The germline HLA-A02B62 supertype is associated with a PD-L1-positive tumour immune microenvironment and poor prognosis in stage I lung cancer

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A B S T R A C T

Background: Germline HLA class I molecule supertypes are shown to correlate with response to anti-PD-1 therapy. Here, we investigate the significance of germline HLA-A and HLA-B supertypes in tumour microenvironment of non-small-cell lung cancer.
Methods: Totally 278 NSCLC patients were collected retrospectively. HLA genotyping was conducted using next-generation sequencing. The evaluation of tumour-infiltrating lymphocytes was performed by multiplex immunohistochemistry assay. Correlations among HLA supertypes, tumour infiltrating lymphocytes, and clinicopathological characteristics were assessed.
Results: HLA-A03 and HLA-B62 were the supertypes with the highest proportions, at 69.1% and 52.2%, respectively. HLA-A02 or HLA-B62, but not HLA-A03, associated with higher PD-L1$^+$ tumour and stromal cells levels, CD68$^+$ cells, and CD68$^+$PD-L1$^+$ cells. Patients with both HLA-A02 and HLA-B62 supertypes displayed significantly higher PD-L1$^+$ cells, CD68$^+$ cells, and CD8$^+$ cells levels than patients with other supertypes ($P = 0.0301$, $P = 0.0479$, $P = 0.0192$). These cells collectively constitute a hot but immunosuppressive tumour microenvironment. Accordingly, patients with both HLA-A02 and HLA-B62 supertypes had short progression-free survival after surgery (HR = 2.27, $P = 0.0373$).
Conclusions: The HLA-A02B62 supertype could serve as a possible indicator of poor prognosis in early-stage lung cancer. However, it may also act as a favorable prognostic factor for immunotherapy, given its association with a PD-L1-positive tumour microenvironment.

1. Introduction

Worldwide, lung cancer exhibits the highest rates of incidence and mortality rates among all types of cancer. Non-small-cell lung cancer (NSCLC) comprises approximately 85% of all lung cancer diagnosis, making it the most common variant. Notably, immunotherapies, particularly those targeting the PD-1/PD-L1 signaling pathway, have demonstrated sustained clinical efficacy and survival benefits in NSCLC [1]. Nevertheless, the benefits of these therapies remain limited to a subset of patients, especially when

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administrated as monotherapy. The identification of clinically relevant biomarkers capable of predicting response to immunotherapy could enable the early identification of individuals who are likely to respond positively and facilitate the timely intervention with treatment strategies.

Currently, the patient selection principle for PD-1/PD-L1 targeted therapy in NSCLC predominantly rely on the assessment of PD-L1 expression in tumour tissue [2,3]. Besides PD-L1, several biomarkers have been used to indicate efficacies of PD-1/PD-L1 targeted therapy, such as microsatellite instability (MSI), tumour mutational burden (TMB), tumour-infiltrating lymphocytes (Tils) and human leukocyte antigen (HLA) supertype [4–12]. However, there is a scarcity of HLA-related studies compared to other biomarkers. Recently, Chowell et al. has shown that patients with a HLA-B44 supertype exhibited prolonged survival, in contrast, those with a HLA-B62 supertype has gained poor clinical benefits after PD-1 or CTLA-4 targeted therapy in melanoma [4]. Additionally, Correale et al. has shown that HLA-A01-positive patients demonstrated extended progression-free survival (PFS) and overall survival (OS) after PD-1/PD-L1 targeted therapy [13]. These studies suggest that the identification of class I HLA allele holds significant implications to predict the response of immunotherapy.

The proficient presentation of tumour antigens through HLA class I (HLA-I) is crucial for $CD8^+$ T-cell-mediated elimination of tumours. However, the impact of HLA-I genotypes on the antitumour immune response in early-stage lung cancer remains unclear. Here, we firstly assessed the HLA-I genotype in 278 stage-I NSCLC patients who underwent radical surgery. Subsequently, we assessed the presence of $PD-L1^+$, $CD68^+$, $CD8^+$, and $PD-1^+$ Tils in tumour tissue samples and explored the correlations between HLA supertypes, Tils, and clinicopathological characteristics. Our findings revealed significant associations between the germline HLA-A02B62 supertype and the presence of PD-L1-positive immunosuppressive tumour microenvironment and poor prognosis in stage I lung cancer.

2. Materials and methods

2.1. Patients and samples

We enrolled 278 stage I NSCLC patients that underwent radical surgery at The First Hospital of Lanzhou University between November 2018 and November 2021. This study included patients that ≥18 years old and had a confirmed stage I NSCLC. All participants underwent radical surgery as their primary treatment. To ensure data quality and accuracy, patients with histories of other malignancies or incomplete medical records/missing data were excluded. Additionally, patients with stage II-IV disease or those for whom second-generation sequencing could not be conducted due to insufficient sample quantity were also excluded from the study. Relevant clinical and pathological information, including age, sex, smoking status, tumour pathological type, clinical stage, and therapy, were collected. Peripheral blood leukocytes were collected to perform HLA genotyping using next-generation sequencing. Paraffin-embedded tumour tissues from patients were obtained for Til infiltration evaluation by multiplex immunohistochemistry assays. The tissue blocks were stored at −80 °C before use. This study was carried out following the approval of the ethics committees at The First Hospital of Lanzhou University (ID: LDYYLL2022-477), and each patient signed written informed consent.

