Research article

Establish a novel immune-related gene prognostic risk index (IRGPRI) associated with CD8+ cytotoxic T lymphocytes in non-small-cell lung cancer (NSCLC)

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ABSTRACT

Background: The aim of this study is to create an index called IRGPRI (immune-related gene prognostic risk index) that can be utilized for predicting the prognosis and assessing the efficacy of immune checkpoint inhibitors (ICIs) therapy in patients with non-small-cell lung cancer (NSCLC).

Methods: Distinguishing gene expression patterns (DEGs) were detected in CD8+ cytotoxic T lymphocytes (CTLs) compared to other cellular types such as CD4 T cells, B cells, plasma cells, and CD8 Tex using the advanced technology of Single-cell RNA Sequencing (scRNA-seq). The construction of IRGPRI was accomplished by employing LASSO Cox regression analysis. We conducted a comparative analysis on clinical characteristics and molecular features, such as pathway enrichment and gene mutation, among the distinct subgroups of IRGPRI. Furthermore, we explored the correlation between immunological characteristics and IRGPRI subgroups to comprehensively assess the effectiveness of ICIs in NSCLC patients.

Results: A total of 109 genes were identified by intersecting immune-related genes with DEGs obtained from single-cell RNA sequencing data (GSE131907), specifically comparing CTLs to other cell types. From these, we selected 7 prognosis-related genes, namely TRBC1, HLA-DMA, CTSH, RAC1, CTSL, ANXA2, and CEBPB. These genes were used to construct the IRGPRI. The prognosis of patients diagnosed with NSCLC was found to be significantly better in the low-risk group compared to the high-risk group, as demonstrated by Kaplan-Meier (K-M) survival analysis. This observation was further confirmed through the utilization of data from the GEO cohort. The low-risk group demonstrated an increase in pathways linked with immune response, whereas the high-risk group exhibited a higher prevalence of pathways related to cancer. Furthermore, it was noted in the TCGA cohort that there existed a significant rise in the mutation frequency of every gene within the high-risk group as opposed to the low-risk group. Missense variation emerged as the most prevalent form of mutation. According to the analysis of immune cell

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infiltration and function, the comprehensive findings suggest that the group with a low risk is characterized by an increased presence of plasma cells, CTLs, T cells follicular helper, Tregs, and Dendritic cell resting. Additionally, they exhibit a higher score in terms of immune function for B cells, CD8$^+$ T cells, checkpoint activity, T cell inhibition and stimulation. Moreover, this low-risk group demonstrates greater efficacy when treated with ICIs therapy compared to the high-risk group.

Conclusions: Our research effectively developed and verified a unique IRGPRI, showcasing its association with immune-related characteristics. As a result, the potential of IRGPRI as a valuable biomarker for predicting prognosis and evaluating the effectiveness of ICIs treatment in cancer is evident.

1. Introduction

Lung cancer is a primary malignant tumor that has a high prevalence globally, leading to a significant number of deaths each year, approximately 1.6 million [1]. NSCLC, which accounts for around 85 % of all cases, can be classified into three main subtypes based on the cellular origin: lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), and large cell carcinoma [2]. Due to the absence of clear clinical manifestations, patients with NSCLC frequently receive a late-stage diagnosis, resulting in missed opportunities for receiving the most effective treatment. Despite various anticancer approaches such as surgery, radiation therapy, and chemotherapy being employed for NSCLC management, there remains a considerable need for effective curative or palliative strategies, especially in individuals with advanced-stage disease [3]. Recent advancements in therapeutic approaches, such as targeted therapy and immunotherapy, have demonstrated significant advancements in enhancing the overall survival (OS) and quality of life among individuals diagnosed with NSCLC [4,5]. The preference for chemotherapy as the primary treatment option for all NSCLC patients is no longer universally endorsed.

Tumor cells exploit various immune checkpoint pathways to evade detection by the immune system and facilitate tumor progression. Immunotherapies targeting immune checkpoints, such as PD-1, PD-L1, and CTLA-4 inhibitors, have received regulatory approval for treating diverse cancer types like melanoma, breast cancer, and NSCLC [6–9]. Immunotherapy is commonly employed in managing advanced and metastatic NSCLC. Encouraging outcomes have been observed in NSCLC with the use of PD-1 inhibitors like pembrolizumab and nivolumab, showing an objective response rate (ORR) ranging from 40 % to 45 % [6,7]. In a clinical trial called IMpower-110, investigators analyzed individuals diagnosed with advanced NSCLC who had not received any prior therapeutic interventions. The analysis focused on individuals with either ≥50 % PD-L1 tumor cells or ≥10 % immune cells infiltrating the tumor. The findings revealed that atezolizumab significantly extended OS by 7 months compared to chemotherapy in this group [10]. Targeting CTLA-4 using ipilimumab has shown up to a 0.84-year improvement in progression-free survival (PFS) among NSCLC patients [11].

