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Correlation between RAR-β expression in lung squamous cell carcinoma tissues and prognosis

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Abstract. – OBJECTIVE: To explore the retinoic acid receptor-β (RAR-β) expression in lung squamous cell carcinoma (LSCC) tissues and its prognosis.

PATIENTS AND METHODS: SP assay was used to detect the RAR-β expression in 100 cases of surgically resected LSCC tissues and 20 cases of peritumoral normal lung tissues, and prognosis follow-up was conducted.

RESULTS: The overall positive expression rate of RAR-β was 54.00%, which was not correlated with age, gender, phase and pathological type ($p>0.05$). Stratified analysis showed that the prognosis of patients with positive IRAR-β expression in phase I was significantly better than that of those with negative IRAR-β expression, in which the median survival times were 31 and 22 months respectively ($p=0.022$). In contrast, the prognosis of patients with negative RAR-β expression was better than that of those with positive RAR-β expression in phase II and III A. The median survival times were 23 and 16 months respectively in phase II $p = 0.008$, and 19 and 7 months respectively in phase III A ($p=0.019$).

CONCLUSIONS: RAR-β is expressed in LSCC tumor tissues. RAR-β expression, which is not significantly correlated with the clinicopathological characteristics of patients, affects the postoperative survival of LSCC patients in phase I and II-III A dually. RAR-β expression state is one of the independent factors for the prognosis of LSCC patients.

Key Words
Lung squamous cell carcinoma, RAR-β, Immunohistochemistry, Correlation.

Introduction

Lung cancer is the most common primary malignant lung tumor, about 80% of which is lung squamous cell carcinoma (LSCC)[1]. In the absence of typical early clinical symptoms, most lung cancer patients have entered advance stage upon diagnosis. Currently, surgery is the major therapeutic protocol for early (phase I and II) and locally advanced (phase III A) LSCC[2]. Despite of surgery, radiotherapy, chemotherapy and other comprehensive treatment measures, the 3-year survival rate of LSCC remains rather low. Some patients benefit from adjuvant chemotherapy[3]. However, an effective treatment method is still in need to enhance therapeutic effects, and to reduce adverse reactions. Retinoic acid receptor (RAR) is closely correlated with lung cancer[4]. However, there are different subtypes in RAR, the functions and pathogenesis of which are not the same. RAR-β is absent in different degrees in a variety of malignant tissues, but many scholars believe that its expression level may be associated with the occurrence of tumor[5]. Studies have found that RAR-β gene expression is down-regulated in lung cancer and its cell lines, but RAR-β gene in lung lesions has rarely been reported[6].

Based on the current *status quo* of lung cancer and gene-related research, this study used immunohistochemical methods to explore the RAR-β expression in LSCC tissues and its significance, and its relationship with survival time, aiming to provide theoretical basis for clinical medication and to realize an individualized future treatment.

Patients and Methods

Patients

The paraffin specimens of 100 patients with LSCC in phase I to III A who underwent radical surgery in our hospital from January 2010 to December 2010 with complete clinical and long-term follow-up data were randomly selected. The patients included 62 males and 38 females aged between 30 and 60 years old with a median age of

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45; there were 52 cases of squamous carcinoma, 37 cases of adenocarcinoma and 11 cases of adeno-squamous mixed carcinoma. This study was approved by the Ethics Committee of the Ninth Hospital of Xi'an. Signed written informed consents were obtained from all participants before the study. With regards to the tumor node metastasis (TNM) staging standard, there were 48 cases in phase I, 20 in phase II and 32 in phase III A. LSCC chemotherapy adopted a cisplatin-based combination chemotherapy regimen. The postoperative routine follow-up continued to December 2012 or death of patients. The postoperative median follow-up time was 22 months (8 to 36 months).

Reagents

RAR-β monoclonal antibody kit and SP hypersensitivity kit were purchased from Shanghai Jianglai Biological Technology Co., Ltd (Shanghai, China).