2.2. DNA extraction and targeted next-generation sequencing

Each patient provided a blood sample of 2 ml for the purpose of DNA extraction., and we used TGuide S32 Magnetic Blood Genomic DNA Kit (TIANGEN, China, DP348-02) to extract peripheral blood lymphocyte DNA, enabling HLA genotyping. The DNA libraries were established using the custom-designed Genescope panel (Genecast, Beijing, China), covering a genomic region of 1.7 Mb, including HLA-A, HLA-B, and HLA-C genes. The collected samples underwent pair-end sequencing in Illumina NovaSeq 6000 platform (California, America). The average sequencing depth of blood cells was 240 × . Following data quality control by Trimmomatic (v0.36) [14], reference mapping to the hg19 genomic assembly was performed using the BWA aligner (v0.7.17) [15], and duplicated reads were masked using Picard (v2.23.0). Processed BAM files were generated and used for subsequent analysis. The processed BAM files were imported into VarDict (v1.5.1) [16]/FreeBayes (v1.2.0) software for the identification of SNV, INDEL and complex mutation calling. All variants were annotated using ANNOVAR [17] and filtered based on the following criteria: 1) VAF ≥2%, total support reads ≥6, and absence of strand bias; 2) variants located in exonic regions and annotated as nonsynonymous; 3) allele frequencies ≤0.002 in both databases of Exome Aggregation Consortium (ExAC) [18] and Genome Aggregation (gnomAD, MacArthur Lab).

2.3. Germline HLA-I genotyping

By identifing HLA-I alleles, reads within the HLA-I regions were extracted from BAM files derived from blood cell samples. Subsequently, these extracted reads were imported into HLA-HD software (v1.2.0.1) [19] using the following parameters: minimum tag size = 50 and rate of cutting = 0.95. The HLA-A/B alleles were then classified based on the established supertype definition, as previously described [20].

2.4. Immunohistochemical staining

In this study, two tissue sections were employed for each case. Section 1 underwent traditional DAB staining targeting PD-L1. Section 2 was subjected to multiple fluorescence co-staining, simultaneously detecting PD-L1, PD-1, CD68, and CD8. The data were confirmed by two pathologists, ensuring reliability and accuracy.

For conventional DAB immunohistochemical staining, paraffin-embedded tumour tissue samples were sectioned at 4 μm and

transferred onto coated glass slides. The analysis of PD-L1 expression was carried out according to the guidelines outlined in the Dako 22C3 kit (Cat. No SK006). To evaluate tumour PD-L1, we utilized the tumour proportion score (TPS), which represents the proportion of viable tumour cells displaying either partial or complete membrane staining. Based on the TPS, PD-L1 expression was categorized into three groups: PD-L1 High, determined when ≥50% tumour cell displayed staining, PD-L1 Low, determined when 1%–49% tumour cell displayed staining, and PD-L1 Negative, determined when <1% of tumour cell displayed staining; Additionally, we calculated the combined positive score (CPS) by dividing the counts of PD-L1-stained cells by the total number of viable tumour cells, and the results was multiplied by 100.

For the multiplex fluorescence immunohistochemical staining, 3 μm serial slices were obtained from paraffin-embedded tissue blocks. These slices were processed according to instructions Opal 7-Colour IHC Kit (NEL797B001KT, PerkinElmer) [21]. In this study, primary antibody information were as follow: anti-CD8 (#144 B, ab17147) and anti-PD-1 antibody (#CAL20, ab237728) from abcam, anti-CD68 antibody (#KP1, ZM-0060) from Zsbio, and anti-PD-L1 antibody from (#SP142) Ventana. To visualize tyramide signal amplification (TSA), we utilized DAPI, Opal 520, Opal 570, Opal 620 and Opal 690 labelling nucleus, CD8, CD68, PD-L1, and PD-1, respectively. The slices were then processed by a PerkinElmer Vectra system (Vectra 3.0.5, PerkinElmer) and multispectral images were separated utilizing by inForm Advanced Image Analysis software (inForm 2.3.0, PerkinElmer). The "tumour mask" feature of the inForm software was utilized to delineate the tumour compartment. The levels of each type of cells were assessed based on the ratio of positive cells to total cells within tumour compartment.

2.5. Statistical analyses

Statistical analysis was conducted by using SPSS (v22.0, SPSS, Inc., Chicago, IL, USA), R software (v3.5.1, http://www.r-project.org), and GraphPad Prism (v8.2.0, La Jolla, CA, USA). PFS was determined as the duration from initiation of therapy to the occurrence of disease progression, with 95% confidence intervals determined by Kaplan-Meier analysis. Comparison of two survival curves was conducted by log rank test. The chi-square test was employed to compare multiple rates or multiple constituent ratios between groups. Differences was analyzed by Mann-Whitney test between two groups and by Kruskal-Wallis test in multiple groups. All statistical tests were performed with a two-sided approach, and statistically significance was determined by a $P < 0.05$ was considered, unless stated otherwise.