While it has been proven that anti-PD-1/PD-L1 and anti-CTLA-4 therapies are effective in treating NSCLC, there is indication that their advantages may be restricted to a particular subgroup of patients. This underscores the challenges associated with implementing ICIs therapy for NSCLC. The immune response against tumors is influenced by the tumor microenvironment (TME), leading to a range of immune reactions. The TME can be categorized into three groups based on the infiltration of immune cells: immune desert, immune excluded, and immune inflamed [12]. Immune inflamed phenotypes are characterized by the presence of various subtypes of immune cells including CD4$^+$ T cells, CTLs, CD20$^+$ B cells, among others. Activated CTLs selectively recognize and eliminate host cells expressing intracellular antigens from infection or cancer to play an essential role in anti-cancer immunity [13]. Some patients receiving ICIs therapy may experience adverse effects; hence better strategies are needed to reduce their risk in future studies [14]. Patients who develop resistance to ICIs therapy have poor prognoses after treatment; therefore identifying predictive biomarkers is critical for indicating which patients will most likely benefit from this treatment approach [15].

Our investigation aimed to explore potential biomarkers that can predict the response to ICIs treatment and evaluate the therapeutic value of ICIs in NSCLC. To achieve this, we utilized scRNA-seq technology to identify DEGs between CTLs and other cell types. The IRGPRI was constructed using genes associated with prognosis, and we further investigated the molecular and immunological characteristics of IRGPRI while assessing its predictive ability for both prognosis and the efficacy of ICI therapy. Our findings suggest that the IRGPRI shows significant promise as a reliable biomarker for accurately predicting prognosis and response to ICIs treatment in cancer patients.

2. Materials and methods

2.1. Data sources and data processing

We collected RNA-seq data, clinical information, and somatic mutation variation data from the TCGA database (https://portal.gdc.cancer.gov), which included 1153 NSCLC samples comprising of 1043 NSCLC tissues and 110 para-cancer tissues. As a validation cohort, we also retrieved a microarray dataset (GSE101929) [16] consisting of 66 NSCLC patients from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). To calculate the TMB, we utilized a Perl script that determined the total number of somatic mutation variations per million bases. In order to identify immune-related genes, we conducted searches in both ImmPort (https://www.immport.org/shared/home) and InnateDB (https://www.innateDBdb.com/) databases [17]. For our analysis on DEGs, scRNA-seq

data was obtained from the Tumor Immune Single-cell Hub 2 (TISCH2) database (http://tisch.comp-genomics.org/), an online platform providing comprehensive and freely accessible scRNA-seq information globally [18].

2.2. Identification and biological function analysis of intersection genes

We employed the "Filter" package in R software to generate a list of DEGs between CTLs and other cell types. Our criteria for DEGs selection were |log2(Fold Change)|>1.3 and a false discovery rate (FDR) < 0.05. The genes at the intersection were analyzed for enrichment in Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways using Sangerbox3.0, with FDR< 0.1 and $P < 0.05$.

