ORIGINAL ARTICLE

WILEY

Downregulation of PD-L1 and HLA-I in non-small cell lung cancer with ALK fusion

Di Mu[1,2,3,4] | Jingjing Guo[1,2,3,4] | Wenwen Yu[1,4,5] | Jiali Zhang[1,4,5] | Xiubao Ren[1,2,3,4,5] | Ying Han[1,2,3,4,5]

[1]National Clinical Research Center for Cancer, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China

[2]Key Laboratory of Cancer Prevention and Therapy, Tianjin, China

[3]Tianjin's Clinical Research Center for Cancer, Tianjin, China

[4]Key Laboratory of Cancer Immunology and Biotherapy, Tianjin, China

[5]Department of Biotherapy, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China

Correspondence
Ying Han, Department of Immunology and Biotherapy, Key Laboratory of Cancer Immunology and Biotherapy, Tianjin Medical University Cancer Institute and Hospital, Huanhu Xi Road, Hexi District, Tianjin 300060, China.
Email: tampa3810@163.com

Abstract

Background: Early clinical trials indicate that patients with anaplastic lymphoma kinase (ALK)-driven non-small cell lung cancer (NSCLC) have a lower response rate to programmed cell death protein 1 (PD-1) antibody therapy. However, the specific mechanism underlying this remains unclear. To further explore the characteristics of the tumor microenvironment and determine the potential mechanism of immunotherapy resistance in patients with ALK, we selected another important immune-related molecule, major histocompatibility complex class I (HLA-I), as the focus of our study.

Methods: We collected the biopsy samples of 140 patients with NSCLC. The number of CD8+ T cells and HLA-I/programmed cell death 1 ligand 1 (PD-L1) expression were determined by immunohistochemistry. Disease-free survival (DFS) and overall survival (OS) were analyzed using the Kaplan–Meier method, and their relationship with patient clinical characteristics analyzed using Cox proportional hazards regression. In addition, we treated ALK-positive lung cancer cells with ALK inhibitors in vitro to observe changes of HLA-I.

Results: ALK positivity was associated with low membrane PD-L1 and HLA-I expression. However, these two indicators were not associated with the prognosis of patients with stage I–IIIa NSCLC. Inhibition of ALK could upregulate HLA-I membrane expression to a certain extent.

Conclusion: Patients with ALK fusion showed downregulation of PD-L1 and HLA-I expression on the tumor cell membrane. Inhibition of ALK and its downstream signaling pathway can reverse it. These results suggest that the appropriate combination therapy should be considered for patients with ALK fusion and using targeted therapy at the proper time may increase patient benefits.

KEYWORDS
ALK, human lymphocyte antigen class I, immunotherapy, NSCLC, programmed death-ligand 1

INTRODUCTION

Several clinical trials have shown that patients with driver mutations do not respond well to immunotherapy, regardless of the level of programmed cell death 1 ligand 1 (PD-L1) expression.[1–3] So far, few studies have provided a full explanation. First, many researchers have explored the influence of anaplastic lymphoma kinase (ALK) fusion on PD-L1 expression. According to the results of a preclinical study,[4] the echinoderm microtubule associated protein like 4 (EML4-ALK) fusion protein induces the synthesis of PD-L1 through a series of downstream pathways, including mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3 kinase (PI3K)-protein kinase B (Akt) signaling. By reducing the phosphorylation of proteins in related signaling pathways, ALK inhibitors can reduce the expression of PD-L1 and, to some extent, restore antitumor T cell function. However, some clinical studies have

demonstrated the opposite effect. A meta-analysis[5] integrated the results of several clinical studies. Most of these studies have showed that PD-L1 expression is reduced in patients with ALK fusion. However, a significant shortcoming of previous studies is the small sample size of patients with ALK fusion. Some researchers have also explored the effect of PD-L1 alterations on CD8+ T cell function. Recently, a study collected 33 ALK-positive lung cancer samples,[6] and the results of immunohistochemistry showed that PD-L1 expression in these patients was very low. Based on this result, the researchers further found that although the number of tumor-infiltrating CD8 + T cells was not reduced in patients with ALK fusion, their ability to kill the tumor cells was significantly inhibited. These results suggest that ALK mutations in tumor cells may also affect the expression of other molecules in addition to PD-L1 and promotes immune escape.

To further investigation of changes downstream of ALK fusion in tumor cells, we selected another key molecule that affects the interaction between tumor cells and immune cells, the major histocompatibility complex class I (HLA-I). HLA-I plays a crucial role in the process of tumor neoantigen presentation and CD8 + T cells activation. Previous studies have confirmed that most tumor cells can downregulate the expression of HLA-I on the cell surface through various mechanisms, thus promoting immune escape.[7] Furthermore, lack of HLA-I expression is also one of the reasons for the poor efficacy of immunotherapy.[8] According to some studies,[9,10] oncogenes and activation of related pathways have been identified as the important mechanisms leading to the downregulation of HLA-I expression. For example, epidermal growth factor receptor (EGFR), the most commonly mutated protein in lung cancer, can reduce the membrane expression of HLA-I through the MAPK pathway.[11] The reduction of HLA-I caused by oncogenes is usually reversible, and the expression and function of HLA-I can be restored to a certain extent by restraining oncogenes and their downstream molecules.[12]

In our study, we collected surgical samples from 140 patients with lung adenocarcinoma, including 70 patients with ALK fusion. We assessed the expression of PD-L1/HLA-I and the number of tumor-infiltrating CD8+T cells by immunohistochemical staining. The influence of these factors on the prognosis of patients with early-stage non-small cell lung cancer (NSCLC) was analyzed in combination with the patients' follow-up data. We also conducted a series of cell experiments to determine whether the inhibition of ALK can alter the expression of PD-L1/HLA-I in a manner that is therapeutically desirable.

METHODS

Patients and samples

Patients diagnosed with stage I–IIIa lung adenocarcinoma who underwent surgical resection between July 2016 and December 2019 at the Tianjin Medical University Cancer Hospital were included in this analysis. The patients did not receive neoadjuvant therapy nor chemotherapy before surgery. All samples were evaluated by a pathologist at our hospital. ALK status was determined using Ventana ALK-D5F3 immunohistochemistry (IHC). EGFR status was determined by genetic testing. A total of 140 patients underwent tissue biopsy and met our inclusion criteria. All patients received adequate follow-up until October 2021. Of these patients, 70 were ALK-positive. The remaining 70 patients were negative for EGFR, kirsten rat sarcoma viral oncogene (KRAS), and ALK. Overall survival (OS) was defined as the period from surgery to death or to the time of last follow-up. Disease-free survival (DFS) was defined as the period from surgery to recurrence.