3. Results

3.1. Patient characteristics

Totally 278 NSCLC subjects were enrolled in this retrospective study (Table 1). The median age of the cohort was 57 years (range 18–87). Among the patients, 62% were female (n = 173). There were few smokers, accounting for only 26% (n = 72). Lung adeno-carcinoma (ADC) accounted for 96% of the cases (n = 266). All patients were diagnosed with clinical stage I disease and underwent radical surgery as the initial treatment.

Patients with the HLA-A02 or B62 supertype show higher levels of PD-L1 expression in tumour microenvironment.

In this study, identification of HLA-A and HLA-B gene alleles and classification of supertypes were performed for all patients based on NGS. The analysis revealed that the most prevalent supertypes in terms of HLA-A and HLA-B genes were HLA-A03 and HLA-B62, accounting for 69.1% and 52.2%, respectively (Fig. 1a). To assess the infiltration of Tils in the tumour compartment among patients

Table 1
Patient characteristics.

Characteristic	NSCLC (n = 278)	
	n	%
Age (years)		
Median	57	
Range	18–87	
Sex		
Male	105	38%
Female	173	62%
Smoking history		
Smoker	72	26%
Never smoker	193	69%
Missing	13	5%
Pathological type		
ADC	266	96%
SQCC	12	4%
Clinical stage		
I	278	100%
Therapy		
Surgery	278	100%

ADC, adenocarcinoma. SQCC, squamous cell carcinoma.

with different HLA supertypes, IHC and mIHC detection were performed on tumour tissues and the content of PD-L1$^+$, CD68$^+$, CD68$^+$PD-L1$^+$, CD8$^+$, PD-1$^+$, and CD8$^+$PD-1$^+$ cells was compared. Representative images of the IHC and mIHC detection are shown in Fig. 1b and a landscape of the HLA supertypes and Til infiltration at the individual patient level is presented in Fig. 1c. Further analysis revealed elevated levels of PD-L1$^+$, CD68$^+$, and CD68$^+$PD-L1$^+$ cells in tumours from HLA-A02 supertype patients compared to other supertype patients ($P = 0.0419$, $P = 0.0092$, $P = 0.0285$) (Fig. 1e–g). Similar results were observed when comparing HLA-B62 supertype patients with other supertype patients, showing higher levels of PD-L1$^+$ tumour cells and CD68$^+$ cells in the tumours ($P = 0.0312$, $P = 0.0409$) (Fig. 1d and f). Although not statistically significantly, the infiltration levels of CD8$^+$, PD-1$^+$, and CD8$^+$PD-1$^+$ cells also appeared to be higher in the tumours of HLA-A02 or HLA-B62 supertype patients (Fig. 1h–j). However, no similar associations were found when comparing other supertypes (Figs. S1–S3). Collectively, these results suggest that the tumour microenvironment of HLA-A02 supertype or HLA-B62 supertype patients might exhibit a bias towards an immunosuppressive state.