2.3. Construction and validation of the IRGPRI

To identify genes linked with prognosis, we conducted univariate Cox regression analysis using R software packages such as 'survival' and 'survminer', setting a significance threshold of $P < 0.05$. We utilized the 'glmnet' package in R to perform LASSO Cox regression analysis, which helped us identify relevant variables and reduce dimensionality. Subsequently, we employed multivariate Cox regression analysis using the selected variables. In order to compute the IRGPRI for each sample in our risk model, we applied the subsequent formula: IRGPRI = [Expression level (prognosis-related genes) × gene coefficient]. Reclassifying NSCLC patients from the TGCA cohort into low-risk and high-risk groups according the median score of IRGPRI, we performed K-M survival curve analysis to assess the prognostic significance of IRGPRI. To validate our findings, we utilized another cohort from GEO to evaluate the prognostic value of IRGPRI. Furthermore, we employed the R software packages "survival" to examine the correlations between risk scores and clinical characteristic such as age, gender, stage, and OS in patients from the TGCA cohort using both univariate and multivariate Cox regression analyses. We conducted statistical analyses to determine hazard ratios (HR), 95 % confidence intervals (CI), and P-values, which helped us identify significant independent prognostic factors. To visually compare clinical characteristics between low-risk and high-risk groups, we utilized R software packages such as "Biocmanager," "Complex Heatmap," and "ggplot2" to generate graphical representations like heatmaps and circular graphs.
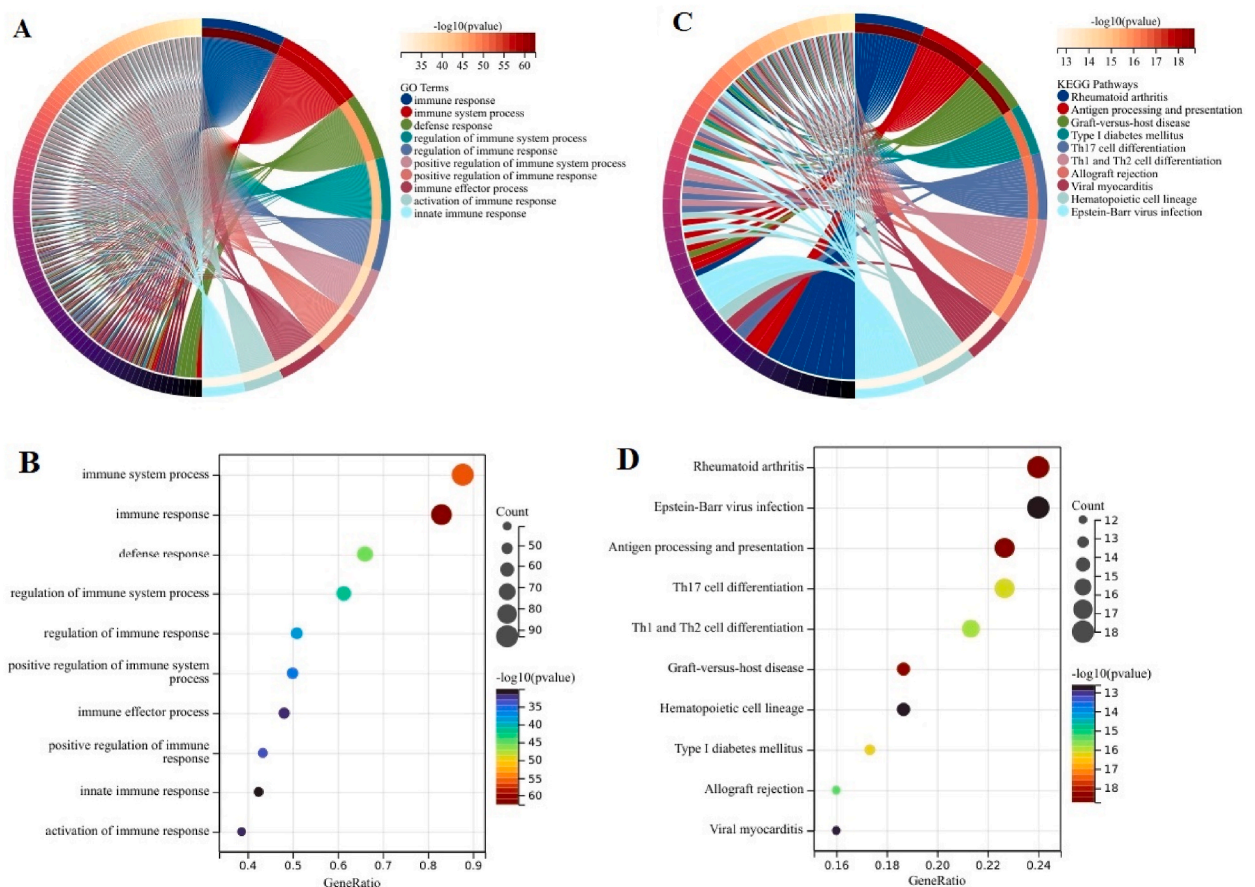

Fig. 1. Gene function and pathways enrichment analysis. (A, B) GO analysis for the intersection genes. (C, D) KEGG pathway analysis for the intersection genes.