Experimental Methods

The RAR-β expression in LSCC tumor tissues was detected using SP immunohistochemistry assay. 20 cases of peritumoral normal lung tissue (>5 cm away from the mass) were taken for control analysis. Surgical paraffin sections were first dewaxed with conventional dimethylbenzene three times, rehydrated with gradient concentrations of ethanol, and rinsed with distilled water three times (5 min each time). They were washed with 0.2 mol/L phosphate buffered solution (PBS) three times (5 min each time), spin-dried, and then they were dropwise added with endogenous peroxidase blocker, and incubated at room temperature for 30 min. Next, they were washed three times with PBS (5 min each time), and dropwise added with normal serum blocking liquid, incubated at room temperature for 30 min to remove extra liquid. Then, they were dropwise added with RAR-β McAb (1:200 dilution) and placed in a 4°C refrigerator overnight. Thereafter, they were washed with 0.2 mol/L PBS three times (5 min each time), dropwise added with biotin-labeled secondary antibody, incubated at room temperature for 30 min, and washed with 0.2 mol/L PBS three times, 5 min for each time. They were dropwise added with streptavidin-peroxidase solution, incubated at room temperature for 5 min, and washed with 0.2 mol/L PBS three times, 10 min for each time. After being dropwise added with DAB chromogenic agent, they were incubated at room temperature for 30 min; counterstained with hematoxylin, and rinsed with tap water to be back to blue. The following steps were taken: dehydration, dimethylbenzene transparency, resinene mounting and microscope observation. Positive sections of lung cancer known were taken as positive control, and the primary antibody was replaced with PBS as negative control.

Determination Standard of Immunohistochemical Results

Single-blind reading method was used (observer did not know the patients' clinical data). RAR-β was mainly stained in cell nucleus. Five visual fields were randomly selected under a ×200 optical microscope, and 100 cells were counted in each field to analyze the immunohistochemical expression levels according to the Kim et al[7] method.

Statistical Analysis

Groups were compared using the x^2 test. Log-rank for differences test, and Cox multivariate analysis was performed. All statistical tests were two-sided probability ones with the defined level $\alpha = 0.05$. The data were analyzed and processed by SPSS 15.0 (SPSS Inc., Chicago, IL, USA).

Results

RAR-β Distribution

RAR-β was uniformly distributed mainly in the nucleus, and also expressed in the cytoplasm of some tumor cells (Figure 1).

Correlation between RAR-β Expression and Clinical Characteristics

The overall positive expression rate of RAR-β was 54.00%, which was not correlated to age, gender, phase and pathological type ($p>0.05$) (Table I).

Correlation between RAR-β Expression and Survival Time

The prognosis of negative RAR-β expression was better. The 1-, 2-, and 3-year survival rates of patients with negative RAR-β expression were 97.2%, 68.5% and 48.7% respectively, the median survival time of which was 25 months. The 1-, 2- and 3-year survival rates of patients with positive RAR-β expression were 88.5%, 52.6% and 26.7% respectively, the median survival time of which was 16 months. Log-rank test showed $p=0.002$ (Figure 2).

