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Original Article

Abnormal β-catenin expression and reduced tumor-infiltrating T cells are related to poor progression in non-small cell lung cancer

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Abstract: Objective: This study was to identify the relationship between the expression of β-catenin and CD8+ T cells infiltration in non-small cell lung cancer (NSCLC). Methods: A total of 100 NSCLC patients undergoing lobectomy and lymph node dissection were enrolled in this study. The baseline demographics, histopathologic data, recurrence-free survival (RFS) period, and pathologic specimens preserved in paraffin were available for them. Immunohistochemistry (IHC) was carried out with resected, paraffin-embedded NSCLC tissues. Evaluation of tumor-infiltrating CD8+ T cells immunostaining was performed using a four-tiered scale according to the visual estimation on lymphocytes. Results: A significant association was found between reduced β-catenin expression and nodal involvement (P=0.047), but no association with other characters. The RFS of patients with reduced β-catenin expression was potently worse than that of patients with preserved β-catenin expression (P=0.026). Meanwhile, no significant association was observed between depressing CD8+ T cells levels and all characters. The RFS of NSCLC patients containing low CD8+TILs was remarkably worse than those with high CD8+ T cells (P=0.003). Multivariate analysis revealed that only CD8+TILs was an independent predictor of RFS (P=0.024). Moreover, CD8+ T cells level was negatively correlated with abnormal β-catenin expression (P=0.016). Conclusion: Abnormal β-catenin expression might suppress antitumor activity by decreasing tumor-infiltrating CD8+ T cells. Inhibition of β-catenin expression and/or activity might be used as a component of anti-cancer immunotherapy in the future.

Keywords: Non-small cell lung cancer, β-catenin, tumor-infiltrating T cells, recurrence-free survival, immunotherapy

Introduction

Lung cancer is the main cause of malignancy-related death worldwide [1] and non-small cell lung cancer (NSCLC) occupies nearly 85% of all lung cancer cases [2]. The therapeutic strategy of NSCLC patients has changed toward personalized therapy according to the genetic status of cancer cells [3-5]. Nevertheless, clinical limitations such as resistance to molecular targeted modalities exist in most cases [6]. Moreover, alternative therapeutic approaches other than cytotoxic chemotherapy, are needed for NSCLC patients instead of medication alterations. Thus, novel strategies for NSCLC are urgent, and note worthily, cancer immunotherapy has emerged as a promising approach for achieving better treatment.

In immunotherapies, the most attractive targets are programmed death-1 (PD-1) and its ligand PD-L1. There is extensive evidence confirming that inhibiting PD-1/PD-L1 pathway can elevate the endogenous antitumor immunity by recovering the action of T lymphocytes [7, 8]. Therefore, manipulating PD-1/PD-L1 axis is considered to be an effective treatment manner for NSCLC patients. However, only a part of the patients respond to this immunotherapy. And its benefit is preferentially gained in patients with a pre-existing T-cell response against their tumor, which was proved by a

Table 1. The relationship between patient characteristics and β-catenin expression, CD8+ T cells

Characteristics	β-catenin expression			CD8 density		
	Abnormal	Normal	*P*	High	Low	*P*
Number	75	25		36	64	
Age (year)	68±9	66±10	0.235	69±10	67±10	0.182
Gender						
Male	48	13	0.287	26	39	0.256
Female	27	12		10	25	
Histology						
Adenocarcinoma	47	20	0.580	26	41	0.209
Squamous cell carcinoma	28	15		8	23	
Primary tumor (pT)						
T1	36	13	0.773	18	31	0.689
T2	29	10		15	24	
T3-4	10	2		3	9	
Nodal involvement (pN)						
N0	47	22	0.047	27	42	0.316
N1	10	2		5	7	
N2	18	1		4	15	
Disease stage						
IA	27	11	0.183	15	23	0.220
IB	18	9		12	15	
II (A+B)	14	2		6	10	
III (A+B)	17	2		3	16	

baseline CD8+ T cells infiltration in the tumor microenvironment. Analogously, increased total tumor infiltrating lymphocytes (TILs) have been related to longer survival in NSCLC patients [9, 10]. Thus, understanding the mechanisms of the presence or absence of antitumor T cells can improve the therapeutic efficacy in patients who lack T cell infiltration [11].