2.4. Comprehensive analysis of molecular and immunological features in distinct IRGPRI subgroups

We utilized the gene set enrichment analysis (GSEA) method to investigate the enrichment of pathways in various subcategories of IRGPRI. Statistical testing was conducted using a significance level of $P < 0.05$. To generate a gene mutation map for the two sub-groups of IRGPRI, we utilized the "maftools" R software package. The R software package 'limma' was utilized to conduct a
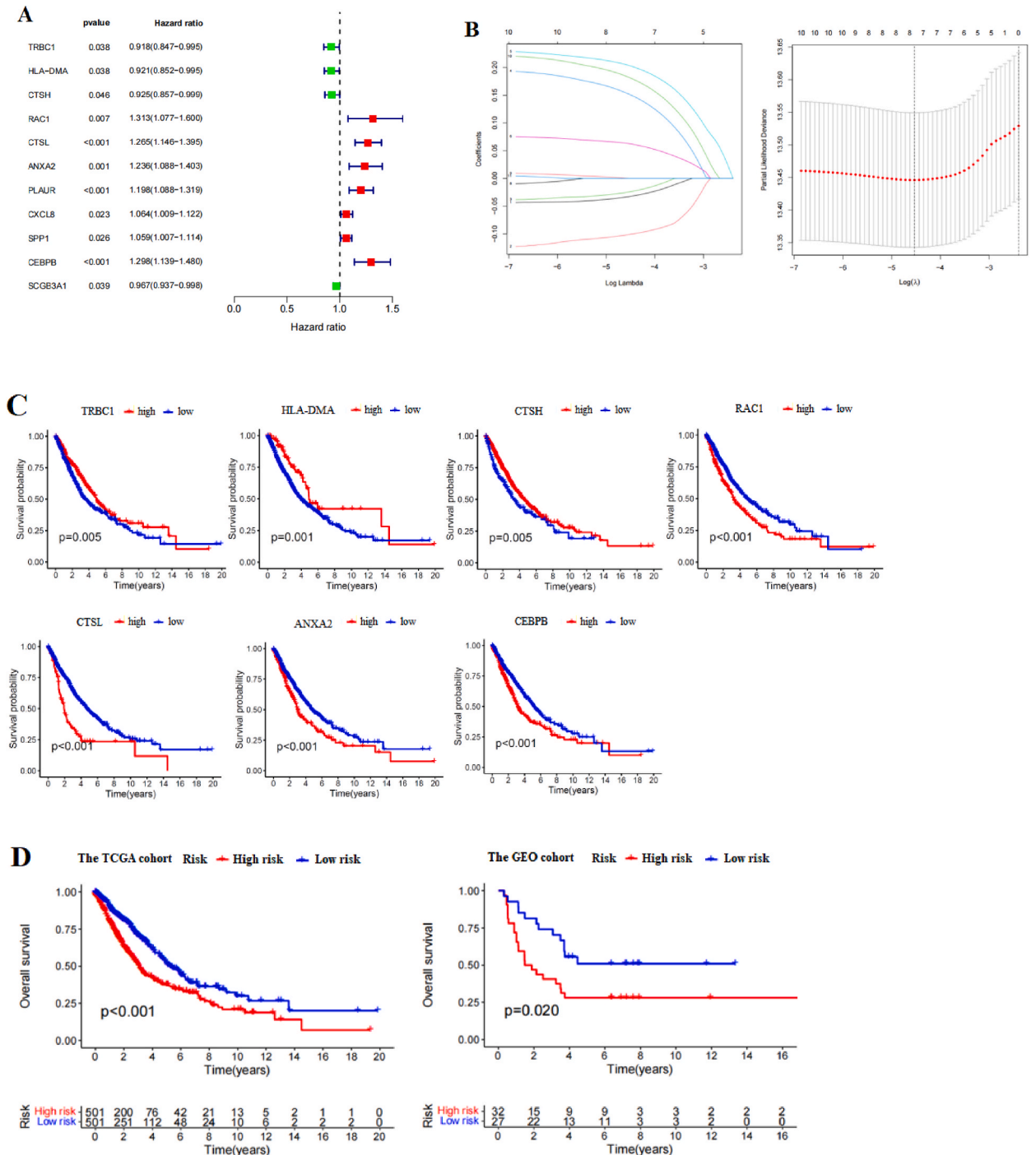

Fig. 2. Construct and validate the IRGPRI. (A) Forest diagram illustrating the predictive significance of immune-related genes in NSCLC through hazard ratios. (B) Seven prognostic-related genes were identified by LASSO regression analysis. (C) Application of K-M survival analysis on a set of 7 genes associated with prognosis. (D) K-M survival analysis was conducted on the subgroups of IRGPRI in both TCGA cohort and GEO cohort.

comparative analysis of checkpoint genes expression between the low-risk and high-risk groups. The relative distributions of 22 types of immune cells were assessed using the CIBERSORT algorithm (https://cibersortx.stanford.edu/) and visualized through box plots. To investigate immune function related to distinct IRGPRI subgroups, we conducted single sample gene set enrichment analysis (ssGSEA). Comparisons between the high-risk and low-risk groups were made using both the stat_compare_means function and Wilcoxon test. We evaluated the prognostic significance of IRGPRI in NSCLC patients receiving immunotherapy by analyzing data from IMvigor210 immunotherapy cohorts and generating K-M survival curves. Additionally, we examined how immunotherapy impacted the two subgroups defined by IRGPRI using three R software packages: "limma", "ggpubr", and "pROC".

