Journal of Cancer Research and Clinical Oncology (2019) 145:2663–2674
https://doi.org/10.1007/s00432-019-03025-8

ORIGINAL ARTICLE – CANCER RESEARCH

Expression and clinical significance of PD-L1 and c-Myc in non-small cell lung cancer

Cuiling Zhou[1] · Gang Che[1] · Xiaobin Zheng[2] · Junlan Qiu[3] · Zhinan Xie[4] · Yunyan Cong[1] · Xiaofeng Pei[1] · Hongyu Zhang[1] · Huanhuan Sun[1] · Haiqing Ma[1]

Received: 22 April 2019 / Accepted: 11 September 2019 / Published online: 20 September 2019

Abstract

Background It is known that there are insufficient prognostic factors for non-small cell lung cancer (NSCLC). It was reported that PD-L1 was a prognostic factor for NSCLC,and c-Myc regulated the expression of PD-L1. Herein, we investigated c-Myc and PD-L1 expression and their association with overall survival (OS) in NSCLC.

Methods Formalin-fixed paraffin-embedded specimens were obtained from 128 patients with surgically resected primary NSCLC. Immunohistochemistry was used to assess the expression of PD-L1 and c-Myc in this study. Pearson's Chi squared test or Fisher's exact test was used to analyze the correlation of the expression of PD-L1 and c-Myc with clinicopathologic features. The relationship between OS and the expression of PD-L1 and c-Myc was evaluated by the Kaplan–Meier method and Cox proportional hazards model, respectively.

Results Positive expression of PD-L1 was detected in 59 patients (46.1%). Patients with negative expression of PD-L1 had remarkably longer OS than those with positive expression of PD-L1. The positive expression rate of c-Myc in NSCLC accounted for 58.6% (75/128) and its expression was significantly more frequent in males ($p=0.002$) and patients with lymph node metastasis ($p=0.029$). PD-L1 expression was positively correlated with c-Myc expression ($r=0.459$, $p<0.001$). The PD-L1 and c-Myc double-positive group had a worse prognosis than other subgroups ($p<0.05$), and the PD-L1 and c-Myc double-negative group had a better OS than other subgroups ($p<0.05$).

Conclusion Conjoint analysis of the expression of PD-L1 and c-Myc was a better prognostic approach for NSCLC patients.

Keywords Non-small cell lung cancer · Programmed cell death ligand 1 · c-Myc · Prognosis

Introduction

Lung cancer is the most common cause of death by cancer worldwide (Siegel et al. 2012). Every year, 1.82 million people are diagnosed with lung cancer, and 1.6 million people die of this disease (Ferlay et al. 2015). The 5-year survival rate is less than 15%. Although new targeted therapies are being developed for lung cancer, the literature contains limited data regarding c-Myc and programmed cell death ligand-1 (PD-L1) in lung cancer.

The Myc oncogene family of transcription factors contains c-Myc, N-Myc, and L-Myc. The c-MYC protein is encoded by the c-Myc gene, which plays a crucial role in cell proliferation, differentiation, tumorigenesis, and apoptosis (Nesbit et al. 1999). Recent work indicated that the proto-oncogene product of c-Myc can activate and repress the transcription of discrete gene sets, resulting in changes to the cellular state that can provide feedback on all RNA

Cuiling Zhou and Gang Che have contributed equally to this work.

✉ Huanhuan Sun
sunhuanh3@mail.sysu.edu.cn

✉ Haiqing Ma
mahaiqing@mail.sysu.edu.cn

1 Department of Oncology, The Fifth Affiliated Hospital of Sun Yat-sen University, Zhuhai 519000, Guangdong, China

2 Department of Respiration, The Fifth Affiliated Hospital of Sun Yat-sen University, Zhuhai 519000, Guangdong, China

3 Department of Oncology, Suzhou Science and Technology Town Hospital, The Affiliated Suzhou Hospital (West District) of Nanjing Medical University, Suzhou 215153, China

4 Department of Equipment Management, The Fifth Affiliated Hospital of Sun Yat-sen University, Zhuhai 519000, Guangdong, China

production and turnover (Sabo et al. 2014). Even so, the mechanism of c-Myc in human cancer is not entirely clear. Myc was strongly proved to be a prognostic marker in patients with early-stage lung adenocarcinoma (Iwakawa et al. 2011). Additionally, expression of c-Myc continually appears in human cancers and is correlated with aggressive, poorly differentiated tumors and poor clinical outcome (Nesbit et al. 1999; Lin et al. 2012; Dang 1999; Henriksson and Luscher 1996). Previous studies reported c-Myc target genes in various tumor cells (Dang et al. 2006; Kim et al. 2006; Schlosser et al. 2005). Moreover, c-Myc was also considered as a representative target for future therapeutic intervention of non-small cell lung cancer (NSCLC) (Rapp et al. 2009).

Patients with advanced lung cancer are often resistant to existing therapies in NSCLC. Recently, researchers focused their attention on programmed death 1/programmed cell death ligand-1 (PD-1/PD-L1). After a considerable amount of research was performed, it was discovered that PD-L1 was expressed in various tumors, including NSCLC, bladder cancer, renal cell carcinoma, and melanoma (Chae et al. 2016; Wu et al. 2016; Choueiri et al. 2014; Merelli et al. 2014). Previous studies (Cooper et al. 2015; Velcheti et al. 2014) had emphasized PD-L1 expression as a favorable prognostic factor for overall survival (OS) in NSCLC, as opposed to two meta-analyses (Wang et al. 2015; Pan et al. 2015). c-Myc inactivation in mouse tumors downregulated CD47 and PD-L1 expression and enhanced the anti-tumor immune response. There has been growing interest in analyzing their contribution to the prognosis of NSCLC.

Materials and methods

Patients and samples

Samples were collected from the archives of the Institute of Pathology, the Fifth Affiliated Hospital of Sun Yat-sen University. Surgical resection with curative intent was performed on 128 patients who were enrolled between September 2002 and December 2012. Formalin-fixed paraffin-embedded blocks were retrieved, and the corresponding slides from all samples were re-reviewed by very prestigious respiratory pathologists in China who were blinded as to the outcome. Clinicopathologic characteristics collected from the original pathology reports included sex, age at diagnosis, tumor histology, tumor differentiation, and pathologic tumor–node–metastasis (TNM) stage, in accordance with the 7th edition of the Union International Cancer Control (UICC) TNM staging system for lung cancer.

Immunohistochemistry

For the immunohistochemistry analysis, 2-μm-thick pathological tissues were deparaffinized in xylene and rehydrated with graded ethanol, and were then unmasked with antigen retrieval buffer (MVS-0099, Maxim ethylenediaminetetraacetic acid (EDTA) buffer, pH 8.0) in an autoclave for 15 min at 120 °C. Subsequently, 3% H_2O_2 was used to block endogenous peroxidase activity for 30 min. Tissues were incubated at 4 °C in rabbit monoclonal PD-L1 antibody E1L3 N (#13684, Cell Signaling Technology, Danvers, USA) at 1:200 dilution, and then in mouse monoclonal c-Myc antibody 9E10 (#sc-40, Santa Cruz Biotechnology, California, USA) at 1:100. Tissues were subsequently washed with phosphate-buffered saline (PBS) three times for 5 min each time. According to the manufacturer's instructions, tissue sections were incubated with secondary antibodies of the Envision Plus kit detection system, followed by incubation with enhanced polymer 3,3′-diaminobenzidine (DAB) from a detection kit (Maxim Biotech, Fuzhou, China). Tissues were then counterstained with hematoxylin. After washing, slides were dehydrated using graded ethanol and then mounted with coverslips.