3.2. Patients with both the HLA-A02 supertype and HLA-B62 supertype have poor postoperative prognosis

Survival analysis showed that patients with HLA-A02 supertype, who underwent surgical resection, had a shorter PFS compared to those in other supertype groups (HR = 2.00, $P = 0.0628$) (Fig. 2a). Similarly, patients with the HLA-B62 supertype exhibited comparable results, although without statistically significance (HR = 1.23, $P = 0.5893$) (Fig. 2b). Given the similarities in Til infiltration profiles and tendencies towards poorer prognosis in both HLA-A02 supertype and HLA-B62 supertype patients, and considering that a substantial proportion (33.1%) of patients had both the HLA-A02 supertype and HLA-B62 supertype (HLA-A02B62 supertype)
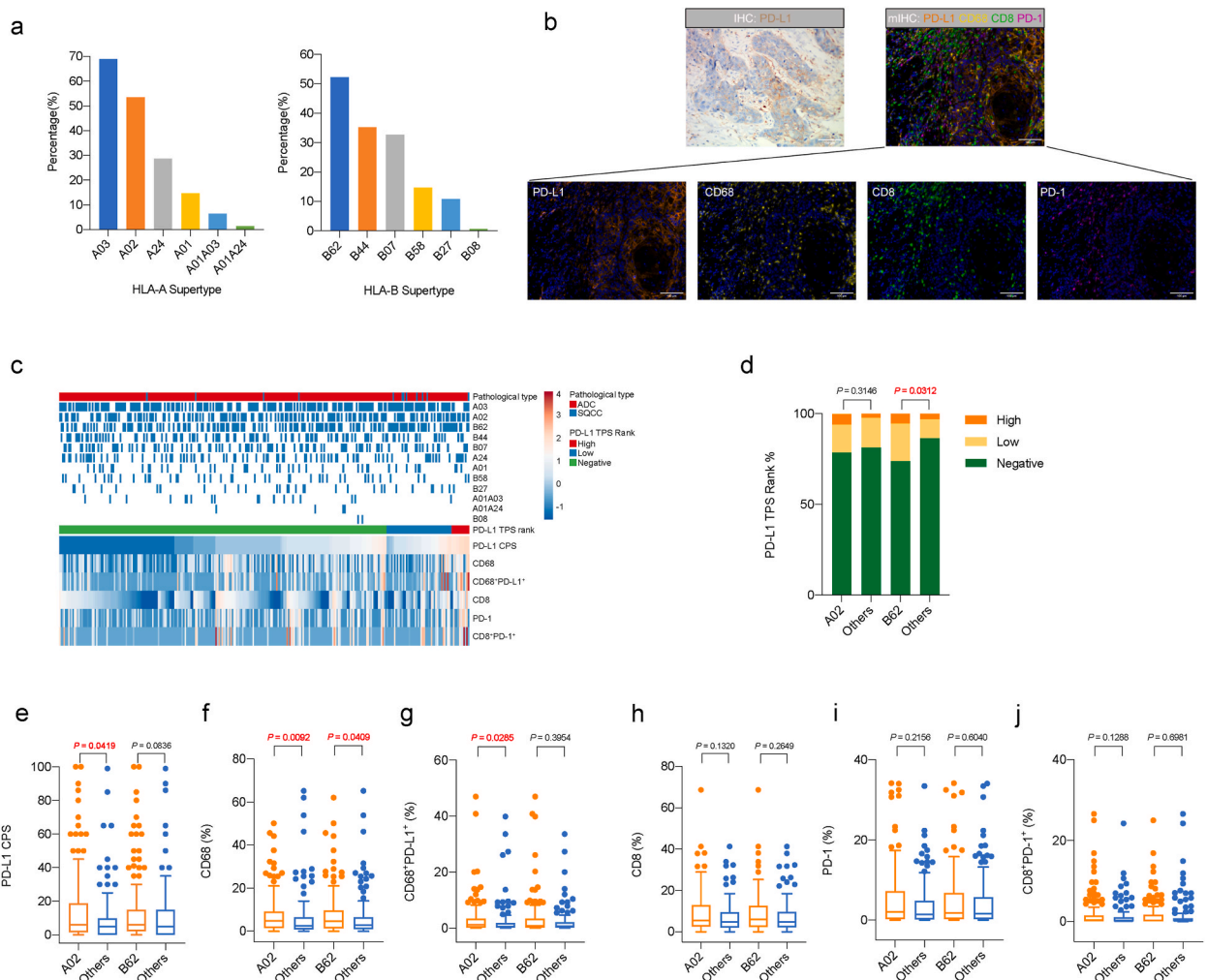

Fig. 1. Evaluation of the HLA supertype and related immune microenvironment.
(a) Frequency distribution of each HLA-A and HLA-B gene supertype. (b) Representative images of IHC (PD-L1) and mIHC (PD-L1, CD68, CD8, PD-1) detection in sections from a lung adenocarcinoma sample; Scale bar = 100 μm. (c) Landscape of HLA supertypes and Til infiltration. (d–j) Differences in PD-L1 TPS rank (d), PD-L1 CPS (e), CD68$^+$ cells (f), CD68$^+$PD-L1$^+$ cells (g), CD8$^+$ (h), PD-1$^+$ (i), and CD8$^+$PD-1$^+$ cells (j) between the HLA-A02 or HLA-B62 supertype group and the other supertype group. Chi-square test. Mann-Whitney U test.
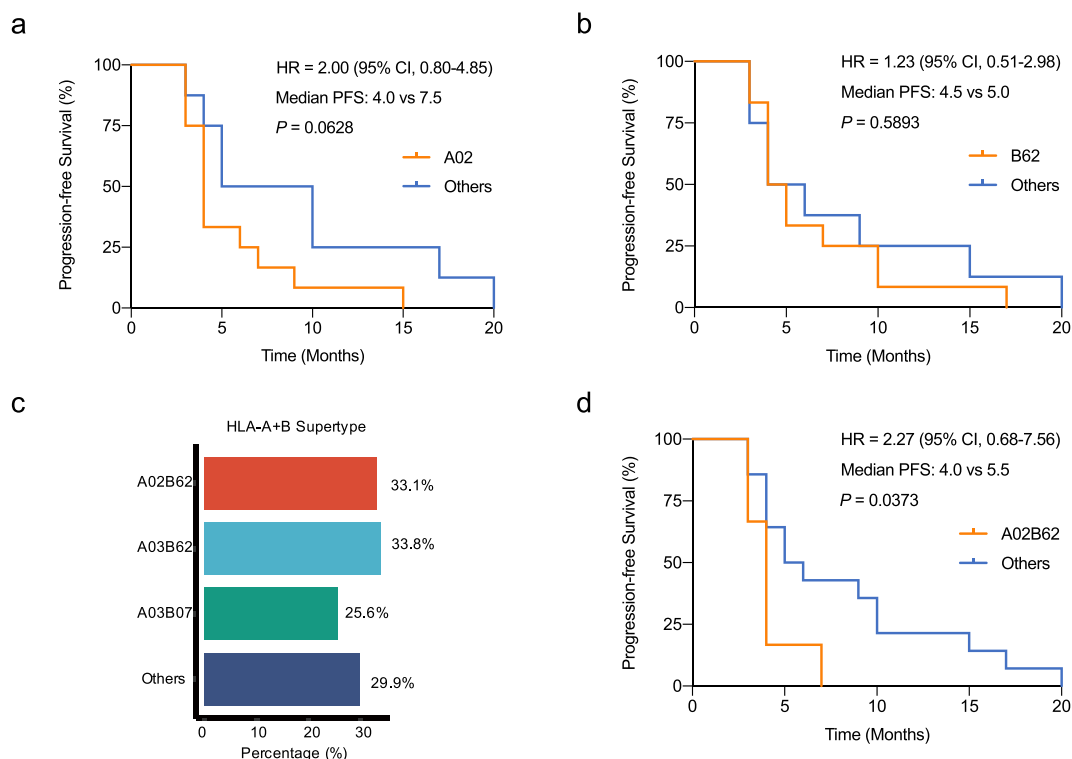
Fig. 2. HLA supertype-related survival analysis.
(a) Kaplan-Meier survival graph comparing differences in progression-free survival time after surgery between the HLA-A02 supertype group and the other supertype group. The log-rank test was performed. (b) Kaplan-Meier survival graph comparing differences in progression-free survival time after surgery between the HLA-B62 supertype group and the other supertype group. The log-rank test was performed. (c) The frequency of each supertype of HLA-A combined with HLA-B genes. (d) Kaplan-Meier survival graph comparing differences in progression-free survival time after surgery between the HLA-A02B62 supertype group and the other supertype group. The log-rank test was performed.