3. Results

3.1. Identification of intersection genes

We acquired scRNA-seq data (GSE131907) from the TISCH2 database, which comprised 44 patients and a total of 203298 cells. Utilizing cell-type annotations based on major-lineage classification, we classified the 25 clusters of cells into 12 distinct cell types. Among these, CTLs accounted for the third highest proportion at 16 % of all cell types. Given their crucial role as central effector cells in mediating the effectiveness of Immune checkpoint blockade (ICB), this suggests that ICB immunotherapy may yield favorable therapeutic outcomes in NSCLC. By analyzing differentially expressed genes (DEGs) between CTLs and other cell types such as CD4 T
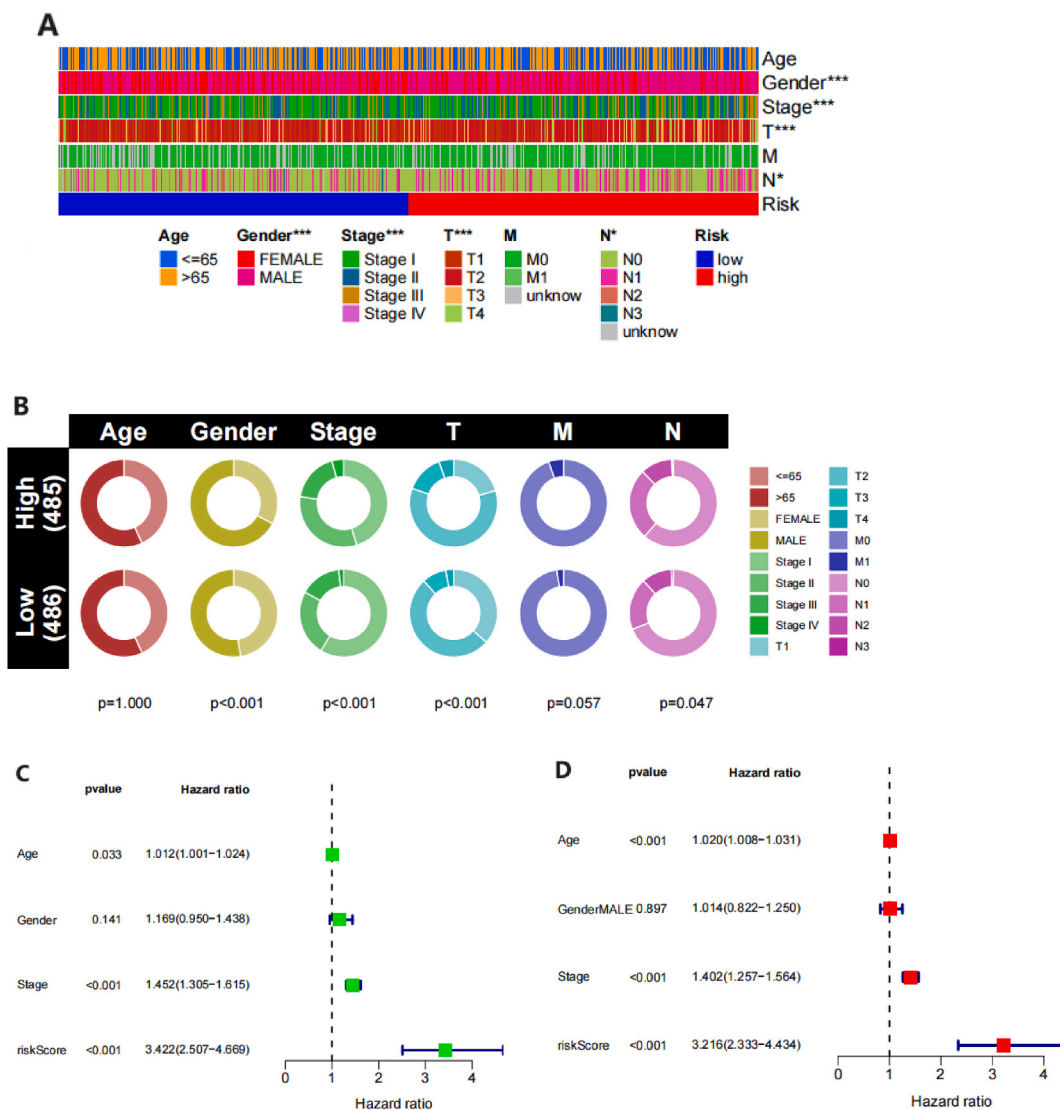

Fig. 3. Clinical characteristics in different IRGPRI subgroups. (A, B) Analyze the differences in Clinical characteristics between the distinct IRGPRI subgroups. (C, D) Analysis of the IRGPRI score and other clinical characteristics using both univariate and multivariate Cox regression.

cells, B cells, plasma cells, and CD8 Tex using scRNA-seq data (GSE131907), we identified a set of immune-related genes through intersection analysis with the aid of "VennDiagram" R software package. This resulted in a total of 109 overlapping genes.