Assessment of c-Myc and PD-L1 expression

Semi-quantitative scoring was used to analyze the expression of PD-L1. According to the dyeing intensity, the grading is as follows: non-dyeing scored 0, light yellow scored 1, brown yellow scored 2, brown scored 3. And five visual fields were randomly selected under high power microscopy (× 400). The mean values were taken to calculate the percentage of positive tumor cells in all the visual fields. The percentage of positive tumor cells in the visual field < 1% scored 0, 1–5% scored 1, 5–50% scored 2, 51–75% scored 3, and 76–100% scored 4. PD-L1 expression was considered positive if the multiplication of the two indices was > = 1. PD-L1 positivity was determined using an expression threshold level of ≥ 1% (Nakashima et al. 2012a). The sample was classified as positive for c-Myc expression when the standardized c-Myc expression was ≥ 40% (Nakashima et al. 2012b).

Statistical analysis

The Pearson's Chi-square test or Fisher's exact test was used to analyze the correlation between the expression of PD-L1/c-Myc and the clinicopathological features, and the correlation between PD-L1 and c-Myc. Overall survival (OS) was defined from the date of the initial operation until the date of death or last follow-up. The association between

PD-L1/c-Myc expression and overall survival was analyzed by the Kaplan–Meier method and the log-rank test. The Cox proportional hazard model was used for univariate and multivariate analyses to assess c-Myc and PD-L1 expression related to overall survival with hazard ratios (HRs) and associated 95% confidence interval (CIs). All calculations were performed using SPSS software version 20.0 (IBM, Armonk, NY, USA), plotted with GraphPad Prism 6.0 software, with $p < 0.05$ as statistically significant. All tests were two sided.

Results

Patient characteristics

The results of c-Myc and PD-L1 immunohistochemical staining, along with the clinicopathologic characteristics, are shown in Table 1. Of 128 enrolled patients, 52 were females and 76 were males, with median age of 55.8 years and mean follow-up time of 43.6 months. There were 79 patients in TNM stage I + II and 48 patients in TNM stage III + IV at the time of their initial surgery; the TNM staging of one patient was unknown. Patients with advanced disease underwent palliative treatment according to the principles of lung cancer guidelines. In view of the medical situation in China (especially 20 years ago), some stage IV patients accepted surgery and some of them did benefit from the surgery as well. According to the pathological type, there were 40 patients with squamous cell carcinoma, 73 patients with adenocarcinoma, and 15 patients with other pathological types (including adenosquamous carcinoma, mucoepidermoid carcinoma, lymphoepithelioma-like carcinoma) of NSCLC. Unknown data were not included in the calculation. The cases enrolled between September 2002 and December 2012 were not treated with anti-PD-1/PD-L1 therapy (for the inhibitor of PD-1/PD-L1 was not approved for use in China until 2018, they were not treated with anti-PD-1/PD-L1 therapy) and were not performed mutation analysis (mutation analysis is an unconventional test for patients with NSCLC, so we did not include mutation analysis in the study). And some of the patients accepted targeted therapy, which would not influence our research. So, these were not analyzed as additional factors.

Table 1 Baseline characteristics of patients in NSCLC

Characteristics	Count ($N = 103$)	
	N	%
Sex		
Male	76	59.4
Female	52	40.6
Age (in years)		
< 55	51	39.8
≥ 55	77	60.2
TNM stage		
I + II	79	61.7
III + IV	48	37.5
Other	1	0.8
Tumor stage		
T1	23	18.0
T2	67	52.3
T3	29	22.7
T4	4	3.1
Other	5	3.9
Lymph node metastasis		
No	76	59.4
Yes	44	34.4
Other	8	6.2
Pathology or histology		
SCC	40	31.5
ADC	73	57.0
Other	15	11.7
Differentiation		
Poor	27	21.1
Moderate	72	56.2
High	12	9.4
Other	17	13.3
PD-L1 expression		
Positive	59	46.1
Negative	69	53.9
c-Myc expression		
Positive	75	58.6
Negative	53	41.4

SCC squamous cell carcinoma, *ADC* adenocarcinoma

Expression of PD-L1 and c-Myc in NSCLC

Among the 128 NSCLC sections, 59 exhibited positive expression of PD-L1 (46.1%) and the positive expression rate of c-Myc in NSCLC was 58.6% (75/128). As shown in Fig. 1, the positive expression of PD-L1 was mainly located in the cell membrane and cytoplasm of tumor cells, and positive c-Myc expression occurred in the cytoplasm of tumor cells or cell nucleus.

Correlation between expression of PD-L1 and c-Myc and clinicopathological features

As shown in Table 2, the expression of PD-L1 in NSCLC was associated with pathological type ($p = 0.008$) and degree of differentiation ($p = 0.021$). Patients with squamous cell carcinoma were more likely to express PD-L1 than patients with lung adenocarcinoma (60.0% vs. 34.2%, $p = 0.008$),