Immunohistochemical staining

Primary antibodies for IHC included those against HLA-I (1:1000, clone#EMR8-5; ab70328, Abcam), PD-L1 (1:10000, clone#2B11D11; 66 248-1-Ig, Proteintech), and CD8 (1:10000, clone#1G2B10; 66 868-1-Ig, Proteintech). Paraffin-embedded sections were deparaffinized in xylene and rehydrated in anhydrous ethanol at different concentrations. They were then subjected to microwave antigen retrieval in citrate buffer for 15 min and cooled to room temperature. After blocking endogenous peroxidase activity with 3% hydrogen peroxide for 20 min, the slides were incubated with primary antibodies for 12 h at 4°C. Next, the slides were cleaned with phosphate-buffered saline (PBS) solution and incubated with horseradish peroxidase (HRP)-linked secondary antibodies. Immunoreactivity was visualized using diaminobenzidine. Slides stained without primary antibody were used as negative controls.

Quantification

Two pathologists who were unaware of the status of the driver gene mutations independently evaluated all the immunohistochemical images. In this study, the H-score was used to assess membrane HLA-I/PD-L1 expression in tumor cells. According to the staining intensities on the surface of tumor cells from light to dark brown, the expression levels were classified as follows: 0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining. The proportion of all tumor cells that expressed HLA-I or PD-L1 was determined and then multiplied by the staining intensity score. The scores range from 0 to 300. Based on the median score, an HLA-I expression score <50.0 was considered low expression, and a score ≥50.0 was considered high expression. According to references using the same antibody,[13] PD-L1 expression score <100 was considered low expression group while ≥100 was considered high expression group. Five fields were selected for each section, and the average values were calculated.

TABLE 1 Correlation between ALK status and clinical characteristics of patients

Variable	Total ($n = 140$)	ALK positive-n (%)	ALK negative-n (%)	p-value
Gender - n (%)				
Male	67 (47.9%)	36 (51.4%)	31 (44.3%)	0.499
Female	73 (52.1%)	34 (49.6%)	39 (55.7%)	
Age at diagnosis - n (%)				
<60	74 (52.6%)	37 (52.9%)	37 (52.9%)	1
≥60	66 (47.4%)	33 (47.1%)	33 (47.1%)	
ECOG - n (%)				
0–1	111 (79.3%)	54 (77.1%)	57 (81.4%)	0.677
2–4	29 (20.7%)	16 (22.9%)	13 (18.6%)	
Smoking status - n (%)				
Current or former smoker	59 (42.1%)	23 (32.6%)	36 (51.4%)	**0.04**
Never-smoker	81 (57.9%)	47 (67.4%)	34 (48.6%)	
T stage - n (%)				
T1	103 (73.6%)	52 (74.3%)	51 (72.6%)	1
T2-4	37 (26.3%)	18 (25.7%)	19 (27.4%)	
Lymph node metastasis - n (%)				
Negative	99 (70.7%)	47 (67.1%)	52 (74.3%)	0.458
Positive	41 (29.3%)	23 (32.9%)	18 (25.7%)	
Pathological stage - n (%)				
I–II	125 (89.3%)	64 (91.4%)	61 (87.1%)	0.586
III	15 (10.7%)	6 (8.6%)	9 (12.9%)	
HLA-I surface expression - n (%)				
High (≥50)	66 (47.1%)	22 (31.4%)	44 (62.9%)	**$p < 0.001$**
Low (<50)	74 (52.9%)	48 (68.6%)	26 (37.1%)	
PD-L1 expression - n (%)				
High (≥100)	34 (24.3%)	7 (10.0%)	27 (38.6%)	**$p < 0.001$**
Low (<100)	106 (76.7%)	63 (90.0%)	43 (61.4%)	

The number of CD8+ tumor-infiltrating lymphocytes (TILs) was recorded at an magnification of 200. Five scanned fields of tumor regions were randomly chosen for each CD8+ TIL count. The density of TILs in the tumor was calculated by dividing the number of TILs by the sum of the area (mm^2) of the viewed fields.[14] TILs were defined as cells positive for CD8, regardless of the staining intensity.

Cell lines and cell culture

All cell lines were purchased from the American Type Culture Collection. H3122 and H2228 cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS). Immortalized human lung bronchial epithelial cells Beas-2B were cultured in specialized medium supplemented with 10% fetal bovine serum. EML4-ALK (V1) was obtained from the Hanbio Corporation. The gene sequence of EML4-ALK (V1) was confirmed by PCR-based sequencing. All cells were maintained in a humidified incubator at 37°C with 5% CO_2.