3.2. Gene function and pathways enrichment analysis

The analysis of GO revealed that the genes expressed in CTLs were mainly associated with processes related to the immune system, including responses to stimuli, defense mechanisms, and regulation of immune system processes (Fig. 1A and B). In addition, the analysis of KEGG pathways revealed that the hub genes played a role in various signaling pathways including rheumatoid arthritis,
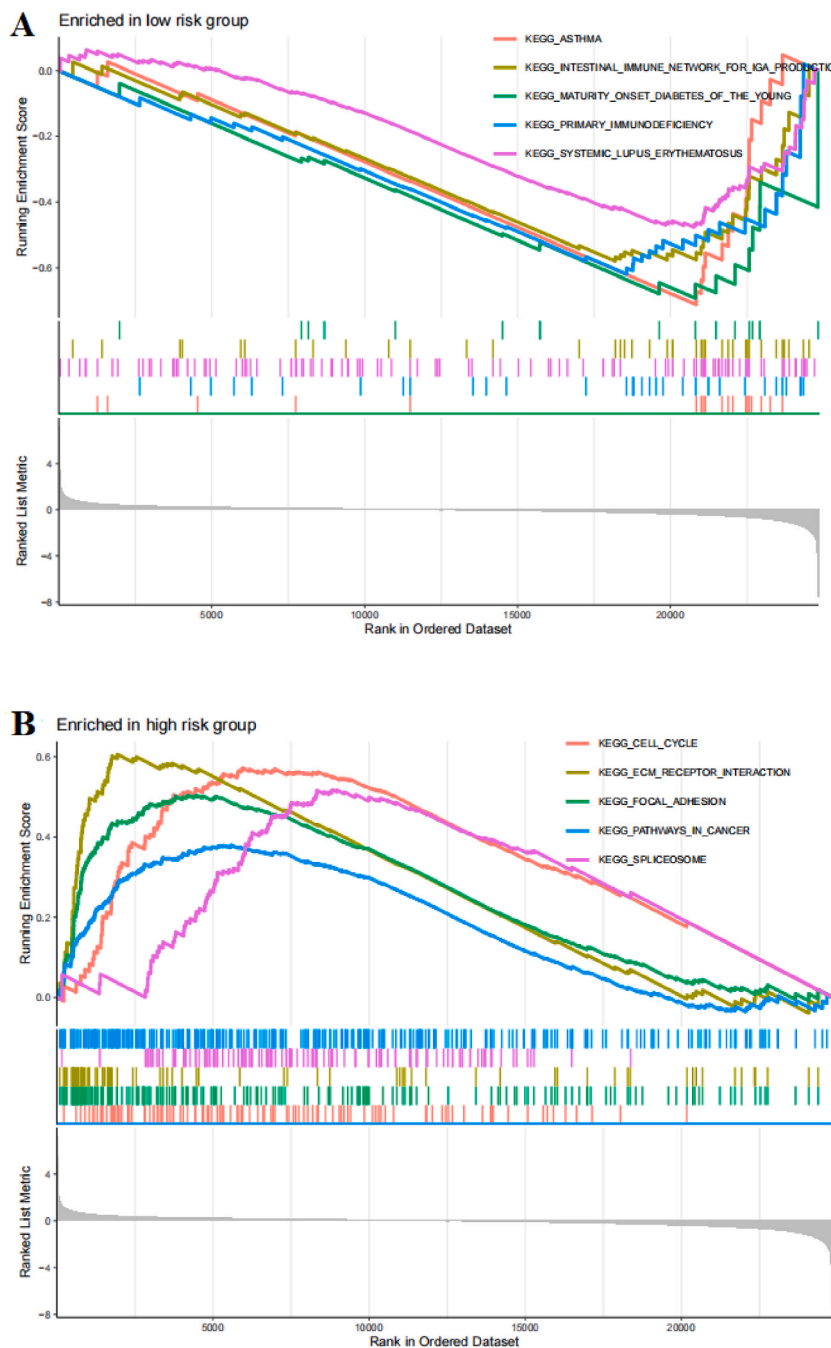