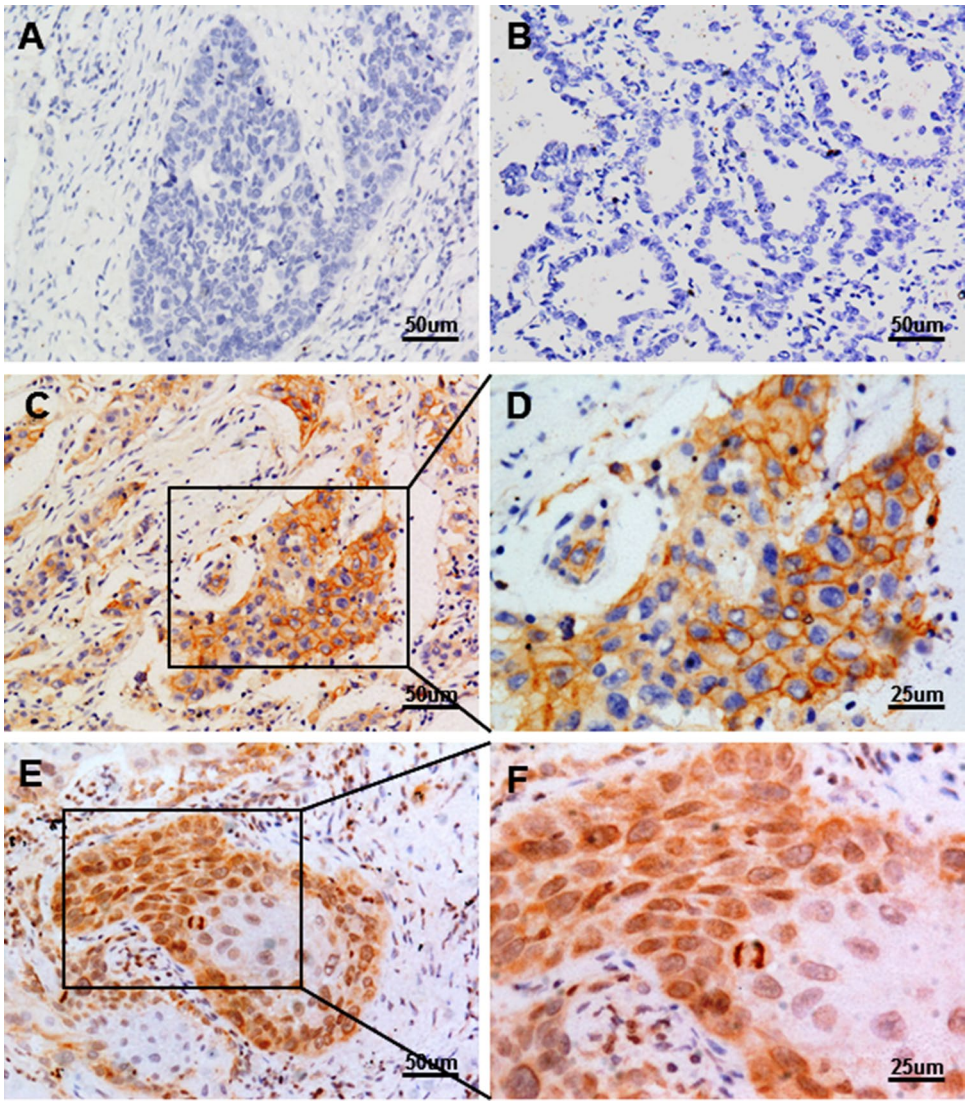

Fig. 1 Representative images of PD-L1 and c-Myc staining in NSCLC. Representative images on NSCLC showed PD-L1 negative staining (**a**), positive staining (**c**), and enlarged positive staining (**d**), respectively, by IHC; representative images on NSCLC showed c-Myc negative staining (**b**), positive staining (**e**), and enlarged positive staining (**f**), respectively, by IHC (The red arrow showed the specific location of positive expression). Original magnification: **a**, **b**, **c** and **e** panels × 200, **d**, **f** panel × 400 (*n*: size of the group)

and poorly differentiated NSCLC was more prone to PD-L1 expression than the moderately or highly differentiated type (66.7%, $p = 0.021$). Conversely, sex ($p = 0.073$), age ($p = 0.795$), TNM stage ($p = 0.445$), T stage ($p = 0.502$), and lymph node metastasis ($p = 0.090$) were irrelevant with PD-L1 expression.

By contrast, c-Myc expression in NSCLC was more common in male patients (69.7%, $p = 0.002$) and patients with lymph node metastasis (70.5%, $p = 0.029$). c-Myc expression was not significantly correlated with age ($p = 0.746$), TNM stage ($p = 0.062$), T stage ($p = 0.256$), pathological type ($p = 0.227$), or differentiation ($p = 0.310$).

Correlation between PD-L1 expression and c-Myc expression in NSCLC

There were 49 tissue sections that exhibited double-positive PD-L1 and c-Myc expression in NSCLC, and 43 sections of double-negative expression. Chi-square test analysis showed that PD-L1 expression was positively correlated with c-Myc expression ($r = 0.459$, $p < 0.001$, Table 3).

Prognostic impact of c-Myc and PD-L1 expression in NSCLC

The median overall survival (OS) of all enrolled 128 patients in NSCLC was 37 months. As shown in Fig. 2a, the Kaplan–Meier curve displayed a significant prolongation of OS in the PD-L1-negative group compared with the PD-L1-positive group (median OS: 96 months vs. 37 months, $p = 0.001$). Similarly, the effect of c-Myc expression on OS in patients with NSCLC was statistically significant. As shown in Fig. 2b, the survival of patients in the c-Myc positive expression group was significantly shorter than that of the negative group (median OS: 42 months *vs.* 98 months, $p = 0.002$). Furthermore, patients with both positive

Table 2 Patients characteristics and their association with PD-L1 and c-Myc expression

Characteristic	PD-L1 expression		*p* value	c-Myc expression		*p* value
	Negative	Positive		Negative	Positive	
Sex			0.073			0.002
Male	36 (47.4)	40 (52.6)		23 (30.3)	53 (69.7)	
Female	33 (63.5)	19 (36.5)		30 (57.7)	22 (42.3)	
Age (years)			0.854			0.746
< 55	28 (54.9)	23 (45.1)		22 (43.1)	29 (56.9)	
≥ 55	41 (53.2)	36 (46.8)		31 (40.3)	46 (59.7)	
TNM stage			0.445			0.062
I + II	45 (57.0)	17 (43.0)		38 (48.1)	41 (51.9)	
III + IV	24 (50.0)	24 (50.0)		15 (31.2)	33 (68.8)	
Tumor stage			0.502			0.256
pT1	11 (47.8)	12 (52.2)		8 (34.8)	15 (65.2)	
pT2	36 (53.7)	31 (46.3)		32 (47.8)	52.2 (35)	
pT3	18 (62.1)	11 (37.9)		12 (41.4)	17 (58.6)	
pT4	1 (25.0)	3 (75.0)		0 (0.0)	4 (100.0)	
Lymph node metastasis			0.090			0.029
No	45 (59.2)	31 (40.8)		38 (50.0)	38 (50.0)	
Yes	19 (43.2)	25 (56.8)		13 (29.5)	31 (70.5)	
Pathology or histology			0.008			0.227
SCC	16 (40.0)	24 (60.0)		15 (37.5)	25 (62.5)	
ADC	48 (65.8)	25 (34.2)		36 (49.3)	37 (50.7)	
Differentiation			0.021			0.310
Poor	9 (33.3)	18 (66.7)		9 (33.3)	18 (66.7)	
Moderate	43 (59.7)	29 (40.3)		33 (45.8)	39 (54.2)	
High	9 (75.0)	3 (25.0)		7 (58.3)	5 (41.7)	

The expression of PD-L1and c-Myc was expressed as a percentage (%)

SCC squamous cell carcinoma, *ADC* adenocarcinoma

Table 3 Association of PD-L1 expression and c-Myc expression

c-Myc expression	PD-L1 expression		Count
	Positive	Negative	
Positive	49	26	75
Negative	10	43	53
Count	59	69	128

Pearson $r = 0.459$, $p < 0.001$

expression of c-Myc and PD-L1 (double-positive expression) had significantly worse prognosis than other subgroups ($p < 0.001$; Fig. 7).

Univariate analysis indicated that the risk of death was significantly higher in those with c-Myc positive expression than in those with c-Myc negative expression (HR = 2.314, 95% CI 1.353–3.957, $p = 0.002$). The hazard ratio of the PD-L1 positive expression group was 2.384-fold greater

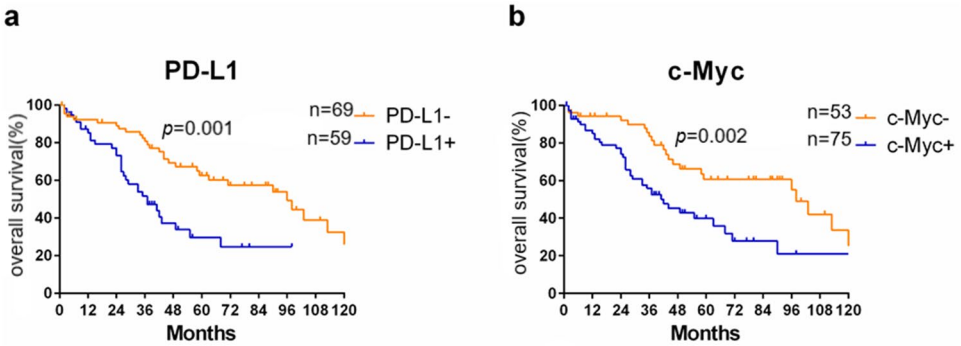

Fig. 2 Kaplan–Meier curves of overall survival according to PD-L1 (**a**) or c-Myc (**b**) expression in NSCLC patients. *OS* overall survival, *N* size of the group (*n*: size of the group)

than that of the PD-L1 negative group (95% CI 1.407–4.042, $p = 0.001$). Similar results were observed in those who were male (HR = 2.179, $p = 0.005$), had a higher TNM stage (HR = 2.624, $p < 0.001$), had lymph node metastasis (HR = 3.439, $p < 0.001$), and had squamous cell carcinoma (HR = 1.744, $p = 0.048$) (Table 4).