Western blot

The cells used in the experiment were cultured in 60 mm dishes. RIPA lysis buffer (Thermo Scientific) combined with PMSF Protease Inhibitor (Thermo Scientific) and phosphatase inhibitors (Roche) was used to lyse cells. Protein concentration was determined using a Pierce BCA Protein Assay Kit (Thermo Scientific). The same amount of protein was loaded in each lane for SDS-PAGE. Proteins were then transferred to a PVDF membrane using a transfer system (Bio-Rad) and blocked with protein free rapid blocking buffer (Epizyme). The membranes were then incubated with primary antibodies against β-tubulin, ALK (D5F3), phospho-ALK(Try1604), p44/42MAPK (ERK1/2), and phospho-p44/42MAPK (ERK1/2) (Thr202/Tyr204) overnight at 4°C. All the above-mentioned antibodies were purchased from Cell Signaling Technology. After three washes with TBST buffer, the membrane was further incubated with HRP-conjugated goat anti-rabbit secondary antibodies (1:3000, Zsbio) at room temperature for 1 h.
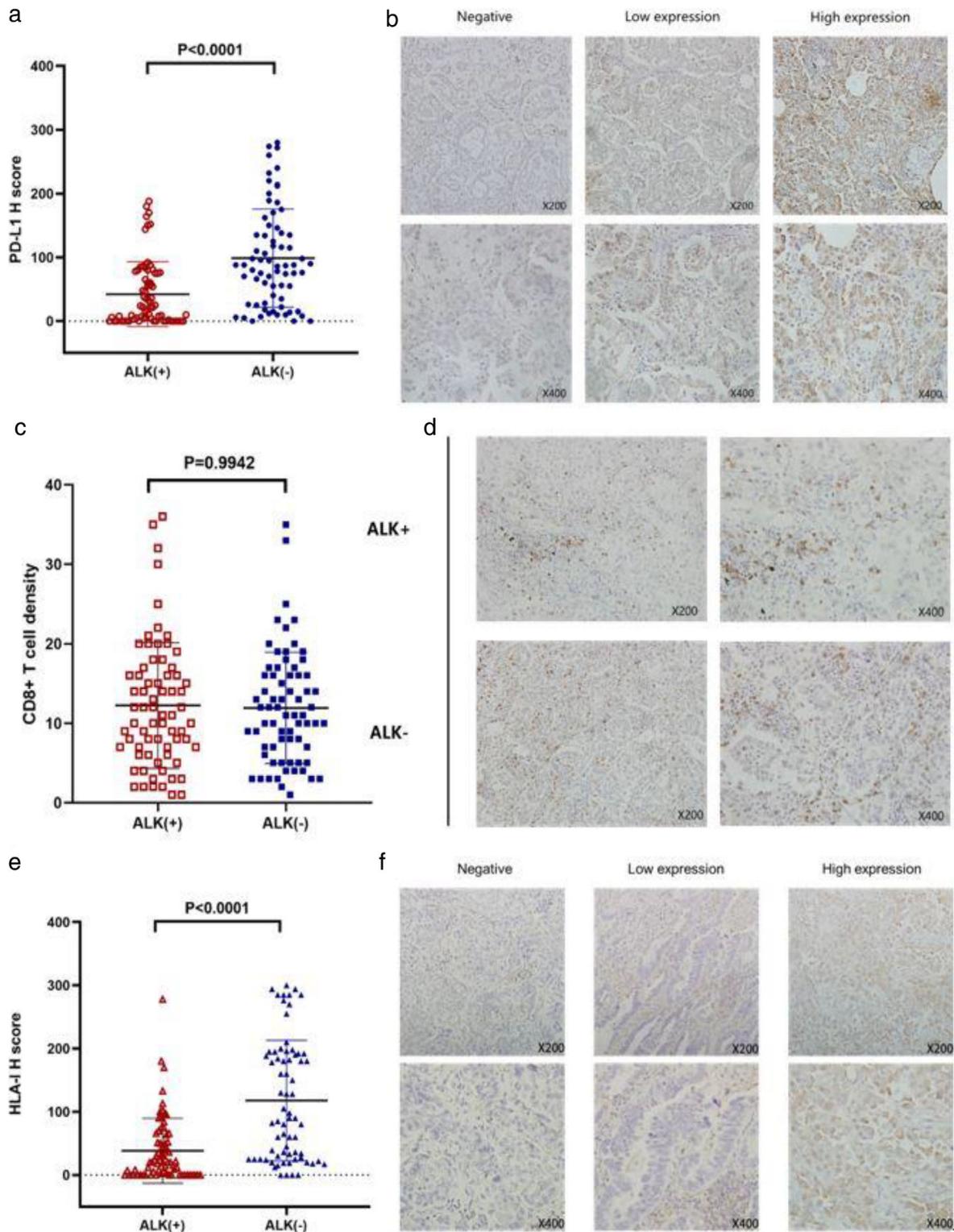
FIGURE 1 Association of ALK fusion with PD-L1/HLA-I expression and the number of tumor-infiltrating CD8 + T cells. The results of IHC showed that (a,b) PD-L1 membrane expression was reduced in patients with ALK fusion, as was HLA-I (e,f). On the other hand, there was no statistical difference in the number of CD8+T cells (c,d)

ALK inhibitor treatment assay

H3122 and H2228 cells were starved without serum for 2 h and treated with the following inhibitors at different doses:

ALK inhibitors (TAE684, crizotinib, and alectinib) and ERK1/2 inhibitor (SCH772984), which were all purchased from Selleckchem.

T A B L E 2 Univariate analysis of the relationship between clinical and survival in patients with lung adenocarcinoma

Variable	n (%)	Univariate analysis			
		Median DFS	*p*-value	Median OS	*p*-value
Gender - n (%)					
Male	67 (47.9%)	55	0.2119	55	0.5934
Female	73 (52.1%)	57		56	
Age at diagnosis - n (%)					
<60	74 (52.6%)	49	**0.0032**	50	**0.0015**
≥60	66 (47.4%)	55		55	
ECOG - n (%)					
0–1	111 (79.3%)	55	0.1978	62	0.1372
2–4	29 (20.7%)	49		46	
Smoking status - n (%)					
Current or former smoker	59 (42.1%)	55	0.5781	55	0.3549
Never-smoker	81 (57.9%)	58		59	
T stage - n (%)					
T1	103 (73.6%)	55	0.545	55	0.3184
T2-4	37 (26.3%)	62		62	
Lymph node metastasis - n (%)					
Negative	99 (70.7%)	55	0.1922	55	0.1838
Positive	41 (29.3%)	62		62	
Pathological stage - n (%)					
I–II	125 (89.3%)	55	0.5442	55	0.9771
III–IV	15 (10.7%)	62		62	
HLA-I surface expression - n (%)					
High (≥50)	66 (47.1%)	62	0.2655	62	0.459
Low (<50)	74 (52.9%)	51		51	
PD-L1 expression - n (%)					
High (≥100)	34 (24.3%)	51	0.3919	51	0.444
Low (<100)	106 (76.7%)	55		62	
ALK fusion - n (%)					
Negative	70 (50.0%)	62	0.3477	62	0.2184
Positive	70 (50.0%)	55		62	

Flow cytometry

Cells (8×10^5) were seeded in a 60 mm dish 1 day before treatment. After 72 h of treatment, cells were harvested, washed with PBS, and incubated at room temperature with appropriate fluorophore-conjugated antibodies for 30 min. Live cells were analyzed on a BD FACScan flow cytometer, and the data were processed with the FlowJo software.

Statistical analysis

Clinical characteristics of the patients with or without ALK fusion were analyzed by one-way analysis of variance (ANOVA) and Chi-square tests. Comparison of PD-L1 and HLA-I results between the groups was performed using the Kruskal-Wallis test. Kaplan–Meier survival curves and log-rank tests were performed to analyze the survival data.

Univariate analyses of associations between continuous variables and survival outcomes were performed using a Cox proportional hazards model. Statistical analyses were performed using the SPSS v25 and GraphPad Prism 8 software. $p < 0.05$ was considered statistically significant. *p*-values are reported as follows* for $p \leq 0.05$,** for $p \leq 0.01$, *** for $p \leq 0.001$, and ****for $p \leq 0.0001$. No symbol indicates that there is no statistical significance ($p > 0.05$).