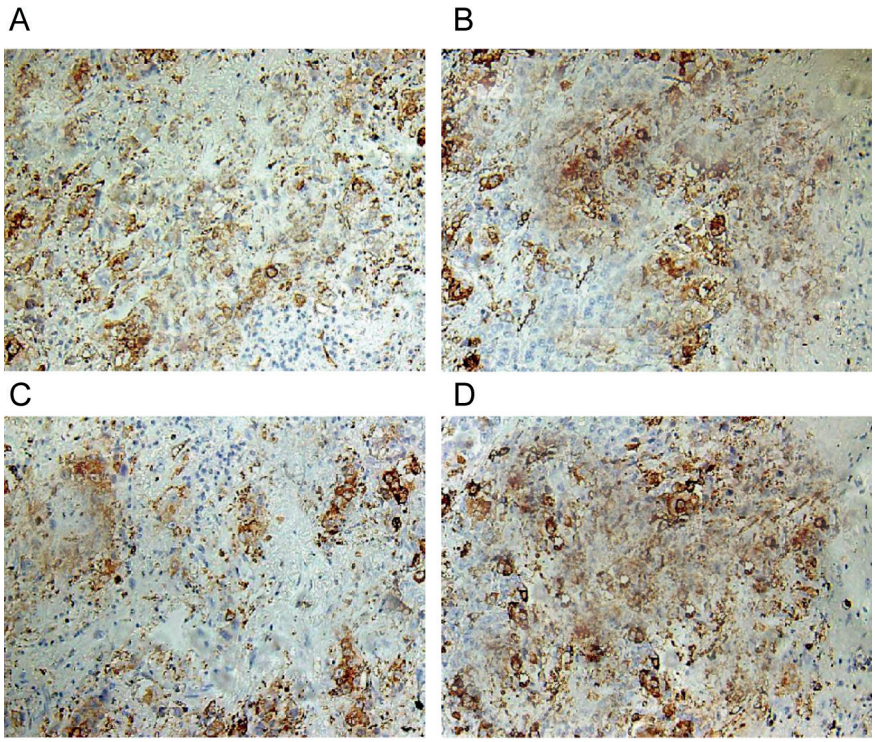

Figure 1. RAR-β expression in LSCC tissues (SP). **A**, Squamous carcinoma expression in cytoplasm (×200); **B**, Adenocarcinoma expression in cell nucleus (×200); **C**, Adenocarcinoma expression in cytoplasm and cell nucleus (×200); **D**, Squamous carcinoma expression in cell nucleus (×200).

Correlation Between RAR-β Expression and Survival Time in Subgroups

Stratified analysis showed that the prognosis of patients with positive IRAR-β expression in phase I was significantly better than that of those with negative IRAR-β expression. The 1-, 2-, and 3-year survival rates of patients with positive IRAR-β expression in phase I were higher than that of those with negative RAR-β expression, but it was contrary in phase II and III A, in which the prognosis of patients with negative RAR-β expression was better than that of those with positive RAR-β expression (Table II and Figure 3).

Cox Regression Analysis of Overall Factors

The variables in the multivariate analysis were TNM staging, age, gender, pathological type and V expression status. TNM staging was an independent prognostic factor (OR=3.153, p=0.004). RAR-β expression status was also an independent factor affecting the prognosis of the patients in this study (OR=3.926, p=0.007).

Table I. Correlation between RAR-β expression and clinical characteristics.

Clinical characteristic	n	Positive RAR-β	x^2	*p*-value
Age				
≥45	52	29 (55.77%)	0.388	0.643
<45	48	25 (52.08%)		
Gender				
Male	62	31 (50.00%)	1.157	0.493
Female	38	23 (60.53%)		
TNM staging				
I	48	28 (58.33%)	1.857	0.318
II	20	11 (55.00%)		
III	32	15 (46.88%)		
Pathological type				
Squamous carcinoma	52	25 (48.08%)	3.750	0.117
Adenocarcinoma	37	22 (59.46%)		
Mixed carcinoma	11	7 (63.64%)		

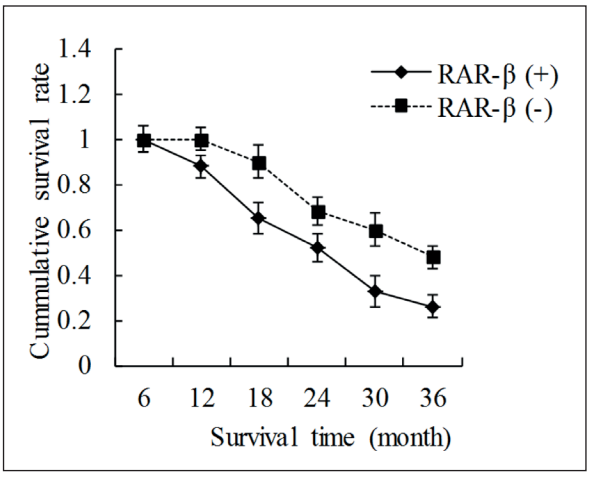

Figure 2. Correlation between RAR-β expression and survival time.

Discussion

The incidence of lung cancer is a complex multi-step biological process with the participation of multiple factors, involving many genic and extragenic changes. In recent study, the role of genetic mechanism is drawing increasing attention of domestic and foreign scholars[8]. The study on lung cancer-related suppressor gene is of great significance to early diagnosis and prognosis of lung cancer. Although some tumor suppressor genes have low frequency for the occurrence of LSCC, the joint detection of these genes can greatly improve the positive rate of the tumor or value in prognostic evaluation[9]. The antitumor mechanism of platinum drugs is that the drugs lead to the death of tumor cells by inhibiting tumor cell division. The drugs covalently combine with tumor cell DNA to form pt2DNA adduct. The interchain or intrachain DNA adducted can inhibit cell replication, finally resulting in DNA damage and DNA replication disorder[10]. Therefore, the difference in repair capacity may be an important factor to decide the efficacy of platinum drugs. Enhancement of DNA repair capacity can repair the damage caused by platinum drugs in attacking DNA in a timely and effective manner. The reduction of pt2DNA adducts and weakens action on tumor cells leading to anti-cancer drug resistance; otherwise, the efficacy is good[11]. DNA excision repair mainly includes 4 ways, i.e. base excision repair (BER), DNA double-strand break repair (DDSBR), mismatch repair (MMR) and NER, of which NER is an important part of human DNA repair system, and the repair of DNA damage caused by platinum drugs is mainly completed by this system, so it is closely related to the resistance of platinum drugs[12]. DNA repair capacity plays a key part in the DDP resistance mechanisms, which is one of the important factors affecting efficacy[13].