Multivariate analysis revealed that PD-L1 positive expression (HR = 2.136, 95% CI 1.194–3.819, $p = 0.011$) and higher TNM stage (HR = 2.685, 95% CI 1.546–4.665, $p < 0.001$) were independent risk predictors for poor prognosis (Table 4).

The Kaplan–Meier method was used to investigate the effect of PD-L1 expression on the survival of patients with different clinicopathological features. The survival analysis and between-group differences were assessed by the stratified log-rank test. As shown in Fig. 3a and c, males with positive PD-L1 expression had a shorter survival compared with these of negative PD-L1 expression (median OS: 33 months vs. 71 months, $p = 0.003$, Fig. 3c). In Fig. 3b, the two survival curves indicated that no statistical difference was seen in different levels of PD-L1 expression in female patients, $p = 0.327$. In the subgroup analysis of age ≥ 55 years with

Table 4 The effect of clinicopathological characteristics, PD-L1 and c-Myc expression on overall survival

Subtype	Univariate analysis		Multivariate analysis[a]		Multivariate analysis[b]	
	HR (95% CI)	*p*	HR (95% CI)	*p*	HR (95% CI)	*p*
Sex						
Female	1					
Male	2.179 (1.261–3.766)	0.005				
Age (years)						
> 55	1					
≥ 55	0.838 (0.503–1.396)	0.497				
TNM stage						
I + II	1		1		1	
III + IV	2.624 (1.580–4.357)	< 0.001	2.68 (1.546–4.665)	< 0.001	2.797 (1.460–5.357)	0.002
Tumor stage						
pT1	1					
pT2	2.228 (0.933–5.321)	0.071				
pT3	3.298 (1.308–8.319)	0.011				
pT4	4.626 (0.925–23.128)	0.062				
Lymph node metastasis						
No	1					
Yes	3.439 (1.972–5.999)	< 0.001				
Pathology or histology						
ADC	1					
SCC	1.744 (1.004–3.031)	0.048				
Differentiation						
Poor	1					
Moderate	1.099 (0.574–2.104)	0.776				
High	0.333 (0.094–1.181)	0.089				
PD-L1 expression						
Negative	1		1			
Positive	2.384 (1.407–4.042)	0.001	2.136 (1.194–3.819)	0.011		
c-Myc expression						
Negative	1					
Positive	2.314 (1.353–3.957)	0.002				
c-Myc, PD-L1 expression						
Double negative	1					
Double positive	3.133 (1.668–5.884)	< 0.0001			2.494 (1.264–4.919)	0.008

SCC squamous cell carcinoma, *ADC* adenocarcinoma

[a]Multivariate analysis without groups of double negative or double positive expression of c-Myc, PD-L1

[b]Multivariate analysis including groups of double negative or double positive expression of c-Myc, PD-L1

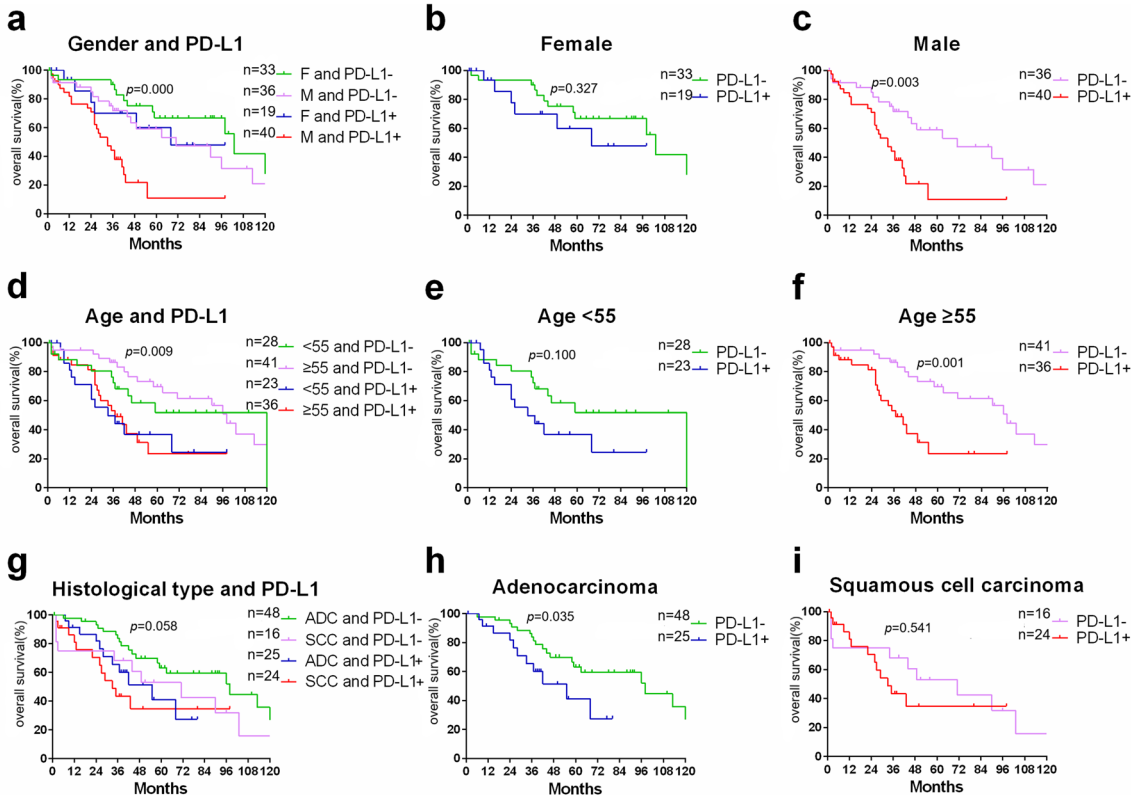

Fig. 3 Survival analyses of NSCLC patients with and without PD-L1 expression according to sex (**a**, **b**, **c**), age (**d**, **e**, **f**), and histological type (**g**, **h**, **i**) (*n*: size of the group)

adenocarcinoma, patients with negative PD-L1 expression had statistically longer survival than those with positive PD-L1 expression, $p=0.001$ (Fig. 3f), $p=0.035$ (Fig. 3h), respectively.

Figure 4b, c shows that the survival of patients with negative PD-L1 expression was significantly longer than that of patients with positive PD-L1 expression, regardless of the TNM stage of lung cancer ($p=0.008$ for early patients, $p=0.023$ for advanced patients, individually). Figure 4f, i shows that patients with positive PD-L1 expression had significantly shorter overall survival than that with negative PD-L1 expression in lymph node metastasis and moderate–high differentiation, separately (Fig. 4f, $p=0.041$; Fig. 4i, $p=0.009$). There were no significant differences in these subgroups for females ($p=0.327$), age < 55 years ($p=0.100$), squamous cell carcinoma ($p=0.541$), normal lymph nodes ($p=0.070$), and poor differentiation ($p=0.164$).