Fig. 4. Association between molecular characteristics and risk signature. (A, B) Conducting KEGG pathway analysis on gene sets that exhibit enrichment in the different subgroups of IRGPRI. (C, D) Mutation waterfall in the distinct IRGPRI subgroups.
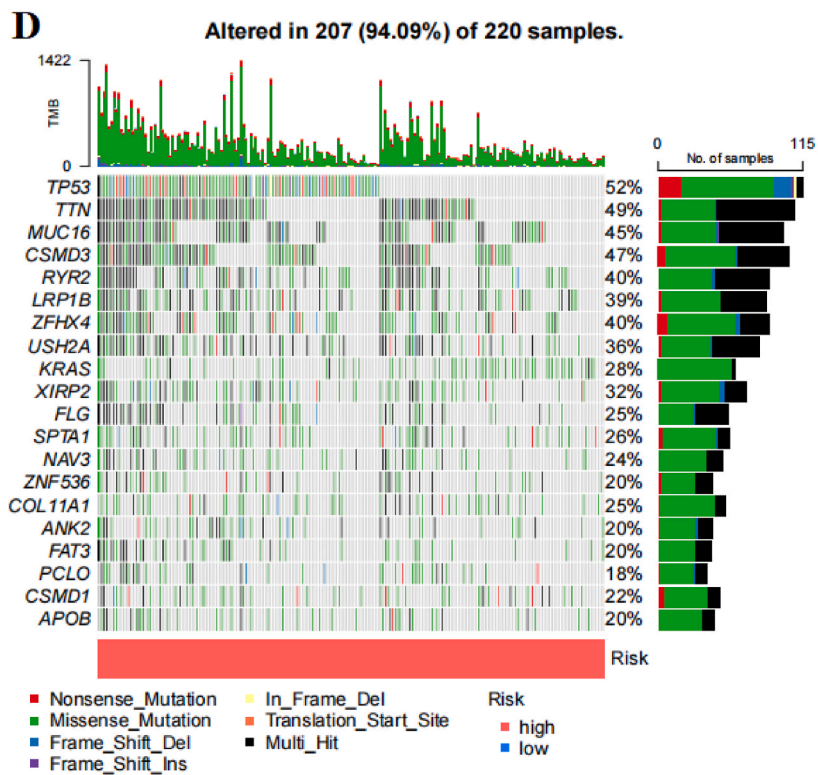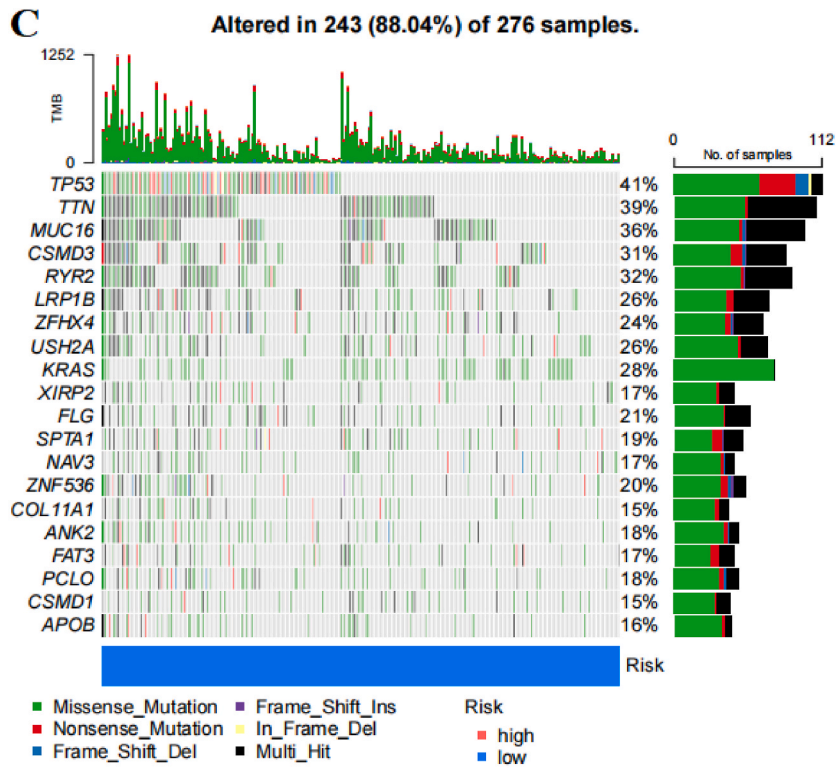
Fig. 4. (continued).

infection caused by Epstein-Barr virus, antigen processing and presentation, differentiation of Th17 cells, as well as differentiation of Th1 and Th2 cells (Fig. 1C and D). By utilizing this information effectively, we conducted further investigations into potential biomarkers for NSCLC.