RESULTS

Correlations between ALK expression and clinicopathological characteristics

The clinical features of the 140 patients with NSCLC are summarized in Table 1. We divided the patients into two groups according to the status of ALK fusion mutations, of
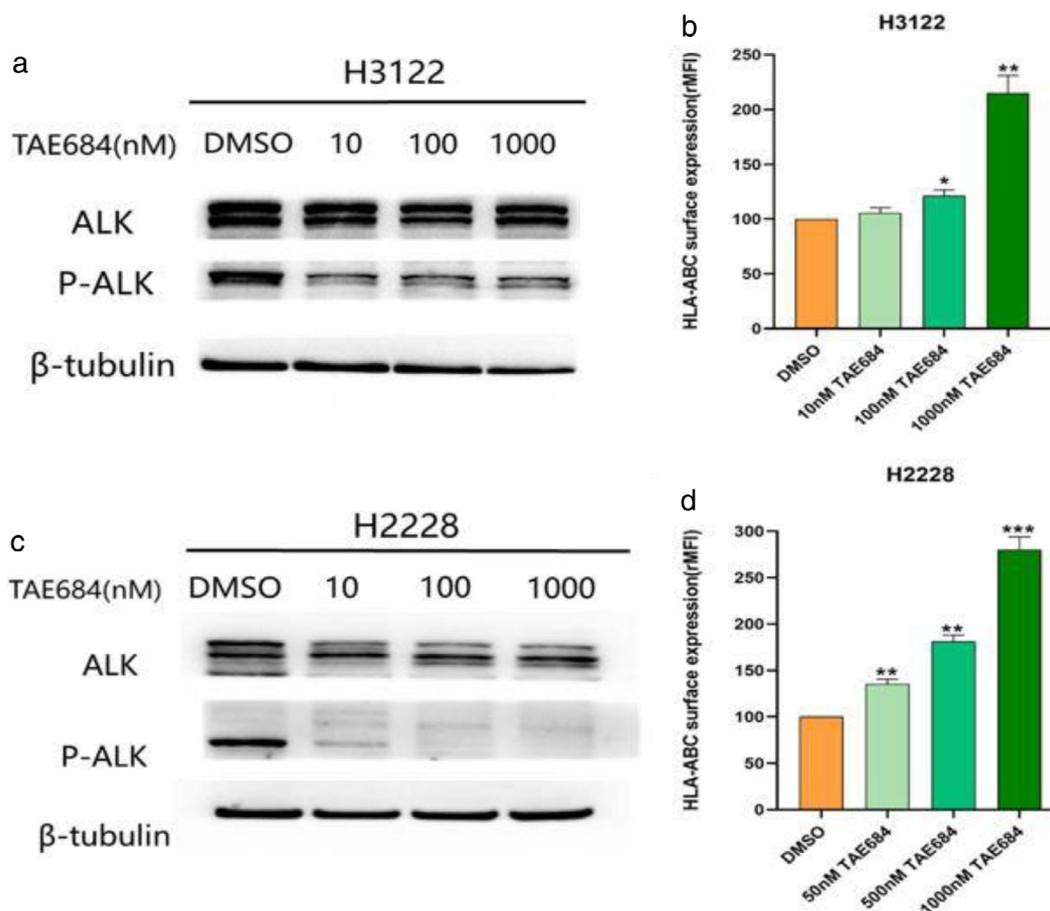
FIGURE 2 ALK inhibition upregulates HLA-I expression level in human NSCLC cells lines. (a,c) H3122 and H2228 cells were treated with ALK inhibitor TAE684 and collected after 4 h for western blot. (b,d) HLA-I surface levels were measured by flow cytometry after 72 h of treatment

which 70 patients were ALK-positive and 70 patients were ALK-negative. Among all patients, ALK expression was not associated with gender, age, pathological grade, nor lymph node metastasis but was related to smoking history ($p = 0.02$). Among the patients positive for ALK fusion, 47 (67.4%) had no smoking history while the proportions of patients without ALK fusion who were non-smokers or smokers were similar (48.6% and 51.4%, respectively).

We initially detected the expression of PD-L1 in tumor samples from these patients. The results of immunohisto-chemistry showed that the membrane expression level of PD-L1 was significantly correlated with ALK mutation ($p < 0.001$; Figure 1a,b). Low PD-L1 expression was more frequently observed in patients with ALK fusion than in those without ALK fusion. This result is in line with the results of previous studies.[6] Based on this, we also considered that changes in PD-L1 could affect tumor-infiltrating T cells and examined the number of CD8+ T cells by IHC. However, as shown in Figure 1c,d, we found that in these samples the number of CD8+ T cells was not significantly different between patients without or with ALK rearrangements ($p = 0.9942$).

Subsequently, HLA-I expression in these patients was evaluated, and we found that it was also associated with ALK mutation ($p < 0.001$; Figure 1e,f). Patients with ALK

mutations tended to have a lower expression of HLA-I on the surfaces of tumor cells. This result might explain why that while the number of CD8+ T cells is not reduced their antitumor function is impaired and why ALK mutations affect the efficacy of immunotherapy in patients with ALK.

On the other hand, Kaplan–Meier survival analysis showed that ALK fusion and PD-L1/HLA-I expression did not affect prognosis in these patients (Table 2). Age at diagnosis was the only prognostic factor associated with both DFS ($p = 0.032$) and OS ($p = 0.015$). Patients younger than 60 years of age at diagnosis had significantly shorter OS and DFS. This result may be due to the fact that most of the samples in this study were from early-stage patients.