Similarly, the Kaplan–Meier method was used to analyze the impact of c-Myc expression on overall survival of patients with different clinicopathological parameters. Figure 5b, c shows that in the subgroup analysis of females or males, c-Myc expression had no significant effect on the overall survival of patients with NSCLC ($p=0.217$ for females, and $p=0.061$ for males). Patients who were over 55 years of age or with c-Myc positive expression had a worse prognosis as compared to patients with c-Myc negative expression ($p=0.003$, Fig. 5f). It is reflected in Fig. 5h that the negative c-Myc expression group with lung adenocarcinoma had a higher survival advantage than the positive c-Myc expression group with lung adenocarcinoma (median OS: 98 months vs. 55 months, $p=0.048$), but no statistical difference was found for patients with squamous cell carcinoma ($p=0.230$, Fig. 5i).

No significant correlation was observed between overall survival and c-Myc expression (Fig. 6b, $p=0.053$; Fig. 6c, $p=0.236$; Fig. 6f, $p=0.352$; Fig. 6e, $p=0.131$), which had no relationship with the staging of lung cancer or lymph node metastasis. According to Fig. 6i, a significantly longer survival was seen in patients with negative c-Myc expression rather than with positive c-Myc expression by the degree of differentiation (median OS: 96 months for moderately differentiated subgroups, 55 months for highly differentiated subgroups, $p=0.020$).

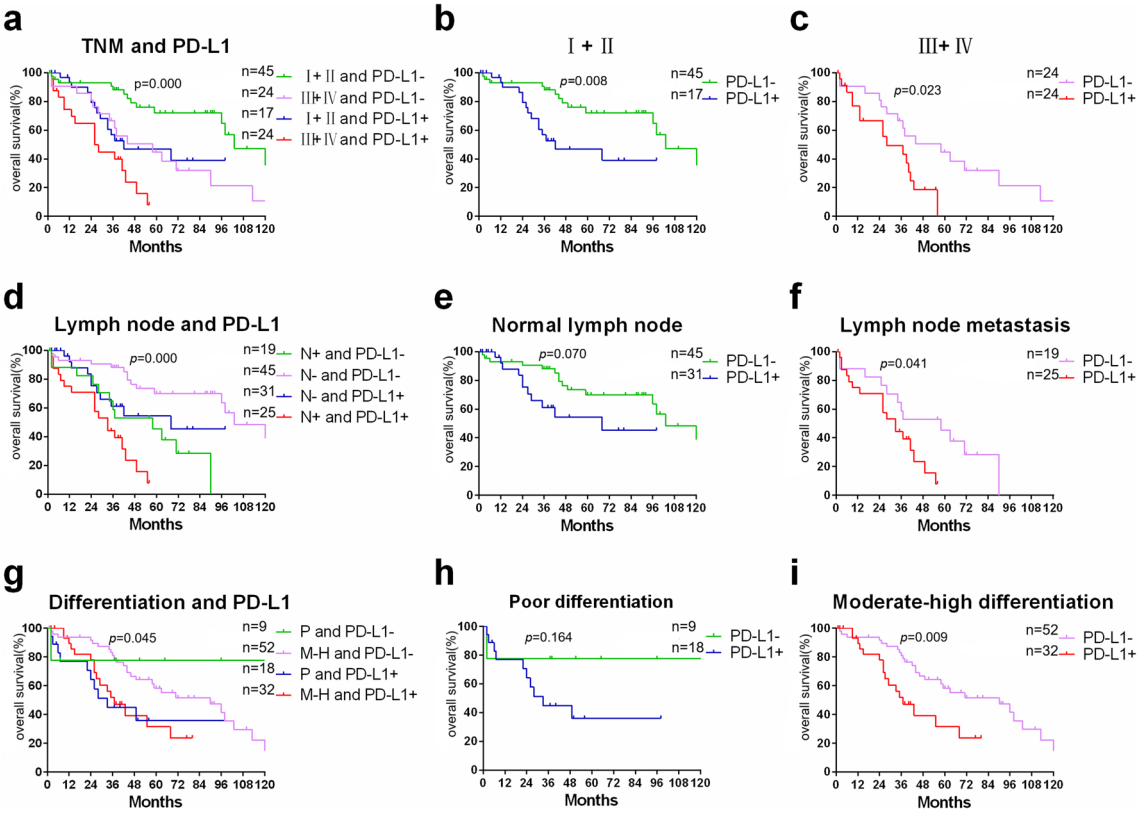

Fig. 4 Survival analyses of NSCLC patients with and without PD-L1 expression according to TNM staging (**a**, **b**, **c**), lymph node metastasis (**d**, **e**, **f**), and differentiation (**g**, **h**, **i**) (*n*: size of the group)

Survival analysis of PD-L1 and c-Myc double-positive expression

To assess the effect of PD-L1 and c-Myc expression on clinical outcomes of patients with NSCLC, the Kaplan–Meier method was used. As shown in Fig. 7a, b, the overall survival of patients with PD-L1 and c-Myc double-positive expression was significantly shorter compared to the other subgroups ($p = 0.000$). As shown in Table 4, the hazard ratio of double-positive expression of PD-L1 and c-Myc group was 3.133-fold greater than that of the double-negative group (95% CI 1.668–5.884, $p < 0.0001$). Multivariate analysis (including double-positive expression of PD-L1 and c-Myc group and double-negative expression of PD-L1 and c-Myc group) revealed that double-positive expressions of PD-L1 and c-Myc (HR = 2.494, 95% CI 1.264–4.919, $p = 0.008$) and *TNM stage (*III + IV; HR = 2.797, 95% CI 1.460–5.357, $p = 0.002$*)*were independent risk predictors for poor prognosis.

In summary, PD-L1 and c-Myc double-positive expression was inferior to PD-L1 or c-Myc single-positive expression, and PD-L1 and c-Myc double-negative expression was superior to PD-L1 or c-Myc single-negative expression for the prognosis of patients with NSCLC.

Discussion

This study found that conjoint analysis of the expression of PD-L1 and c-Myc was a better prognostic approach for NSCLC patients.

There are a number of studies indicating that PD-L1 protein is positively expressed in various tumor tissues, and it is an independent poor prognostic factor, not only in lung cancer tissue (Mu et al. 2011; Zhou et al. 2017), but also in renal cell carcinoma (Thompson et al. 2006), melanoma (Hino et al. 2010), bladder cancer (Nomi et al. 2007), and gastric cancer (Wu et al. 2006). In the current study, the PD-L1-positive group showed significantly shorter OS than the PD-L1-negative group. Oncogenic mutations, such as EGFRm+, can drive PD-L1 expression in NSCLC, which is then downregulated by treatment with EGFR-TKIs and leads to shorter OS (Akbay et al. 2013; Ilie et al. 2016). Guo et al. (2019) demonstrated that HIF-1α and NF-κB played important roles in regulating PD-L1 expression by EGFR mutants in NSCLC cells. However, some studies found that there was no significant association between PD-L1 expression and survival in NSCLC (Konishi et al. 2004; Schmidt et al. 2015), which may have occurred because the sample sizes of their studies were insufficiently large.