(Fig. 2c), we further analyzed the prognosis of patients with HLA-A02B62 supertype and found significantly shorter PFS in these patients compared to other supertypes (HR = 2.27, $P = 0.0373$) (Fig. 2d).

Furthermore, the analysis of the tumour microenvironment showed elevated levels of PD-L1^{+}, CD68^{+}, and CD8^{+} cells in tumours from patients with the HLA-A02B62 supertype compared to those with other supertypes ($P = 0.0301$, $P = 0.0479$, $P = 0.0192$) (Fig. 3b, c and 3e). Moreover, the infiltration levels of PD-L1^{+} tumour cells, CD8^{+}, PD-1^{+}, and CD8^{+}PD-1^{+} cells also seemed to be higher in the tumours of HLA-A02B62 supertype patients, though not statistically significant (Fig. 3a, d, 3f and 3 g). These findings suggest that the poor prognosis might be associated with the immunosuppressive effect caused by the presence of numerous PD-L1^{+} cells in tumour microenvironment.

The homogeneity/heterogeneity of alleles at HLA-A and HLA-B gene loci does not affect the level of Til infiltration.

As illustrated in Fig. 1b, each patient had at least 2 supertypes of the HLA-A gene and -B gene, with the majority of patients having 4 supertypes. However, some patients only had 2 supertypes due to homologous alleles at HLA-A and -B gene loci. Based on this distinction (heterologous or homologous alleles), we categorized all patients into 4 groups. PD-L1 expression and Til infiltration in the heterologous group did not show significant different compared to the homologous group (Fig. 4a). Intriguingly, even in the HLA-A02B62 supertype patients, who displayed higher PD-L1 expression and Til infiltration, the heterologous group did not significantly differ from the homologous group according to PD-L1 expression and Til infiltration (Fig. 4b). Collectively, these results suggest that specific HLA supertypes may hold more significance for the immune response process than the overall HLA supertype complexity, as exemplified by the analysis of HLA-A02B62 supertype in this study.