ALK inhibition upregulates HLA-I expression

Integrating the results of previous studies with our IHC results, we believe that the reduction of HLA-I expression may be one of the reasons why patients with ALK fusion have an impaired antitumor immune response. Considering that the HLA-I downregulation caused by genetic alteration can be reversed by the inhibition of targeted agents, we next selected some commonly used inhibitors to block ALK
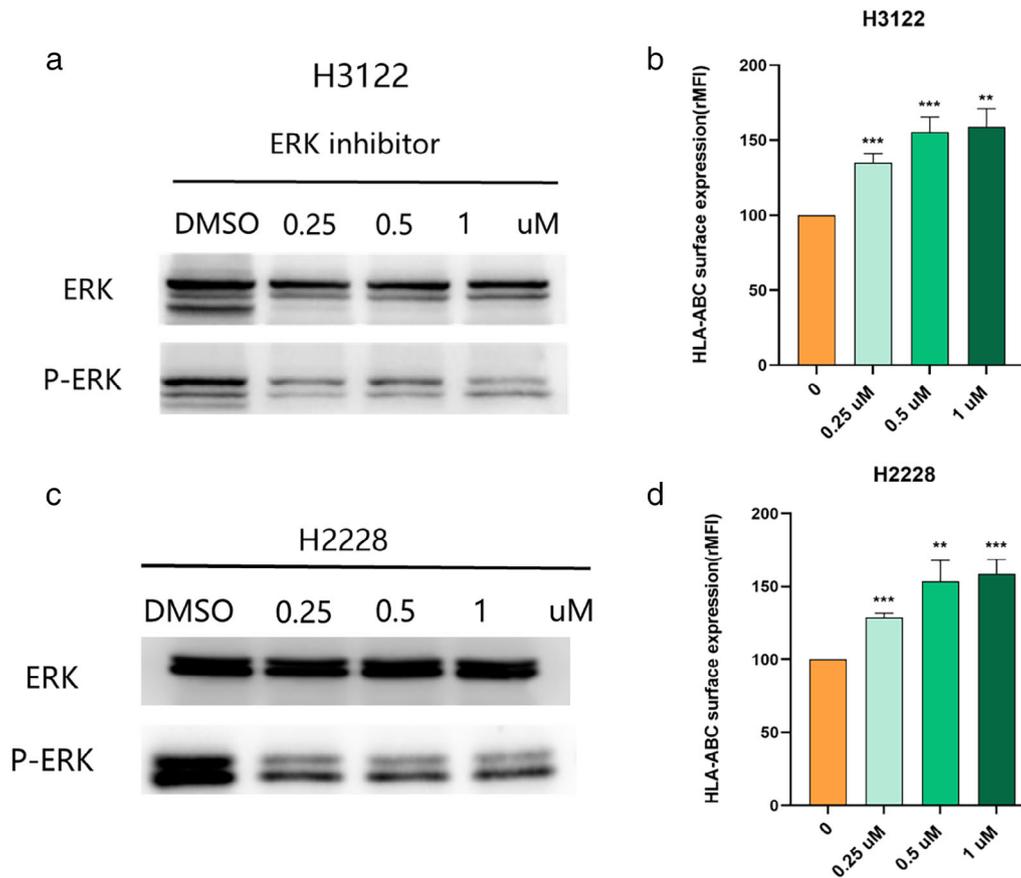
FIGURE 3 ERK inhibition also enhances HLA-I expression level in human NSCLC cells lines. The duration of inhibitor treatment and experimental methods were the same as before. ERK is the loading control [Correction added on 16 March 2022, figure 3 has been replaced.]

activity in vitro to determine whether we could restore HLA-I expression to a certain degree.

TAE684 is an ALK inhibitor that is widely used in cell experiments.[15] It inhibits the phosphorylation of ALK fusion proteins and several downstream signaling proteins, including MAPK, which has been proven to be the main factor affecting HLA-I expression in previous experiments.[16]

Our results indicated that 4 h after the treatment of H3122 cells with different concentrations of TAE684, pALK decreased significantly (Figure 2a); after 48 h of incubation, flow cytometry analysis showed that HLA-I membrane expression was upregulated with increasing concentrations of TAE684 (Figure 2b). The same results were obtained for another ALK-positive lung cancer cell line, H2228 (Figure 2c,d). These results preliminarily demonstrate that inhibition of ALK and its downstream pathways can upregulate HLA-I in ALK-positive NSCLC cell lines.

We comprehensively compared the signaling pathways activated by ALK fusion[17] and the related regulatory pathways that dictate HLA-I expression.[7] In combination with previous research results showing HLA-I upregulation after ALK inhibition in ALK-positive anaplastic lymphomas (ALCLs),[16] we believe that the MAPK pathway plays a major role in the induction of HLA-I.

To further confirm that MAPK is the main pathway affecting HLA-I expression, we evaluated the effect of ERK

inhibitors on HLA-I. A small molecule kinase inhibitor of ERK1/2 called SCH772984 showed similar effects compared to ALK inhibitors. Treatment of H3122 and H2228 cells with SCH772984[18] for 4 h resulted in a significant reduction in pERK. Furthermore, 72 h of incubation also led to an increase in cell surface HLA-I levels (Figure 3). These results suggest that the inhibition of HLA-I expression by ALK activity is mediated by MAPK signaling.

ALK-tyrosine kinase inhibitors (TKIs) are associated with upregulation of HLA-I in NSCLC