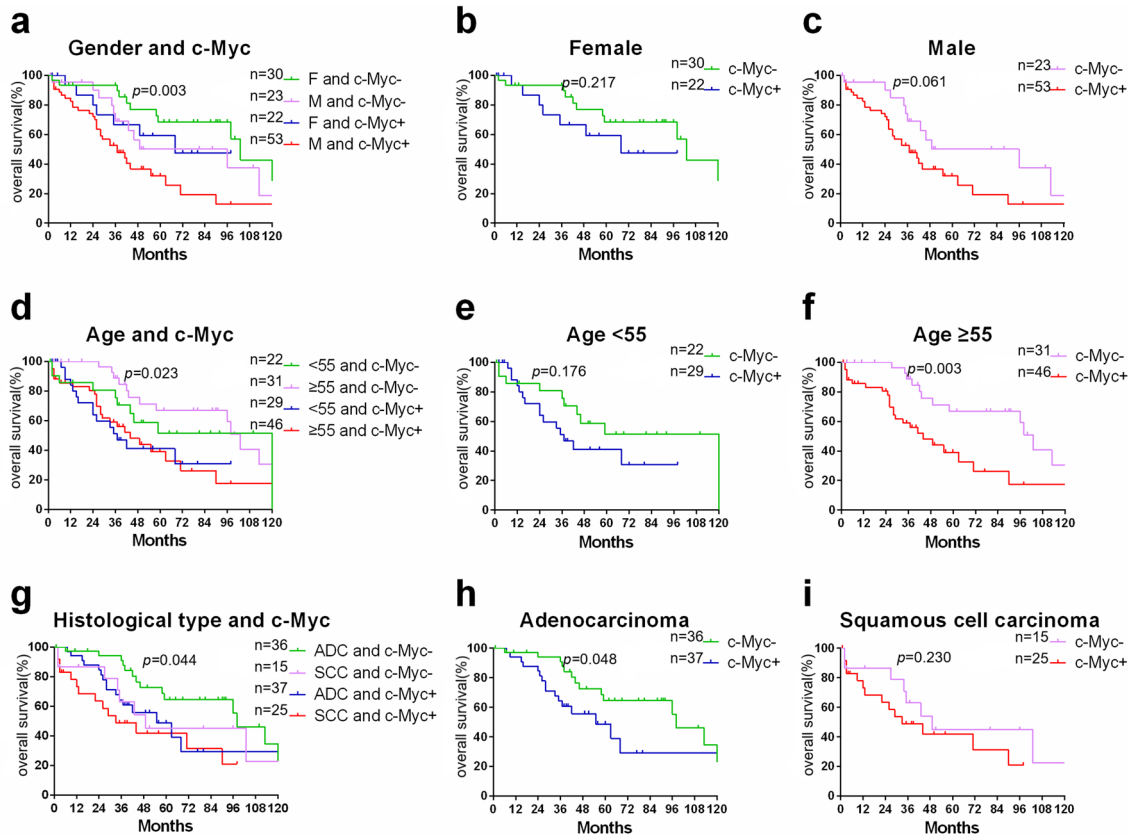

Fig. 5 Survival analyses of NSCLC patients with and without c-Myc expression according to sex (**a**, **b**, **c**), age (**d**, **e**, **f**), and histological type (**g**, **h**, **i**) (*n*: size of the group)

We found that immunohistochemical staining for both proteins showed a higher positive rate than the other studies previously reported. The reasons that immunohistochemical staining for both proteins showed a higher positive rate than the other studies previously reported may be as follows. Firstly, the methods for determining the score of the staining and the antibody for detecting the expression of PD-L1 are not uniform, leading to the inconsistency of the results. Secondly, classical immunohistochemistry and semi-quantitative scoring methods were used to detect the expression of PD-L1. And the corresponding slides from all samples were re-reviewed by very prestigious respiratory pathologists in China who were blinded as to the outcome. And it is unexpected to find that the patients with a poorly differentiated tumor have a better survival in both PD-L1 and c-Myc analysis than moderate-high differentiation. In our view, the small proportion of patients with a poorly differentiated tumor to all patients of the research (27/128) may lead to this phenomenon. In addition, perhaps the patients with a poorly differentiated tumor included contained many other factors that were conductive to survival, such as young age, female or TNM stage I + II etc.

In the current study, we also found that the expression of PD-L1 was positively correlated with the expression of c-Myc. It was confirmed again that the expression of PD-L1 was influenced by c-Myc as well. Casey et al. (2016) confirmed that c-Myc can bind to promoters of PD-L1 and CD47 in different types of tumors (including lymphoma/leukemia, liver cancer, and lung cancer) and directly regulate their expression to achieve immune escape. After knocking down the expression of c-Myc, the expression of PD-L1 decreased. It was further discovered that c-Myc maintained tumorigenesis via regulating immunoregulatory molecules such as $CD4^+$ T cells and macrophages. Similarly, Kim et al. (2017) found that the expression of PD-L1 was positively correlated with the expression of c-Myc in NSCLC cell lines. After knocking down c-Myc, the expression of PD-L1 decreased as well. Melaiu and his colleagues also found that c-Myc may be involved in regulating the expression of PD-L1 in neuroblastoma (Melaiu et al. 2017). In vivo and in vitro experiments showed that c-Myc regulated the expression of PD-L1, knocked down the expression of c-Myc, and inhibited the expression of Myc in neuroblastoma patients using JQ1, a BRD4 inhibitor. The results showed that the expression of PD-L1 decreased (Melaiu

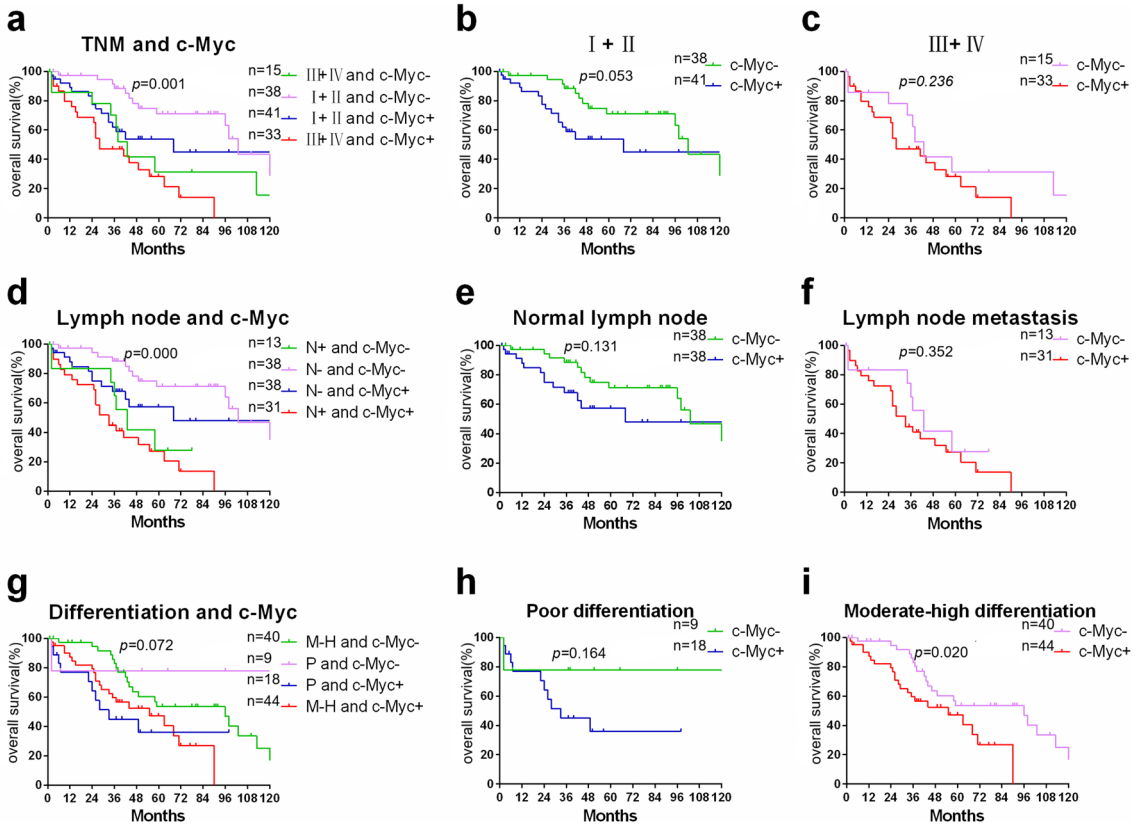

Fig. 6 Survival analyses of NSCLC patients with and without c-Myc expression according to TNM staging (**a**, **b**, **c**), lymph node metastasis (**d**, **e**, **f**), and differentiation (**g**, **h**, **i**) (*n*: size of the group)