RAR-β gene, as a potential tumor suppressor gene, is associated with tumor differentiation and malignant transformation. Many studies show that RRAR-β affects many tumor suppressor genes, so transcriptional gene is silenced and not expressed[14]. Zhao et al[15] observed the transcriptional level changes of retinoic acid receptors α, β and retinoic acid-type receptor α in lung cancer tissues and normal control lung tissues before and after the treatment with cis retinoic acid (9-cis-RA), and proved the abnormal expression of RRAR-β receptor in lung cancer tissues may be related to the occurrence of lung cancer. Other studies have shown that in the nude mouse model of lung cancer induced by exposure of relevant environmental factors, RRAR-β abnormality is an early and common change. These findings suggest that abnormal change of RRAR-β can be used as an indicator for the early detection of lung cancer[16].

Table II. Correlation between RAR-β expression and survival time in I-III A phases.

Variable	n	Survival rate (%)			MST (month)	*p*-value
		1 year	2 year	3 year		
Phase I						
RAR-β (+)	26	100.00	95.86	90.34	31	0.022
RAR-β (-)	22	96.90	68.32	57.71	22	
Phase II						
RAR-β (+)	11	81.49	41.68	26.71	16	0.008
RAR-β (-)	9	89.93	60.27	49.68	23	
Phase III						
RAR-β (+)	17	53.89	30.16	16.83	7	0.019
RAR-β (-)	15	70.65	52.92	39.60	19	

MST: median survival time.