4. Discussion

Our study focused on early-stage lung cancer, aiming to assess the potential of PD-1/PD-L1 targeted therapy as an earlier treatment option due to its rapid progress, long-term response, and beneficial characteristics. Trials investigating PD-1/PD-L1 targeted therapy for early-stage lung cancer have shown encouraging results [22–25]. However, similar to late-stage patients, not all early-stage lung cancer patients respond effectively to anti-PD-1/PD-L1 therapy. In late-stage patients, biomarkers have been utilized to select those who may obtain benefits, and the same approach should be applicable to early-stage patients. A recent study in early-stage oesophageal cancer patients demonstrated that those with high Til infiltration in the tumour microenvironment are more prone to respond to
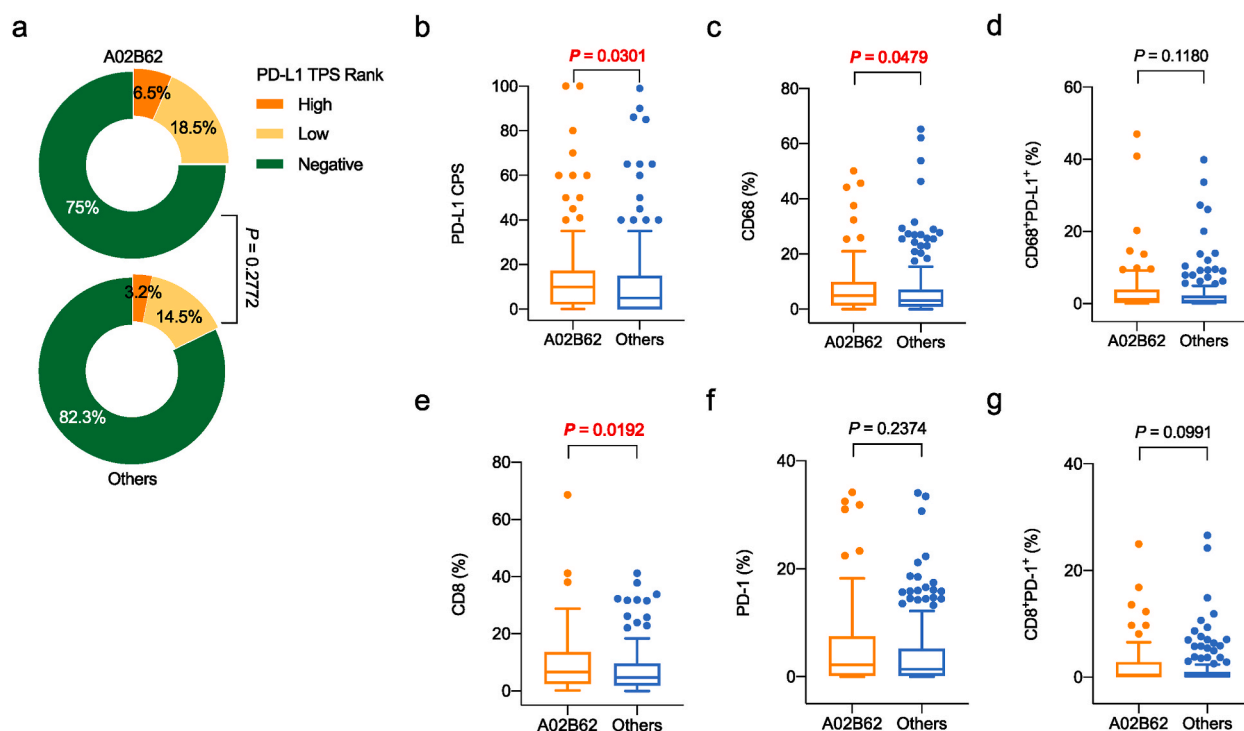
Fig. 3. Evaluation of the HLA-A02B62 supertype-related immune microenvironment.
(a–g) Comparison between the HLA-A02B62 supertype group and other supertype groups in terms of programmed cell death ligand 1 tumour proportion score (PD-L1 TPS rank) (a), PD-L1 combined positive score (CPS) (b), CD68$^+$ cells (c), CD68+PD-L1+ cells (d), CD8$^+$ cells (e), PD-1+ cells (f), and CD8+PD-1+ cells (g). Chi-square tests and Mann–Whitney U tests were performed.

anti-PD-1 therapy [26]. The HLA supertype, which is the target biomarker of our study, has been established as a predictive factor for anti-PD-1/PD-L1 therapy in advanced tumours [4]. However, as no similar studies have been conducted in early-stage lung cancer, our findings contribute valuable knowledge to this field of investigation.

One of the key findings of this study is that patients with the HLA-A02B62 supertype exhibited higher levels of PD-L1$^+$, CD68$^+$, CD68$^+$PD-L1$^+$, CD8$^+$, PD-1$^+$, and CD8$^+$PD-1$^+$ cells in the tumour microenvironment (Fig. 3). These cells all participate in the tumour immune response. Tumours often harbour numerous mutations and neoantigens, which are processed by antigen-presenting cells and presented on the cell surface by HLA-I molecules. CD8$^+$ cells recognize these antigens and become activated, possessing tumour-killing capabilities. Activation of CD8$^+$ cells is commonly marked by PD-1expression, thus leading to increased CD8$^+$PD-1$^+$ cells in the microenvironment [27]. During this immune response process, antigen-presenting cells and activated CD8$^+$ cells release cytokines or inflammatory factors, triggering the activation of other immune cells within the microenvironment. This manifests as an overall increase in PD-1$^+$ cells. Concurrently, the activation of immune cells, particularly CD8$^+$ cells, prompts a counter-regulatory response by tumour cells and supporting cells, resulting in upregulation of PD-L1 expression on tumour cells to evade immune cell-mediated killing, and on immune cells (primarily CD68$^+$ cells) to prevent collateral damage to normal tissues caused by the immune response. Consequently, the levels of PD-L1$^+$ cells and CD68$^+$PD-L1$^+$ cells increase in the microenvironment [27,28]. HLA-I molecules play a pivotal role in this entire process, as their antigen-presenting capacities can vary among different types of HLA molecules when presenting viral antigens [29], and a similar phenomenon may occur during the presentation of the tumour antigens [30]. Furthermore, the tumour antigen repertoire differs across various tumours types. Thus, although neoantigens are generated, it remains uncertain whether an individual will mount a subsequent tumour immune response. Based on these considerations, our study aimed to identify a subgroup of early-stage lung cancer patients with a tumour immune response from the perspective of the HLA supertype. The HLA-A02B62 supertype subgroup in early-stage lung cancer exhibited a tumour microenvironment that was characterized as both inflamed and immunosuppressive, and therefore, may have a higher probability of responding to PD-1/PD-L1 targeted therapy.