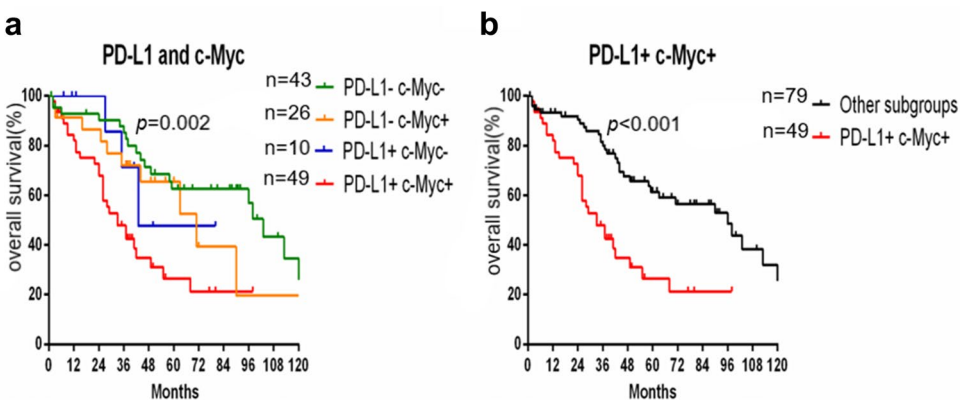

Fig. 7 Kaplan–Meier survival curves of PD-L1 and c-Myc expression in NSCLC patients. The prognosis of patients with double-positive expression of PD-L1 and c-Myc was poor, $p < 0.05$ (*n*: size of the group)

et al. 2017). Atsaves showed that c-Myc and STAT3 regulated the expression of PD-L1 at the transcriptional level in ALK-negative anaplastic large cell lymphoma (Atsaves et al. 2017). These studies have shown that c-Myc can regulate PD-L1, and perhaps is involved in regulating the immune response effector of tumors.

The Kaplan–Meier curve showed that the total survival time of the c-Myc-negative group was significantly longer than that of the c-Myc-positive group, and the difference was statistically significant. Kaplan–Meier analysis showed that the prognosis of the PD-L1 and c-Myc double-positive expression group was worse than that of each subgroup, and the difference was statistically significant, which was consistent with the results of the Kim et al. (2017). Cox multivariate regression analysis showed that the positive expression of c-Myc had no significant effect on the clinical prognosis of lung cancer patients. Statistically speaking, this may be affected by mixed factors such as PD-L1 expression, gender, and TNM stage. However, what was clear was that Myc, as a proto-oncogene, was expressed in a variety of

human cancer cells, which was related to the occurrence and development of a variety of tumors. The negative effects of Myc on the clinical benefit of lung cancer patients cannot be negated by the difference in the statistical results.

Recently, researchers used fluorescence in situ hybridization (FISH) to detect c-Myc gene expression in patients with lung adenocarcinoma, suggesting that c-Myc gene expression is an independent predictor of poor overall survival and progression-free survival in patients with lung adenocarcinoma (Seo et al. 2014). Therefore, in order to compensate for the shortcomings of this experiment, in the next step of our study, we will consider increasing the sample size and adopting more sensitive methods, such as FISH, to examine the expression of c-Myc.

In conclusion, conjoint analysis of the expression of PD-L1 and c-Myc was a better prognostic approach for NSCLC patients. It showed greater differences in survival and HR by two indices than by single one. Double staining of PD-L1 and c-Myc deserves further exploration in predicting the prognosis of patients with NSCLC in clinical practice in the future.

Acknowledgements This study was funded by the National Natural Science Foundation of China (81872308, 81500030), the National Science Foundation of Guangdong Province (2017A030313573, 2016A030313272, 2016A030313277), project of the Suzhou industrial technology innovation (applied basic research of the people's Livelihood technology and applied health care) (SYSD2016062), and Youth Project of Health and Family Planning Bureau of Suzhou High-tech Zone (2017Q005).

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest for this article.

References

Akbay EA, Koyama S, Carretero J, Altabef A, Tchaicha JH, Christensen CL et al (2013) Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. Cancer Discov 3(12):1355–1363. https://doi.org/10.1158/2159-8290.Cd-13-0310

Atsaves V, Tsesmetzis N, Chioureas D, Kis L, Leventaki V, Drakos E et al (2017) PD-L1 is commonly expressed and transcriptionally regulated by STAT3 and MYC in ALK-negative anaplastic large-cell lymphoma. Leukemia 31(7):1633–1637. https://doi.org/10.1038/leu.2017.103

Casey SC, Tong L, Li Y, Do R, Walz S, Fitzgerald KN et al (2016) MYC regulates the antitumor immune response through CD47 and PD-L1. Science 352(6282):227–231. https://doi.org/10.1126/science.aac9935

Chae YK, Pan A, Davis AA, Raparia K, Mohindra NA, Matsangou M et al (2016) Biomarkers for PD-1/PD-L1 blockade therapy in non-small-cell lung cancer: is PD-L1 expression a good marker for patient selection? Clin Lung Cancer. https://doi.org/10.1016/j.cllc.2016.03.011

Choueiri TK, Fay AP, Gray KP, Callea M, Ho TH, Albiges L et al (2014) PD-L1 expression in nonclear-cell renal cell carcinoma. Ann Oncol 25(11):2178–2184. https://doi.org/10.1093/annonc/mdu445

Cooper WA, Tran T, Vilain RE, Madore J, Selinger CI, Kohonen-Corish M et al (2015) PD-L1 expression is a favorable prognostic factor in early stage non-small cell carcinoma. Lung Cancer 89(2):181–188. https://doi.org/10.1016/j.lungcan.2015.05.007

Dang CV (1999) c-Myc target genes involved in cell growth, apoptosis, and metabolism. Mol Cell Biol 19(1):1–11

Dang CV, O'Donnell KA, Zeller KI, Nguyen T, Osthus RC, Li F (2006) The c-Myc target gene network. Semin Cancer Biol 16(4):253–264. https://doi.org/10.1016/j.semcancer.2006.07.014

Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M et al (2015) Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 136(5):E359–E386. https://doi.org/10.1002/ijc.29210

Guo R, Li Y, Wang Z, Bai H, Duan J, Wang S et al (2019) HIF-1alpha and NF-kappaB play important roles in regulating PD-L1 expression by EGFR mutants in non-small cell lung cancer cells. Cancer Sci. https://doi.org/10.1111/cas.13989

Henriksson M, Luscher B (1996) Proteins of the Myc network: essential regulators of cell growth and differentiation. Adv Cancer Res 68:109–182

Hino R, Kabashima K, Kato Y, Yagi H, Nakamura M, Honjo T et al (2010) Tumor cell expression of programmed cell death-1 ligand 1 is a prognostic factor for malignant melanoma. Cancer 116(7):1757–1766. https://doi.org/10.1002/cncr.24899