To further investigate the possible role of ALK-MAPK signaling in regulating HLA-I expression, we treated H3122 and H2228 cells with the other two small molecule tyrosine kinase inhibitors the Food and Drug Administration (FDA) approved for the treatment of mutated ALK-positive NSCLC. Flow cytometric analysis of HLA-I levels after a 72 h incubation of H3122 cells with crizotinib or alectinib showed an inverse dose–response. Decreasing levels of pERK were associated with increased surface expression of HLA-I (Figure 4a,b). By contrast, H2228 cells were less sensitive to ALK-TKIs, but we still observed that high concentrations of crizotinib and alectinib could upregulate HLA-I on the cell surface (Figure 4c,d).
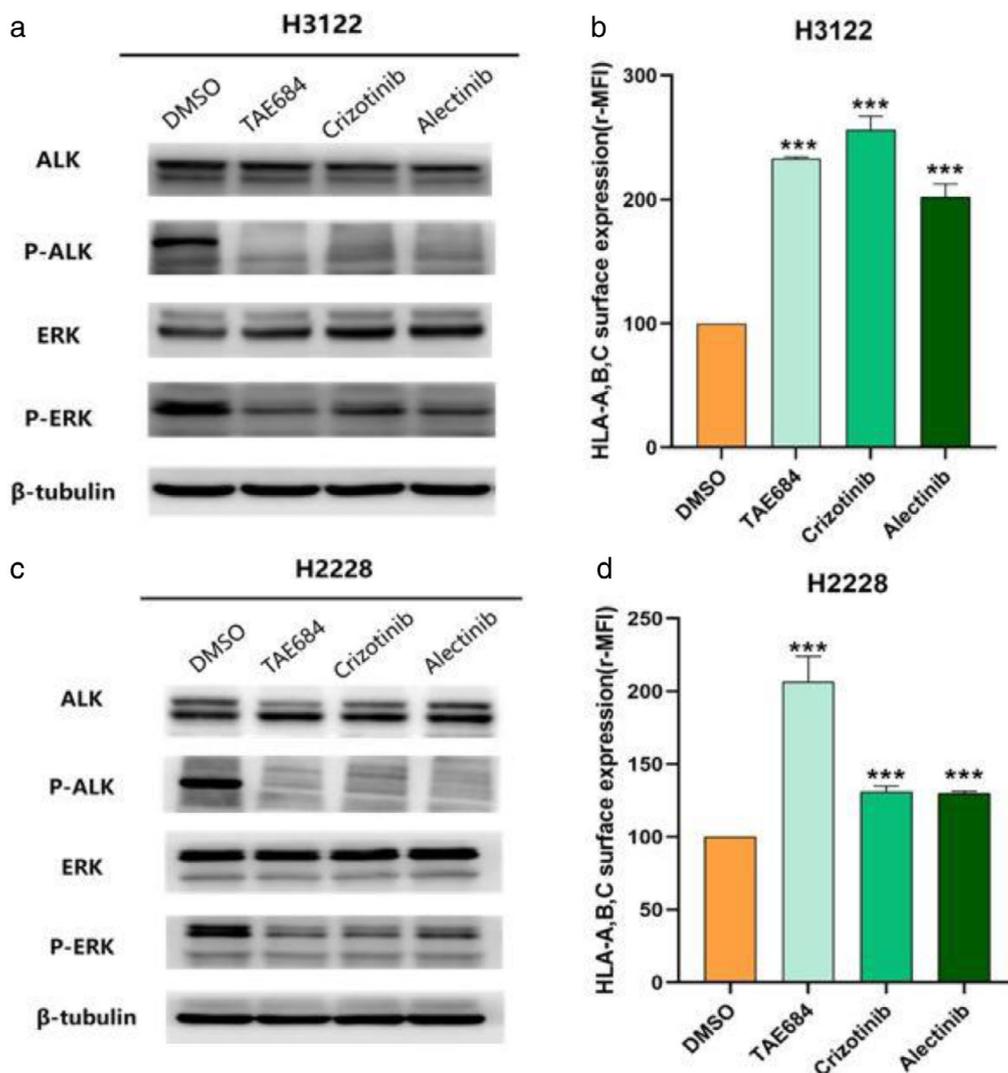
FIGURE 4 ALK TKIs led to upregulation of HLA -I in human NSCLC cells lines. H3122 cells were treated with TAE684, crizotinib or alectinib (1 μM) for the same time and their effects compared. (a) Proteins were collected 4 h after treatment to observe the changes in signaling pathways. (b) The membrane expression of HLA-I was observed after 72 h of incubation with inhibitors. (c,d) H2228 cells performed the same

Overexpression exogenous EML4-ALK changed HLA-I expression

A EML4-ALK (V1) overexpression lentiviral vector was constructed and used to transduce immortalized human lung Beas-2B cells. Consistent with our previous findings, we found that the expression of HLA-I was also influenced by exogenous EML4-ALK (V1), and the MAPK pathway was overactivated in comparison to control cells. These cells were also treated with ALK inhibitors, TAE684 and Crizotinib. After 4 h, proteins were extracted, and the changes in related molecules were detected; after 72 h, the membrane expression level of HLA-I was also detected. We observed a reduction in surface HLA-I levels in cells overexpressing the EML4-ALK (V1) fusion protein, and HLA-I was upregulated by ALK inhibition in these cells (Figure 5). This is consistent with our findings for tumor cell lines. Taken together, these results indicate that the inhibition of ALK can reverse HLA-I downregulation.

DISCUSSION

EML4-ALK fusion is one of the common driver mutations in NSCLC and is second only to mutations of *EGFR* or *KRAS* in incidence, and it is also an important target in the treatment of NSCLC. ALK fusion proteins are abnormally activated and regulate cell proliferation and apoptosis by activating MAPK, PI3K/AKT, signal transducer and activator of transcription 3 (STAT3), and other signaling pathways, which ultimately leads to the occurrence of lung cancer.[17] Previous studies have pointed out that the expression of various molecules in the tumor microenvironment, such as PD-L1 and HLA-I, is regulated by oncogenic mutations and eventually affects clinical outcomes.[1,2,8,19] Multiple
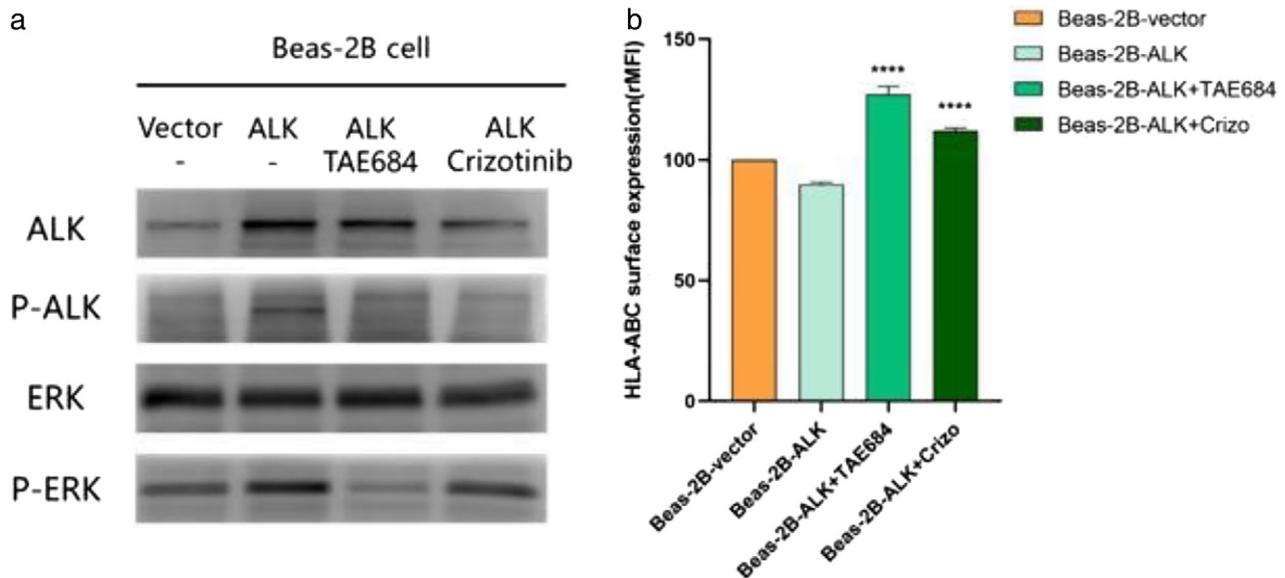
FIGURE 5 Overexpression of exogenous EML4–ALK changed HLA–I expression. (a) Exogenous EML4 - ALK (V1) was successfully expressed in Beas - 2B cells and activated the MAPK pathway. Similarly, these effects can be reduced by ALK inhibitors. (b) After 72 h treatment with ALK inhibitors TAE684 or crizotinib (1 μM), flow cytometry analysis showed that HLA -I expression levels were also upregulated