As previously reported, lung cancer with a high rate of PD-L1 positivity in tumour cells correlates with poor prognosis following surgery [31]. In our study, we observed that HLA-A02B62 supertype patients with early-stage lung cancer had a shorter PFS after surgery (Fig. 2). This observation might be attributed to the immunosuppressive tumour microenvironment characterized by PD-L1 expression in these HLA-A02B62 supertype patients. As a result, we reasonably speculate that the presence of HLA-A02B62 supertype could serve as an indicator of poor prognosis in early-stage lung cancer. Interestingly, it might also be a favorable prognostic factor for immunotherapy owing to its association with a PD-L1-positive tumour microenvironment. These two assumptions are intriguing as they offer the potential to predict the postoperative prognosis of patients and guide appropriate treatment options for patients who may face a poor prognosis. Once successfully validated, the findings could hold significant implications for further
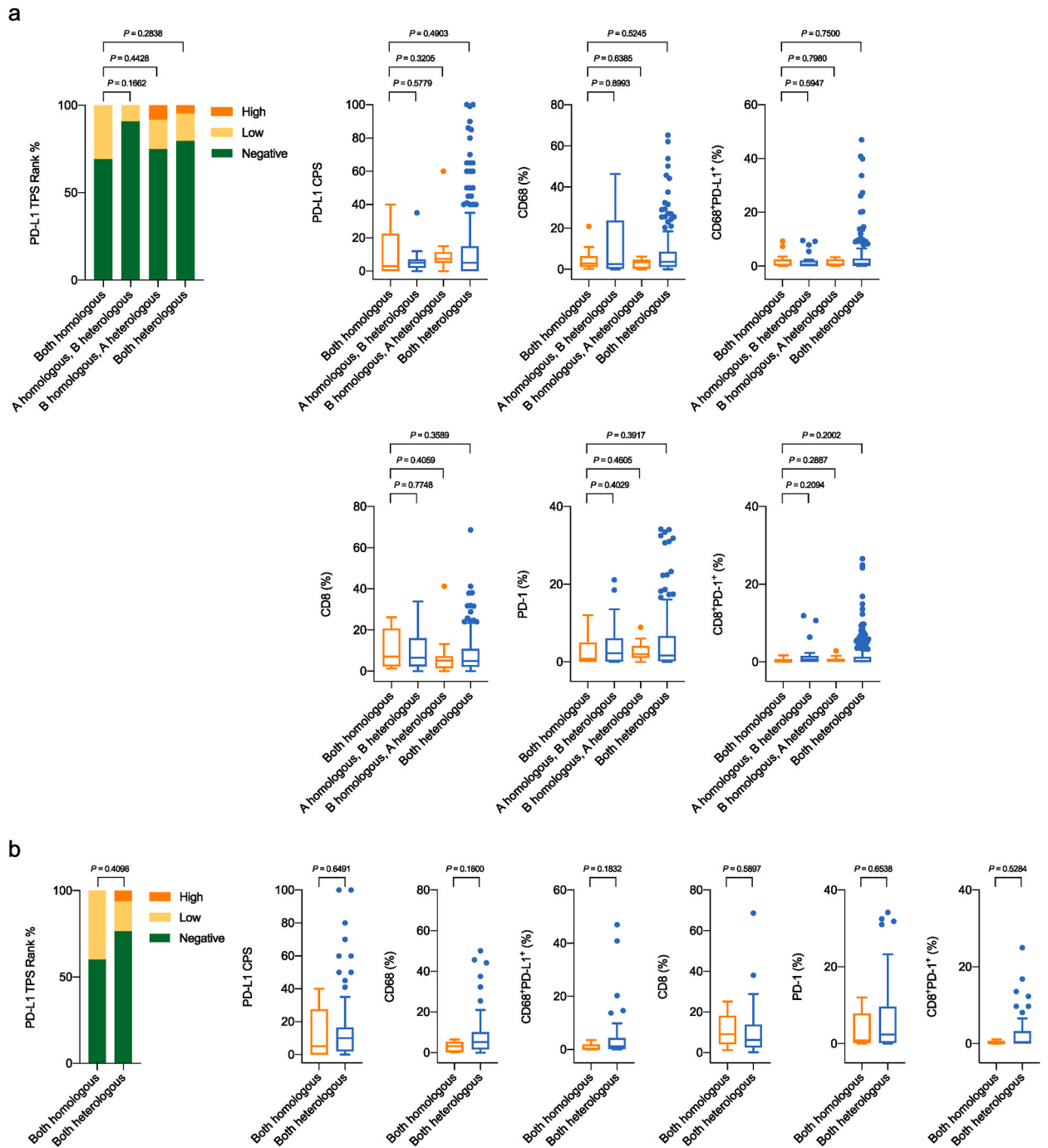
Fig. 4. Evaluation of the HLA supertype diversity-related immune microenvironment.
(a) Comparison between the HLA homologous group and heterologous group in all patients in terms of programmed cell death ligand 1 tumour proportion score (PD-L1 TPS rank), PD-L1 combined positive score (CPS), CD68+ cells, CD68+PD-L1+ cells, CD8+, PD-1+, and CD8+PD-1+ cells using the chi-square test and Mann−Whitney U test. (b) Comparison between the HLA homologous group and the heterologous group in HLA-A02B62 supertype patients in terms of PD-L1 TPS rank, PD-L1 CPS, CD68+ cells, CD68+PD-L1+ cells, CD8+, PD-1+, and CD8+PD-1+ cells using the chi-square test and Mann−Whitney U test.

