK1 protein in the groups of adenocarcinoma, squamous cell carcinoma and adenosqumous carcinoma, compared with the normal lung tissue. Thus, our current results demonstrate that IRAK1 might be an oncogene for lung cancer independent of the pathology type. However, there was no variation of the IRAK1 expression in different subtypes of adenocarcinoma, compared to normal lung tissues. The samples are needed to expand to explore the relationship between IRAK1 expression and the histological subtype of adenocarcinoma.

Then we went further to investigate the relationship between the expression of IRAK1 protein and diverse clinicopathological parameters. The significantly higher IRAK1 expression was found in the groups of advanced stage, larger tumor and lymph node metastasis, compared to the corresponding groups in the lung cancer population. Spearman correlation also showed the positive relationships between IRAK1 protein level and clinical TNM stage, tumor size and lymph node metastasis. The

parallel trend could be observed in NSCLC, which showed an additional positive correlation with distal metastasis. These results indicated that the expression of IRAK1 might play a vital role in the progression via influencing tumor cell growth, lymph node metastasis and distal metastasis. Since the aforementioned clinicopathological parameters represent partly the deterioration and progress of lung cancer, IRAK1 might be a tumor promoter in lung cancer and assists the development of lung cancer. However, no association between IRAK1 expression and the clinicopathological parameters in SCLC was identified. This inconsistence between NSCLC and SCLC could be partially due to the altered role of IRAK1 in the progression of different types of cancer. Further large scale studies of SCLC are required to verify the current findings.

Despite the lack of study of IRAK1 on lung cancer, its expression and relevant mechanism have been investigated in other tumors. For instance, IRAK1 was strongly expressed in the melanoma cell lines, such as Malme-3M, SK-MEL-2 and A375. Overexpressing IRAK1 increased the levels of various cytokines/chemokines, including VEGF, CXCL1, G-CSF and IL-12p40, which promote cell survival and proliferation [20]. IRAK1 has been reported to play a central role in TLR-mediated signaling. Recent studies have demonstrated that TLRs pathway molecules, such as TLR2, TLR4, TRAF6, IRAK1 could be potential biological markers of the aggressiveness of tumor because of their influence on the regulation of the secretion of cytokines with proinflammatory activity and also the indirect determination of proliferation, apoptosis, metastasis, angiogenesis and cell differentiation processes in neoplasm diseases [26-28]. Unfortunately, the statistical differences in the mean values of mRNA TLR2, TLR4 and TRAF6, IRAK1 levels between normal control and laryngeal squamous cell carcinoma group have not been noticed [24]. Moreover, laryngeal squamous cell carcinoma group with lower IRAK1 mRNA expression in purified PBMCs frequently demonstrated a more disseminated mode of infiltration. TLR2, TLR4 and IRAK1 active in peripheral blood immunocompetent cells can determine tumor invasiveness, estimated on the basis of pathological features of the edge of laryngeal cancer infiltration [24]. Furthermore, increasing stromal expression of IRAK1 protein were adverse prognostic factors and predicted clinical outcome in prostate cancer [29]. Therefore, IRAK1 may play an important role in neoplasm diseases by activating TLRs pathway. Further studies on the mechanism of IRAK1 in lung cancer are warranted *in vitro* and *in vivo*.

In addition, IRAK1 protein is associated with microRNAs. IRAK1 is one of cancer-related genes, down-regulated by miR-146a [30]. IRAK1 can inhibit Ik-Ba, leading to the activation of NF-kB pathway [31]. IRAK1 is upstream of NF-kB signaling pathway and is involved in progression of breast cancer [32]. Our previous study also found that IRAK1 was a target of miR-146a to activate NF-kB signaling pathway, which might play an important role in lung cancer [7]. Consistent with our preliminary experimental results, Wang et al [33] revealed that the expression of IRAK1 protein significantly downregulated by miR-146a, which inhibited cell proliferation in bladder cancer cells. In pancreatic cancer cells, IRAK1 expression was significantly inhibited by miR-146a transfection [34] as well. Consequently, IRAK1, as a target of miR-146a, might be a key role in the development of lung cancer through activating NF-kB signaling pathway.

In summary, for the first time, we examined IRAK1 protein levels by using immunohistochemistry and we found that in lung cancer the IRAK1 expression was significantly higher, compared with normal lung tissue. IRAK1 protein was also correlated with the clinicopathological parameters representing the progression of lung cancer, especially NSCLC. These findings suggest that IRAK1 may participate in the development and deterioration of lung cancer, being considered as a potential biomarker in the diagnosis and prognosis of lung cancer in clinic. However, the molecular mechanism of IRAK1 in lung cancer remains greatly unclear. Further *in vitro* and *in vivo* investigations have been planned to assess the potential function of IRAK1 in the carcinogenesis of and progression in lung cancer.

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Disclosure of conflict of interest

None.

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