β-catenin is a key component of Wnt/β-catenin signal and is an important oncogene involved in the pathogenesis and progression of malignant tumors including lung cancer [12-14]. In lung cancer, there is accumulating evidence that Wnt/c-catenin may be activated [15]. Recently, it has been shown that this pathway plays an important role in lung adenocarcinoma metastasis [16], however, the role of β-catenin signaling in NSCLC initiation/progression is not well understood. Recent research showed that β-catenin is an immunoregulatory molecule that can suppress antitumor activity [17]. And it is shown that intrinsic β-catenin signaling suppresses anti-tumor immunity, indicating an inverse correlation between aberrant β-catenin and CD8+ T cells in metastatic human cutaneous melanoma [18]. However, little work has been performed on CD8+ T cells and β-catenin expression in NSCLC tissues. In the present study, we detected prognostic values of β-catenin expression and tumor-infiltrating CD8+ T cells, and identified the relationship between the expression of β-catenin and CD8+ T -cell infiltration in NSCLC.

Materials and methods

Study population

Participants who had undergone lobectomy and lymph node dissection between January 2010 and December 2011 in Jinling Clinical Medical College of Nanjing Medical University were retrospectively studied for at least 2 years. Patients who received induction therapy or who had another malignancy were excluded. A total of 100 NSCLC patients undergoing lobectomy and lymph node dissection were finally enrolled in this study. The baseline demographics, histopathologic data, recurrence-free survival (RFS) period, and pathologic specimens preserved in paraffin were available for

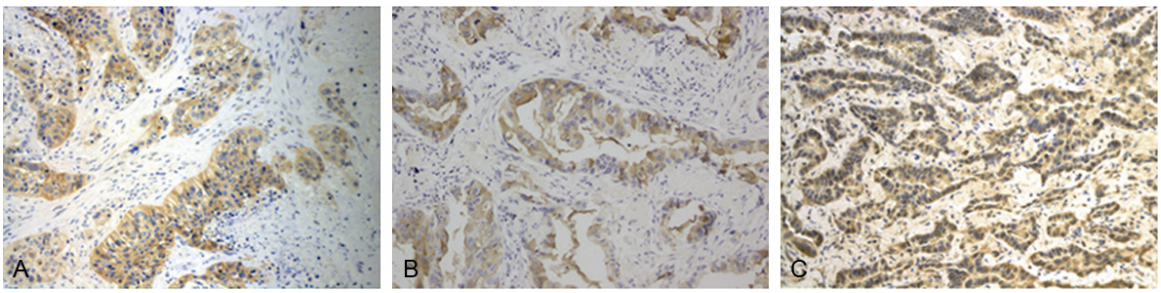

Figure 1. Expression patterns of β-catenin and CD8 in non-small cell lung cancer (×200). A. Membranous β-catenin expression accompanied by weak cytoplasmic expression; B. Cytoplasmic β-catenin expression; C. Nuclear β-catenin expression with cytoplasmic expression.

them. Lung tissue samples were fixed in formalin and, after dehydration, embedded in paraffin. A written informed consent was signed by each patient before surgery, and this study was approved by the institutional Ethics Committee of Jinling Clinical Medical College of Nanjing Medical University.

Immunohistochemistry (IHC)

IHC was carried out with resected, paraffin-embedded NSCLC tissues. After microtome sectioning, 4 μm-thick slides were processed for β-catenin and CD8 staining by an automated immunostainer (Ventana Medical System, Strasbourg, France). Subsequently, the streptavidin-biotin-peroxidase was observed using diaminobenzidine as a chromogen. Primary antibodies were used strictly according to the manufacturer's protocol (β-catenin: DakoCytomation clone CX-294, 1/250 dilution; CD8: Abcam, clone 22510, 1/100 dilution).

Then, slides were measured by two investigators who had no knowledge of the corresponding clinicopathologic data. The internal control was β-catenin localization in normal lung tissues. Although 20% samples declared normal membranous staining, most NSCLC specimens declared aberrant one. More than 90% tumor cells with membranous staining were defined as normal β-catenin expression, which were considered to be positive nuclear expression of β-catenin. Meanwhile, the cells with <90% membranous staining (with or without cytoplasmic staining) were defined as reduced expression, which were scored as abnormal β-catenin expression.