studies have confirmed that EML4–ALK can upregulate PD-L1 by activating the PI3K–AKT and MAPK signaling pathways in NSCLC.[12,19] However, this conclusion alone does not explain the patient's performance in clinical treatment. Some studies have analyzed the number of TILs in patients with ALK+ NSCLC and showed that these patients have fewer CD8+T cells while having more CD4+ T cells.[20] Another analysis based on patient samples showed that the function of tumor-infiltrating CD8+T cells in patients with ALK was also impaired.[6] These results cannot be explained by the changes in PD-L1 expression alone. The tumor microenvironment in ALK-positive NSCLC requires further analysis. We hope to find a new molecule that will lead to a breakthrough in the treatment of patients with ALK fusion.

On the other hand, HLA-I is also an important factor which plays a decisive role in tumor antigen presentation and the function of TILs. HLA-I downregulation has been found in several malignancies[21–23] and often correlates with poor response and prognosis.[8,24,25] Recent research has found strong links between the lack of HLA-I expression in tumor cells and immune evasion.[26,27] Tumors downregulate HLA-I expression to escape the recognition of the immune system. There are several mechanisms that reduce HLA-I expression or impair its function, including genetic, epigenetic, transcriptional, and post-transcriptional alterations that lead to irreversible or reversible changes in HLA-I expression. Among the numerous factors that influence HLA-I expression, activation oncogenic such as MAPK, EGFR, and human epidermal growth factor receptor 2 (HER2) signaling is one of the main reasons.[9,11,28] This oncogenic activity can be reversed by specific inhibitors. Inhibitors that target tyrosine kinase receptors, such as the anti-EGFR antibody nimotuzumab, increase the expression of HLA-I in different cancer cell lines as well as in patients with cancer.[12] This potential therapeutic effect is beneficial for patients, especially those with poor responses to immunotherapy.

Considering that MAPK signaling is a key pathway that can affect the transcription of HLA-I and is overactivated by ALK fusion, we believe that ALK can affect HLA-I expression through this pathway. This connection has been demonstrated in the ALCL.[16] Therefore, we focused on HLA-I as the key molecule of this study.

Through immunohistochemical analysis of lung cancer samples from patients, we identified that EML4–ALK and HLA-I expression in NSCLC were related. However, our samples were all from patients with early-stage resectable lung cancer. Whether there are other molecular characteristics and regulatory mechanisms in patients with advanced lung cancer still requires further study.

Our findings indicate that there is a correlation between ALK and HLA-I expression in NSCLC, and inhibition of ALK can significantly restore its expression level. And the effects of ALK on HLA-I are reversible. We may restore the expression of HLA-I to some extent through targeted therapy. This improvement may restore the function of tumor infiltrating lymphocytes thus providing benefits to the treatment of patients; it also provides the possibility of widespread use of immunotherapy and its combination with targeted therapy. The regulatory mechanisms and possible influencing factors of HLA-I expression in tumor cells still require further detailed studies.

ORCID

Di Mu https://orcid.org/0000-0002-7211-941X
Jingjing Guo https://orcid.org/0000-0002-8962-0980
Ying Han https://orcid.org/0000-0001-5181-5267

REFERENCES

1. Mazieres J, Drilon A, Lusque A, Mhanna L, Cortot AB, Mezquita L, et al. Immune checkpoint inhibitors for patients with advanced lung cancer and oncogenic driver alterations: results from the IMMUNOTARGET registry. Ann Oncol. 2019;30(8):1321–8. https://doi.org/10.1093/annonc/mdz167

2. Garassino MC, Cho BC, Kim JH, Mazières J, Vansteenkiste J, Lena H, et al. Durvalumab as third-line or later treatment for advanced non-small-cell lung cancer (ATLANTIC): an open-label, single-arm, phase 2 study. Lancet Oncol. 2018;19(4):521–36. https://doi.org/10.1016/S1470-2045(18)30144-X Epub 2018 Mar 12.

3. Garassino MC, Cho BC, Kim JH, Mazières J, Vansteenkiste J, Lena H, et al. Final overall survival and safety update for durvalumab in third- or later-line advanced NSCLC: the phase II ATLANTIC study. Lung Cancer. 2020;147:137–42. https://doi.org/10.1016/j.lungcan.2020.06.032

4. Hong S, Chen N, Fang W, Zhan J, Liu Q, Kang S, et al. Upregulation of PD-L1 by EML4-ALK fusion protein mediates the immune escape in ALK positive NSCLC: implication for optional anti-PD-1/PD-L1 immune therapy for ALK-TKIs sensitive and resistant NSCLC patients. Onco Targets Ther. 2015;5(3):e1094598. https://doi.org/10.1080/2162402X.2015.1094598

5. Zhang M, Li G, Wang Y, Wang Y, Zhao S, Haihong P, et al. PD-L1 expression in lung cancer and its correlation with driver mutations: a meta-analysis. Sci Rep. 2017;7(1):10255. https://doi.org/10.1038/s41598-017-10925-7

6. Zeng C, Gao Y, Xiong J, Lu J, Yang J, Wang X, et al. Tumor-infiltrating CD8$^+$ T cells in ALK-positive lung cancer are functionally impaired despite the absence of PD-L1 on tumor cells. Lung Cancer. 2020;150:139–44. https://doi.org/10.1016/j.lungcan.2020.10.009

7. Cornel AM, Mimpen IL, Nierkens S. MHC class I downregulation in cancer: underlying mechanisms and potential targets for cancer immunotherapy. Cancers (Basel). 2020;12(7):1760. https://doi.org/10.3390/cancers12071760

8. Gettinger S, Choi J, Hastings K, Truini A, Datar I, Sowell R, et al. Impaired HLA class I antigen processing and presentation as a mechanism of acquired resistance to immune checkpoint inhibitors in lung cancer. Cancer Discov. 2017;7(12):1420–35. https://doi.org/10.1158/2159-8290.CD-17-0593