enhancing the overall prognosis of early-stage lung cancer patients.

The decrease in the diversity of HLA molecules may result in the failure of some neoantigens to be presented on cell surface, rendering reduced activation of T-cell responses and potential resistance to immune drugs. Chowell et al. previously highlighted that the HLA-I phenotype significantly influences the efficacy of immune drugs. In general, greater diversity of HLAs allows for the

presentation of a wider range of neoantigens, leading to improved efficacy of immune drugs [4]. Our study found that in all patients, as well as in the HLA-A02B62 supertype patients, PD-L1 expression and Til infiltration in the heterologous group did not significantly differ from that in the homologous group (Fig. 4). These results indicate that certain specific HLA supertypes might play a more critical role in tumour immune response process than overall HLA supertype diversity.

The true role of HLA-I supertype or diversity as a biomarker for tumour immunotherapy efficacy or prognosis remains a topic of debate. While Chowell et al. support its significance, there are also conflicting reports suggesting otherwise. Negrao et al. reported that PD-L1 expression, tumour mutational burden, as well as cancer gene mutations hold greater predictive value for immune checkpoint blockade benefit in non-small-cell lung cancer compared to HLA-I genotype [32]. Similarly, According to Chhibber et al. the germline HLA landscape does not serve as a predictor for the efficacy of pembrolizumab monotherapy in various solid-tumour types [33]. In contrast, our findings indicate that HLA-I supertype may indeed be indicative of tumour immunotherapy efficacy or prognosis. we performed a preliminary verification of this perspective based on PD-L1 expression and Til infiltration in the tumour microenvironment, and obtained promising results.

Here, we thoroughly evaluated germline HLA supertypes, their associated tumour immune microenvironment, and their clinical significance in early-stage NSCLC patients. The methodology employed in this study is widely recognized for its reliability and extensive application, and the samples utilized were relatively recent, further ensuring the credibility of the findings. Nevertheless, due to the heterogeneity of tumours and the microenvironment, one limitation is the potential for overestimation or underestimation of biomarkers expression in the entire tissues. Furthermore, the sample size was relatively small, underscoring the need for further exploration and validation of our results. Consequently, these findings should be considered preliminary and warrant further exploration in a larger patient cohort to establish their robustness and clinical significance.

5. Conclusions

In conclusion, our study suggests that the HLA-A02B62 supertype may function as an indicator of the tumour microenvironment characterized by heightened immune activity and immunosuppression. The potential significance of the HLA-A02B62 supertype in predicting the therapeutic efficacy of PD-1 blockade therapy warrants further investigation in future.

Funding information

Ethics approval and consent to participate

The study was conducted with the approval of the ethics committees of The First Hospital of Lanzhou University (LDYYLL2022-477). Each patient signed a written informed consent form.

Author contribution statement

Ruijiang Lin: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper. Minjie Ma: Conceived and designed the experiments. Cuixiang Zhang: Performed the experiments. Xiaohua Chen; Fei Su; Hongbin Wang; Biao Han; Yanhui Chen: Contributed reagents, materials, analysis tools or data.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Abbreviations

NSCLC   Non-small-cell lung cancer
PD-1   Programmed death-1
PD-L1   Programmed death ligand-1
ADC   Adenocarcinoma
SQCC   Squamous cell carcinoma
TMB   Tumour mutational burden
mIHC   multiplex immunohistochemistry
NGS   Next-generation sequencing

TC   Tumour cell
Til   Tumour-infiltrating lymphocyte
CPS   Combined proportion score
EGFR-TKIs   Epidermal growth factor receptor tyrosine kinase inhibitors
TIME   Tumour immune microenvironment
PFS   Progression-free survival

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e18948.

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