Pathological evaluation of TILs

Evaluation of tumor-infiltrating CD8+ T cells immunostaining was performed by a pathologist (DC) using a four-tiered scale according to the visual estimation on lymphocytes in each visual field. A score of 0 suggested virtual absence of TILs, 1+ = low TILs (<30%), 2+ = moderate (30%-60%), and 3+ = significant increases (>60%) in the lymphocytic infiltrate as previous reports [19, 20]. Moreover, patients with TIL scores of 0 to 2+ were taken as the low-TIL group and those categories of 3+ were considered as high-TIL tumor cases.

Statistical analysis

SPSS 17.0 statistical software (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. A statistical analysis testing significant differences among categorized groups and potential relationship between β-catenin expression and clinicopathologic features were performed via the Fisher's exact test or the χ^2 test, as appropriate. The comparison of continuous data was carried out with student's t-test. A logistic regression analysis was applied on multivariate analyses. To detect the correlation between RFS and β-catenin expression or the CD8+TILs count, Kaplan-Meier survival analysis was used to stratify significant predictor variables determined in the Cox proportional hazards model. *P*-value <0.05 was considered as statistically significant.

Results

Relation between β-catenin expression and clinicopathologic characteristics

In order to investigate the significance of β-catenin expression in NSCLC, β-catenin expression was determined by IHC. All characteristics and IHC variables of the 100 NSCLC cases were shown in **Table 1**. Among them, 75 patients markedly exhibited abnormal β-catenin

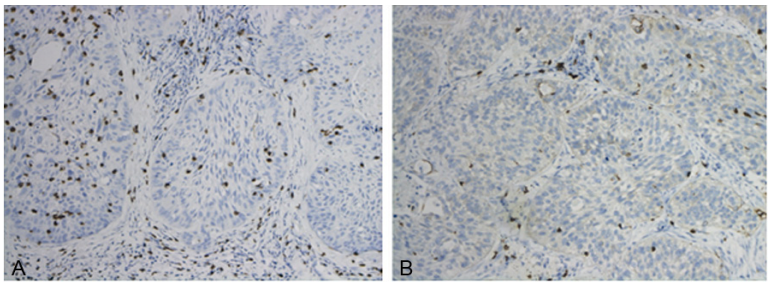

Figure 2. Representative image of CD8+ tumor infiltrating lymphocytes (×200). High expression (A) and low expression (B).

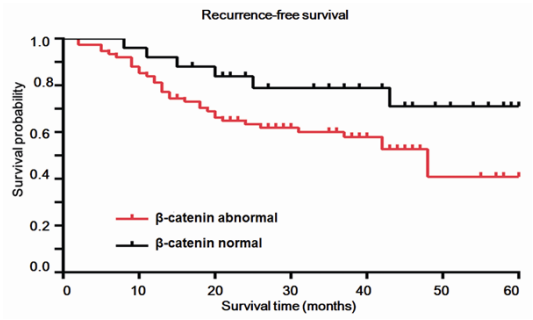

Figure 3. Kaplan-Meier recurrence-free survival curve according to β-catenin expression (*P*=0.026).

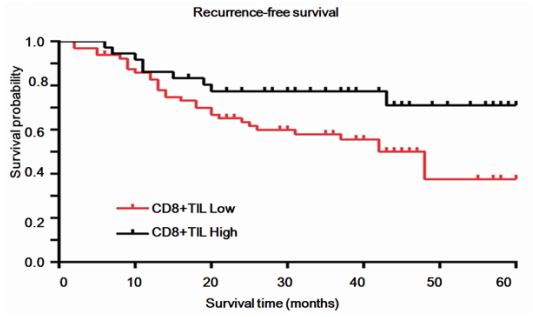

Figure 4. Kaplan-Meier recurrence-free survival curve based on CD8+TIL expression (*P*=0.003).

immunoreactivities in NSCLC cells, whereas the remaining 25 cases showed no abnormal expression in β-catenin. A significant association was found between reduced β-catenin expression and nodal involvement (*P*=0.047), however, no association with age (*P*=0.235), sex (*P*=0.287), histology (*P*=0.580), tumor size (*P*=0.773), or disease stage (*P*=0.183) was revealed. Additionally, membranous, cytoplasmic, and nuclear β-catenin expression patterns were shown in **Figure 1A-C** and representative image of CD8+TILs were in **Figure 2**. The RFS of patients with reduced β-catenin expression was potently worse than that of patients with preserved β-catenin expression (*P*=0.026 according to a log-rank test; **Figure 3**).