9. Maruyama T, Mimura K, Sato E, Watanabe M, Mizukami Y, Kawaguchi Y, et al. Inverse correlation of HER2 with MHC class I expression on oesophageal squamous cell carcinoma. Br J Cancer. 2010;103(4):552–9. https://doi.org/10.1038/sj.bjc.6605772

10. Brea EJ, Oh CY, Manchado E, Budhu S, Gejman RS, Mo G, et al. Kinase regulation of human MHC class I molecule expression on cancer cells. Cancer Immunol Res. 2016;4(11):936–47. https://doi.org/10.1158/2326-6066.CIR-16-0177

11. Watanabe S, Hayashi H, Haratani K, Shimizu S, Tanizaki J, Sakai K, et al. Mutational activation of the epidermal growth factor receptor down-regulates major histocompatibility complex class I expression via the extracellular signal-regulated kinase in non-small cell lung cancer. Cancer Sci. 2019;110(1):52–60. https://doi.org/10.1111/cas.13860

12. Garrido G, Rabasa A, Garrido C, Chao L, Garrido F, García-Lora ÁM, et al. Upregulation of HLA class I expression on tumor cells by the anti-EGFR antibody Nimotuzumab. Front Pharmacol. 2017;8:595. https://doi.org/10.3389/fphar.2017.00595

13. Liu S, Qin T, Jia Y, Li K. PD-L1 expression is associated with VEGFA and LADC Patients' survival. Front Oncol. 2019;9:189. https://doi.org/10.3389/fonc.2019.00189

14. Haratani K, Hayashi H, Tanaka T, Kaneda H, Togashi Y, Sakai K, et al. Tumor immune microenvironment and nivolumab efficacy in EGFR mutation-positive non-small-cell lung cancer based on T790M status after disease progression during EGFR-TKI treatment. Ann Oncol. 2017;28(7):1532–9. https://doi.org/10.1093/annonc/mdx183

15. Galkin AV, Melnick JS, Kim S, Hood TL, Li N, Li L, et al. Identification of NVP-TAE684, a potent, selective, and efficacious inhibitor of NPM-ALK. Proc Natl Acad Sci U S A. 2007;104(1):270–5. https://doi.org/10.1073/pnas.0609412103. Erratum in: Proc Natl Acad Sci U S A. 2007; 104(6): 2025.

16. Oh CY, Klatt MG, Bourne C, Dao T, Dacek MM, Brea EJ, et al. ALK and RET inhibitors promote HLA class I antigen presentation and unmask new antigens within the tumor immunopeptidome. Cancer Immunol Res. 2019;7(12):1984–97. https://doi.org/10.1158/2326-6066.CIR-19-0056

17. Lin JJ, Riely GJ, Shaw AT. Targeting ALK: precision medicine takes on drug resistance. Cancer Discov. 2017;7(2):137–55. https://doi.org/10.1158/2159-8290.CD-16-1123

18. Morris EJ, Jha S, Restaino CR, Dayananth P, Zhu H, Cooper A, et al. Discovery of a novel ERK inhibitor with activity in models of acquired resistance to BRAF and MEK inhibitors. Cancer Discov. 2013;3(7):742–50. https://doi.org/10.1158/2159-8290.CD-13-0070

19. Ota K, Azuma K, Kawahara A, Hattori S, Iwama E, Tanizaki J, et al. Induction of PD-L1 expression by the EML4-ALK oncoprotein and downstream signaling pathways in non-small cell lung cancer. Clin Cancer Res. 2015;21(17):4014–21. https://doi.org/10.1158/1078-0432.CCR-15-0016

20. Jin R, Liu C, Zheng S, Wang X, Feng X, Li H, et al. Molecular heterogeneity of anti-PD-1/PD-L1 immunotherapy efficacy is correlated with tumor immune microenvironment in east Asian patients with non-small cell lung cancer. Cancer Biol Med. 2020;17(3):768–81. https://doi.org/10.20892/j.issn.2095-3941.2020.0121

21. Feenstra M, Veltkamp M, van Kuik J, Wiertsema S, Slootweg P, van den Tweel J, et al. HLA class I expression and chromosomal deletions at 6p and 15q in head and neck squamous cell carcinomas. Tissue Antigens. 1999;54(3):235–45. https://doi.org/10.1034/j.1399-0039.1999.540304.x

22. Romero JM, Jiménez P, Cabrera T, Cózar JM, Pedrinaci S, Tallada M, et al. Coordinated downregulation of the antigen presentation machinery and HLA class I/beta2-microglobulin complex is responsible for HLA-ABC loss in bladder cancer. Int J Cancer. 2005;113(4):605–10. https://doi.org/10.1002/ijc.20499

23. Garrido MA, Rodriguez T, Zinchenko S, Maleno I, Ruiz-Cabello F, Concha Á, et al. HLA class I alterations in breast carcinoma are associated with a high frequency of the loss of heterozygosity at chromosomes 6 and 15. Immunogenetics. 2018;70(10):647–59. https://doi.org/10.1007/s00251-018-1074-2

24. Meissner M, Reichert TE, Kunkel M, Gooding W, Whiteside TL, Ferrone S, et al. Defects in the human leukocyte antigen class I antigen processing machinery in head and neck squamous cell carcinoma: association with clinical outcome. Clin Cancer Res. 2005;11(7):2552–60. https://doi.org/10.1158/1078-0432.CCR-04-2146

25. Watson NF, Ramage JM, Madjd Z, Spendlove I, Ellis IO, Scholefield JH, et al. Immunosurveillance is active in colorectal cancer as downregulation but not complete loss of MHC class I expression correlates with a poor prognosis. Int J Cancer. 2006;118(1):6–10. https://doi.org/10.1002/ijc.21303

26. Burr ML, Sparbier CE, Chan KL, Chan YC, Kersbergen A, Lam EYN, et al. An evolutionarily conserved function of Polycomb silences the MHC class I antigen presentation pathway and enables immune evasion in cancer. Cancer Cell. 2019;36(4):385–401.e8. https://doi.org/10.1016/j.ccell.2019.08.008

27. Yamamoto K, Venida A, Yano J, Biancur DE, Kakiuchi M, Gupta S, et al. Autophagy promotes immune evasion of pancreatic cancer by degrading MHC-I. Nature. 2020;581(7806):100–5. https://doi.org/10.1038/s41586-020-2229-5

28. Lulli D, Carbone ML, Pastore S. The MEK inhibitors trametinib and cobimetinib induce a type I interferon response in human keratinocytes. Int J Mol Sci. 2017;18(10):2227. https://doi.org/10.3390/ijms18102227