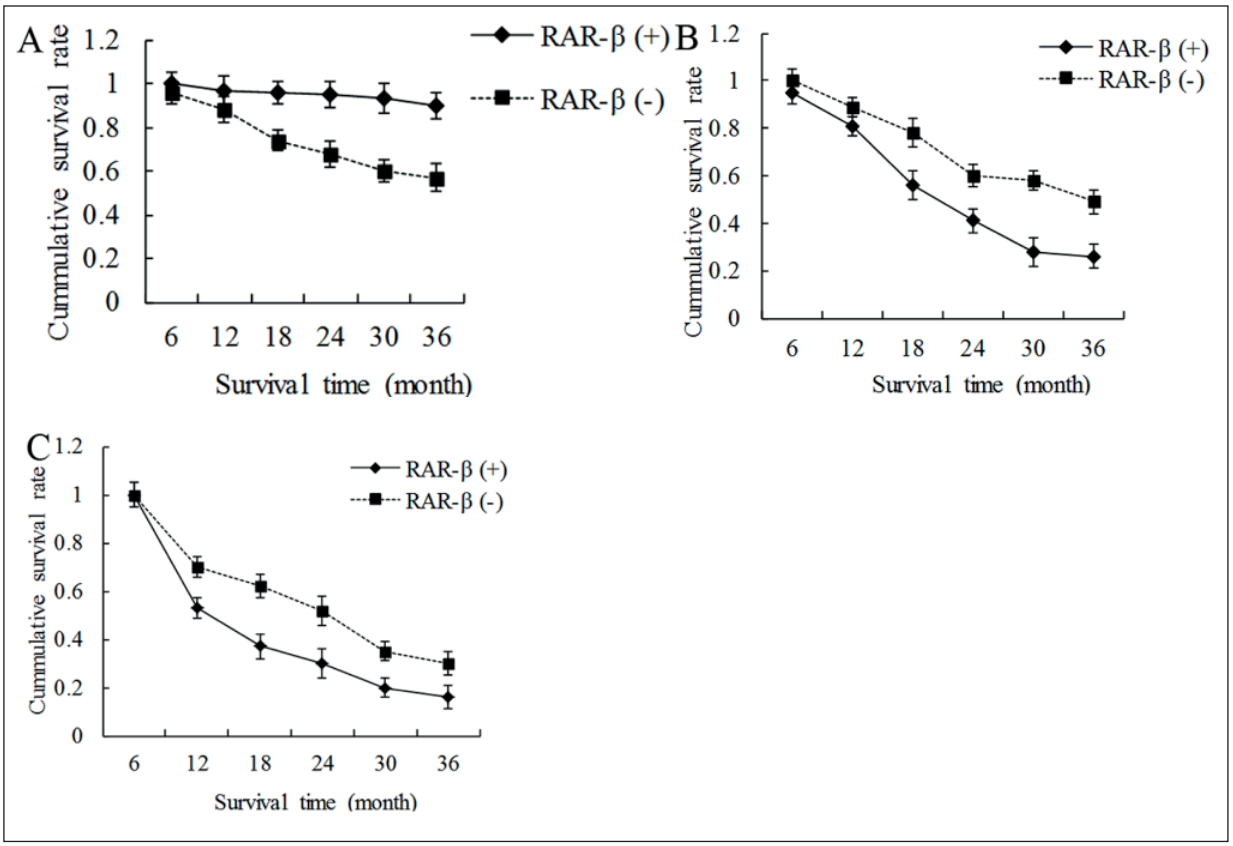

Figure 3. Survival rates of RAR-β (+) and RAR-β (-) patients. A: comparison between the survival rates of RAR-β (+) and RAR-β (-) in phase I; B: comparison between the survival rates of RAR-β (+) and RAR-β (-) in phase II; C: comparison between the survival rates of RAR-β (+) and RAR-β (-) in phase III A.

The results of this study showed that TNM staging and RAR-β expression status is an independent factor affecting the prognosis of patients with LSCC. Thus, the impact of RAR-β expression on survival time of patients plays different roles in patients at early and advanced stages. Stratified analysis found that the survival rate of patients with high expression of RAR-β in phase I was higher than that of those with low expression, while it was contrary in phase II and III A, the prognosis of patients with negative expression of RAR-β was better than that of those with positive expression. This indicates that the expression of RAR-β plays different roles in its impact on the survival of LSCC patients in phase I, II and III A. This is because DNA repair mechanism is very important to protect cell genome against damage from various factors outside and the DNA integrity of the cell genome. The absence of DNA repair genes and non-effective repair of nucleotide damage may cause increased genomic instability, which is the reason for mutation, and it is easy to evade the immune and pro-apoptotic guardianship with proliferative advantage[17]. The DNA repair gene RAR-β, which may represent the intrinsic DNA repair capacity, is a biological marker of the degree of aggregation of tumor DNA repair genes. The damage of nucleotide repair capacity may cause increased genomic instability, and the tumor induced may have more malignant phenotypes. Therefore, the DNA repair capacity may have a certain relationship with the occurrence of lung cancer and its prognosis, so patients with low expression of DNA repair may experience significantly more risk of lung cancer than normal persons[18,19]. It is reported in the literature that low expression of RAR-β is often accompanied by the increased morbidity of lung cancer. This shows that the DNA repair capacity has a dual effect, and patients with poor DNA repair capacity are predisposed to lung cancer[20].

The results of this study also suggest that the influence of RAR-β expression on postoperative survival plays different roles in LSCC patients in phase I, II and III A. Theoretically, the tumor re-

sidual or load of phase I LSCC has been reduced to zero or minimum, so high expression of RAR-β may take more effect in DNA repair and protection. That is why Phase I LSCC patients accompanied by high expression of RAR-β after surgery have significantly longer survival time than those with low expression. While in phase II, III A LSCC, there are still remaining a certain number of tumor cells in the body, and the tumor load is relatively high, so high RAR-β expression as a factor of DNA repair and protection, is weakened or concealed. What is more embodied is increased DNA repair capacity induced by chemotherapy to produce platinum drug resistance. Lung cancer may experience an increased risk of postoperative recurrence, but it has high sensitivity to DDP-based chemotherapy; conversely, high DNA repair capacity may cause low tumor susceptibility, the risk of postoperative recurrence of lung cancer patients is greatly reduced, but DDP resistance occurred.

Conclusions

RAR-β is expressed in LSCC tumor tissues, high expression of RAR-β in phase I LSCC patients predicts a relatively long postoperative survival time, but high expression often means increased drug resistance; low RAR -β expression in LSCC patients in phase II and III A indicates that they are more sensitive to platinum-based chemotherapy, and the prognosis is better than that of high expression. Therefore, the detection on tumor RAR-β expression in LSCC surgical specimens before chemotherapy can help to select patients for adjuvant chemotherapy.

Fund

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Conflict of Interest

The authors declared no conflict of interest.

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