Relation between CD8+TILs expression and clinicopathologic characteristics

To unravel the relation between CD8+ T cells expression and clinicopathologic characteristics, IHC was undertaken. Among the 100 cases enrolled in this study, 64 cases showed low expression of CD8+TILs. As illustrated in **Table 1**, no significant association was observed between a depression in CD8+TILs levels and age (*P*=0.182), sex (*P*=0.256), histology (*P*=0.209), tumor size (*P*=0.689), nodal involvement (*P*=0.316), or disease stage (*P*=0.220). The RFS of NSCLC patients containing low CD8+TILs was significantly worse than that with tumors containing high CD8+TILs (*P*=0.003 according to a log-rank test; **Figure 4**). To sum up, the results indicated that reduced CD8+TILs level might cause a worse survival.

Prognostic values of β-catenin and CD8+TILs

In order to identify whether β-catenin and CD8+TILs were independent risk factors for prognosis, Cox proportional hazard regression model was constructed. A univariate analysis revealed that nodal status (*P*=0.002), β-catenin expression (*P*=0.026), and CD8+ T cells (*P*=0.003) were independent risk factors associated with RFS (**Table 2**). Nevertheless, a multivariate analysis demonstrated that only nodal status was an independent risk factor (*P*=0.005). In the node-negative group (N=69), the RFS of patients with abnormal β-catenin and low CD8+TILs levels were significantly worse than that of patients with normal β-catenin expression (*P*=0.035) or high CD8+TILs (*P*<0.001) (**Figure 5**). Conversely, in the node-positive NSCLC group, the RFS of patients with abnormal β-catenin expression and low CD8+TILs expression levels were not significantly worse than that of patients with normal β-catenin expression (*P*=0.874) or low CD8+TILs expression (*P*=0.679). A multivariate

Table 2. Prognostic values of recurrence-free survival

Variables	Univariate analysis		Multivariate analysis	
	Unfavorable/Favorable	*P*	HR (95% CI)	*P*
Nodal involvement	N1-2/N0	0.002	2.78 (1.40-5.78)	0.005
β-catenin	Positive/negative	0.026	1.75 (0.63-4.37)	0.331
CD8+ T cells		0.003	2.02 (0.86-4.74)	0.118
Node-negative cases				
β-catenin	Positive/negative	0.035	1.92 (0.50-7.39)	0.356
CD8 density	High/Low	<0.001	5.38 (1.38-21.07)	0.024

HR, hazard ratio; CI, confidence interval.

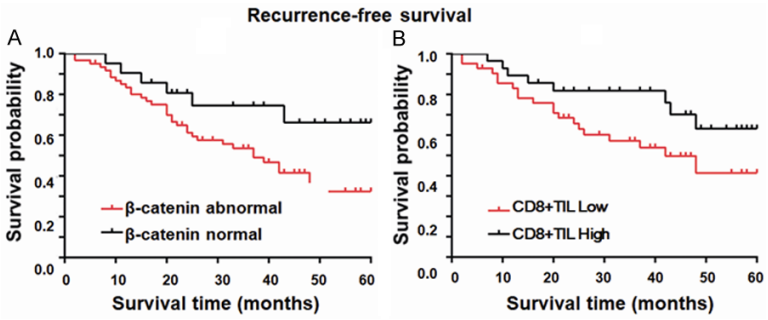

Figure 5. Kaplan-Meier recurrence-free survival curve in node-negative non-small cell lung cancer according to (A) β-catenin expression (*P*=0.035) and (B) CD8+TIL (*P*<0.001).

Table 3. Relationship between β-catenin and CD8+ T cells expression in non-small cell lung cancer

β-catenin	CD8+ T cells		Total	r	*P*
	+	-			
+	22	53	75		
-	14	11	25	-0.241	0.016
Total	36	64	100		

analysis revealed that only CD8+TILs was an independent predictor of RFS (*P*=0.024; **Table 2**). Accordingly, CD8+TILs expression was an independent predictor of RFS.

Relation between β-catenin expression and the CD8+TILs count

To further identify the relationship between β-catenin expression and the CD8+ T cells count, the expression condition of the NSCLC tissues used in this study was studied. As shown in **Table 3**, 53 out of 75 β-catenin reduced samples also exhibited negative staining for CD8+TIL, indicating that the expression of CD8+TIL was negatively correlated with abnormal β-catenin expression (r=-0.241, *P*=0.016).