Ilie M, Hofman V, Dietel M, Soria JC, Hofman P (2016) Assessment of the PD-L1 status by immunohistochemistry: challenges and perspectives for therapeutic strategies in lung cancer patients. Virchows Arch 468(5):511–525. https://doi.org/10.1007/s00428-016-1910-4

Iwakawa R, Kohno T, Kato M, Shiraishi K, Tsuta K, Noguchi M et al (2011) MYC amplification as a prognostic marker of early-stage lung adenocarcinoma identified by whole genome copy number analysis. Clin Cancer Res 17(6):1481–1489. https://doi.org/10.1158/1078-0432.CCR-10-2484

Kim YH, Girard L, Giacomini CP, Wang P, Hernandez-Boussard T, Tibshirani R et al (2006) Combined microarray analysis of small cell lung cancer reveals altered apoptotic balance and distinct expression signatures of MYC family gene amplification. Oncogene 25(1):130–138. https://doi.org/10.1038/sj.onc.1208997

Kim EY, Kim A, Kim SK, Chang YS (2017) MYC expression correlates with PD-L1 expression in non-small cell lung cancer. Lung Cancer 110:63–67. https://doi.org/10.1016/j.lungcan.2017.06.006

Konishi J, Yamazaki K, Azuma M, Kinoshita I, Dosaka-Akita H, Nishimura M (2004) B7-H1 expression on non-small cell lung cancer cells and its relationship with tumor-infiltrating lymphocytes and their PD-1 expression. Clinical Cancer Res 10(15):5094–5100. https://doi.org/10.1158/1078-0432.Ccr-04-0428

Lin CY, Loven J, Rahl PB, Paranal RM, Burge CB, Bradner JE et al (2012) Transcriptional amplification in tumor cells with elevated c-Myc. Cell 151(1):56–67. https://doi.org/10.1016/j.cell.2012.08.026

Melaiu O, Mina M, Chierici M, Boldrini R, Jurman G, Romania P et al (2017) PD-L1 is a therapeutic target of the bromodomain inhibitor JQ1 and combined with HLA class I, a promising prognostic biomarker in neuroblastoma. Clin Cancer Res 23(15):4462–4472. https://doi.org/10.1158/1078-0432.Ccr-16-2601

Merelli B, Massi D, Cattaneo L, Mandala M (2014) Targeting the PD1/PD-L1 axis in melanoma: biological rationale, clinical challenges and opportunities. Crit Rev Oncol Hematol 89(1):140–165. https://doi.org/10.1016/j.critrevonc.2013.08.002

Mu CY, Huang JA, Chen Y, Chen C, Zhang XG (2011) High expression of PD-L1 in lung cancer may contribute to poor prognosis and tumor cells immune escape through suppressing tumor

infiltrating dendritic cells maturation. Med Oncol 28(3):682–688. https://doi.org/10.1007/s12032-010-9515-2

Nakashima N, Liu D, Huang CL, Ueno M, Zhang X, Yokomise H (2012a) Wnt3 gene expression promotes tumor progression in non-small cell lung cancer. Lung Cancer 76(2):228–234

Nakashima N, Liu D, Huang CL, Ueno M, Zhang X, Yokomise H (2012b) Wnt3 gene expression promotes tumor progression in non-small cell lung cancer. Lung Cancer 76(2):228–234. https://doi.org/10.1016/j.lungcan.2011.10.007

Nesbit CE, Tersak JM, Prochownik EV (1999) MYC oncogenes and human neoplastic disease. Oncogene 18(19):3004–3016. https://doi.org/10.1038/sj.onc.1202746

Nomi T, Sho M, Akahori T, Hamada K, Kubo A, Kanehiro H et al (2007) Clinical significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer. Clin Cancer Res 13(7):2151–2157. https://doi.org/10.1158/1078-0432.CCR-06-2746

Pan ZK, Ye F, Wu X, An HX, Wu JX (2015) Clinicopathological and prognostic significance of programmed cell death ligand1 (PD-L1) expression in patients with non-small cell lung cancer: a meta-analysis. J Thorac Dis 7(3):462–470. https://doi.org/10.3978/j.issn.2072-1439.2015.02.13

Rapp UR, Korn C, Ceteci F, Karreman C, Luetkenhaus K, Serafin V et al (2009) MYC is a metastasis gene for non-small-cell lung cancer. PLoS One 4(6):e6029. https://doi.org/10.1371/journal.pone.0006029

Sabo A, Kress TR, Pelizzola M, de Pretis S, Gorski MM, Tesi A et al (2014) Selective transcriptional regulation by Myc in cellular growth control and lymphomagenesis. Nature 511(7510):488–492. https://doi.org/10.1038/nature13537

Schlosser I, Holzel M, Hoffmann R, Burtscher H, Kohlhuber F, Schuhmacher M et al (2005) Dissection of transcriptional programmes in response to serum and c-Myc in a human B-cell line. Oncogene 24(3):520–524. https://doi.org/10.1038/sj.onc.1208198

Schmidt LH, Kummel A, Gorlich D, Mohr M, Brockling S, Mikesch JH et al (2015) PD-1 and PD-L1 expression in NSCLC indicate a favorable prognosis in defined subgroups. PLoS One 10(8):e0136023. https://doi.org/10.1371/journal.pone.0136023

Seo AN, Yang JM, Kim H, Jheon S, Kim K, Lee CT et al (2014) Clinicopathologic and prognostic significance of c-MYC copy number gain in lung adenocarcinomas. Br J Cancer 110(11):2688–2699. https://doi.org/10.1038/bjc.2014.218

Siegel R, Naishadham D, Jemal A (2012) Cancer statistics, 2012. CA Cancer J Clin 62(1):10–29. https://doi.org/10.3322/caac.20138

Thompson RH, Kuntz SM, Leibovich BC, Dong H, Lohse CM, Webster WS et al (2006) Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up. Cancer Res 66(7):3381–3385. https://doi.org/10.1158/0008-5472.CAN-05-4303

Velcheti V, Schalper KA, Carvajal DE, Anagnostou VK, Syrigos KN, Sznol M et al (2014) Programmed death ligand-1 expression in non-small cell lung cancer. Lab Invest 94(1):107–116. https://doi.org/10.1038/labinvest.2013.130

Wang A, Wang HY, Liu Y, Zhao MC, Zhang HJ, Lu ZY et al (2015) The prognostic value of PD-L1 expression for non-small cell lung cancer patients: a meta-analysis. Eur J Surg Oncol 41(4):450–456. https://doi.org/10.1016/j.ejso.2015.01.020

Wu C, Zhu Y, Jiang J, Zhao J, Zhang XG, Xu N (2006) Immunohistochemical localization of programmed death-1 ligand-1 (PD-L1) in gastric carcinoma and its clinical significance. Acta Histochem 108(1):19–24. https://doi.org/10.1016/j.acthis.2006.01.003

Wu CT, Chen WC, Chang YH, Lin WY, Chen MF (2016) The role of PD-L1 in the radiation response and clinical outcome for bladder cancer. Sci Rep 6:19740. https://doi.org/10.1038/srep19740

Zhou C, Tang J, Sun H, Zheng X, Li Z, Sun T et al (2017) PD-L1 expression as poor prognostic factor in patients with non-squamous non-small cell lung cancer. Oncotarget 8(35):58457–58468. https://doi.org/10.18632/oncotarget.17022