Discussion

Deeply understanding the effect of tumor-infiltrating immune cells, particularly T cells, on cancer process has paved the road for novel immune-directed treatment strategies [21]. More and more TILs have been consistently associated with better outcome in various human neoplasms, such as melanoma, colorectal, and triple-negative carcinomas [9, 22]. Analogously, increased total TILs are found to be related to longer survival in NSCLC patients. Higher levels of CD3 and CD8+TILs are suggestive of better outcome in NSCLC, but only CD8 is independent prognostic indicator [20]. In addition, objective measurement of TIL subpopulations is well known to predict response or assess the local immune effect of anticancer immune checkpoint inhibitors [20]. Similar like the previous reports, our studies also showed that the number of tumor-infiltrating CD8+TILs was related to a worse RFS, especially among patients with node-negative NSCLC.

Emerging evidence pointed out that overexpressing of β-catenin was associated with a poor prognosis and a short survival period in NSCLC [23]. To date, a host of investigators have intensively studied the contribution of β-catenin to tumorigenesis. Several mechanisms are thought to mediate the tumorigenic activity of β-catenin as follows: β-catenin was existed in most squamous cell lung cancer samples and occupied a half of adenocarcinomas [24]. It was considered that β-catenin

accumulation in nuclear was related to epidermal growth factor receptor (EGFR) mutations [25], and β-catenin overexpression was indicative of the resistance to gefitinib in NSCLC cell line [26]. Increasing extracellular matrix metalloproteinase inducer (EMMPRIN) levels are proved to be associated with poor prognosis and metastasis in some tumors. High expression of EMMPRIN is proved to activate β-catenin signaling whereas silencing of EMMPRIN suppressed β-catenin signaling pathway, cell proliferation, mi-gration, anchorage-independent growth, as well as xenograft growth in lung cancer cells [27]. Another report found that β-catenin might also be dysregulated in lung cancer by interaction with Wolf-Hirschhorn syndrome candidate [28]. Sulindac could inhibit β-catenin level in lung cancer cells, hence dropping transcriptional targets of β-catenin (c-myc, cyclin D1, and cdk 4), and inhibiting proliferation [29]. Recently, β-catenin is considered as immunoregulatory molecule that can suppress antitumor activity [17]. Our studies also demonstrated that β-catenin expression was associated with a worse RFS, which pointed to poor prognosis and short survival periods in NSCLC.

Of note, the number of tumor-infiltrating CD8+ T cells was positively linked to intratumoral β-catenin expression in this study. To our knowledge, this is the first study demonstrating that tumor-infiltrating CD8+TILs decrease is associated with a poor prognosis and is negatively correlated with intratumoral β-catenin expression in NSCLC patients. Driessens and colleagues argue that β-catenin is constitutively degraded in primary T cells [17]. After the introduction of β-catenin, the proliferation and cytokine secretion of the primary T cells (TCR stimulated) were inhibited, and the differentiation of effector T cells was attenuated [17]. That was related to functional inhibition of IL-2 production and differentiation of effector cell. Yaguchi et al. illustrated that IL-10 is a target of the Wnt/β-catenin signaling pathway in human melanoma [30]. In addition, they point out that the constitutively activated Wnt/β-catenin signaling might contribute to the suppression of both the induction and effector phases of antimelanoma T cell responses via impairing DC and T cell functions partly due to IL-10 production and immunosuppressive microenvironment generation [30]. Spranger and coworkers show that reduced chemokines CCL4 and CXCL1 expression is associated with diminished antitumor T-cell response [18]. In this study, NSCLC tissue was applied, showing a correlation between tumor-infiltrating CD8+ T cells and β-catenin expression in NSCLC, which was similar to that of the previous works. It is possible that future cancer treatments targeting β-catenin and CD8+ T cells might be feasible.

To sum up, the present results indicated that the number of tumor-infiltrating CD8+ T cell was positively correlated with intratumoral β-catenin expression. For patients with node-negative NSCLC, in particular, CD8+ T cells expression was found to be an independent prognostic factor by a multivariate analysis. Thus, β-catenin expression might suppress antitumor activity by down regulating tumor-infiltrating CD8+ T cells. Inhibition of β-catenin expression and/or activity might have future utility as a component of anti-cancer immunotherapy. Further studies for identifying other types of cancer are necessary.

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Disclosure of conflict of interest

None.

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