3.3. Construction and validation of the IRGPRI

The prognosis survival of NSCLC patients was found to be significantly influenced by 11 immune-related genes, as indicated by the statistically significant results obtained from the Univariate Cox regression analysis (Fig. 2A). Subsequently, a 1000-fold cross-validation was performed to conduct LASSO Cox regression analysis on the set of 11 genes. This analysis identified seven genes (*TRBC1*, *HLA-DMA*, *CTSH*, *RAC1*, *CTSL*, *ANXA2*, and *CEBPB*) that significantly influenced the OS of NSCLC patients. These prognostic genes were utilized to construct the IRGPRI score (Fig. 2B and C). Reclassifying patients from the TCGA cohort into low-risk and high-risk groups was based on utilizing the median IRGPRI score as a threshold value. The K-M survival analysis revealed a significant difference in prognosis between individuals classified as high-risk and low-risk groups. Furthermore, the prognostic value of IRGPRI was validated using data from the GEO cohort (Fig. 2D).

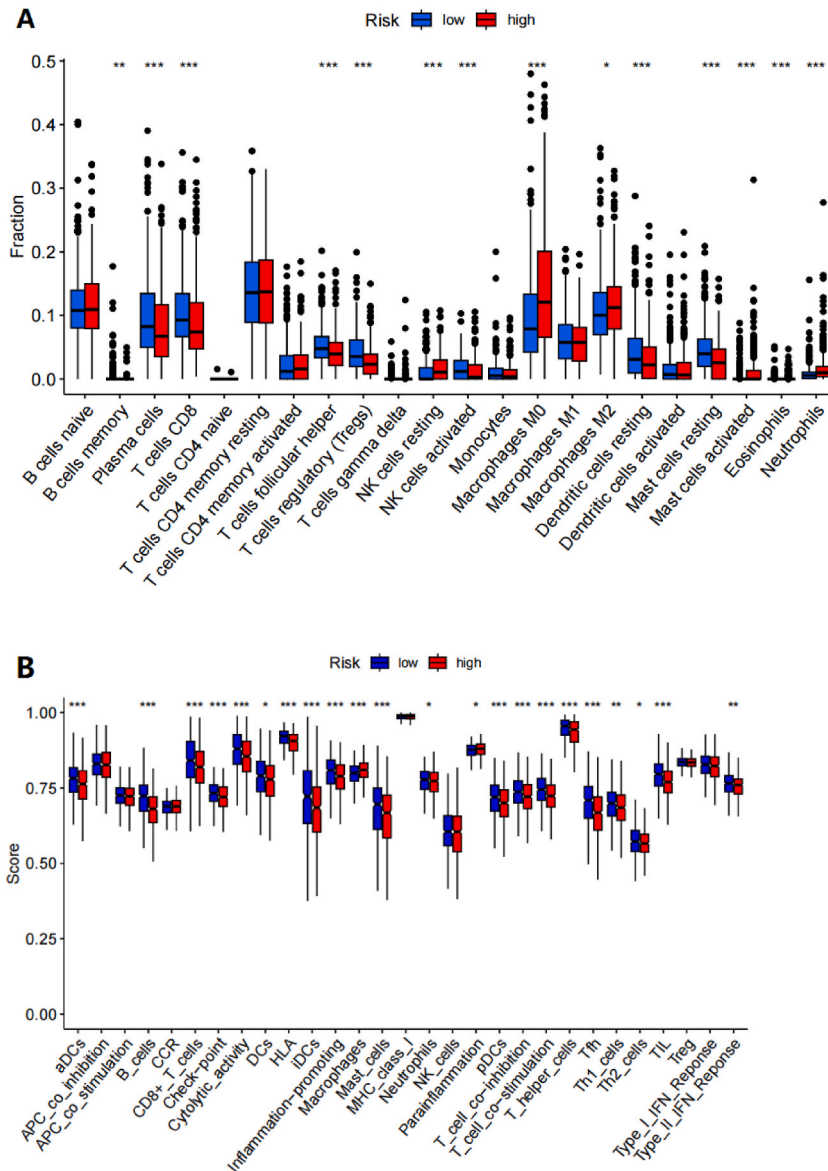
Fig. 5. The assessment of immune cell infiltration and functional score across various subgroups within the IRGPRI. (A) Examining the variation in immune cell composition among different subgroups of IRGPRI. (B) Assessment of the immune cell function score among different subgroups within the IRGPRI. (C) The correlation between the score of IRGPRI and the genes associated with checkpoints.
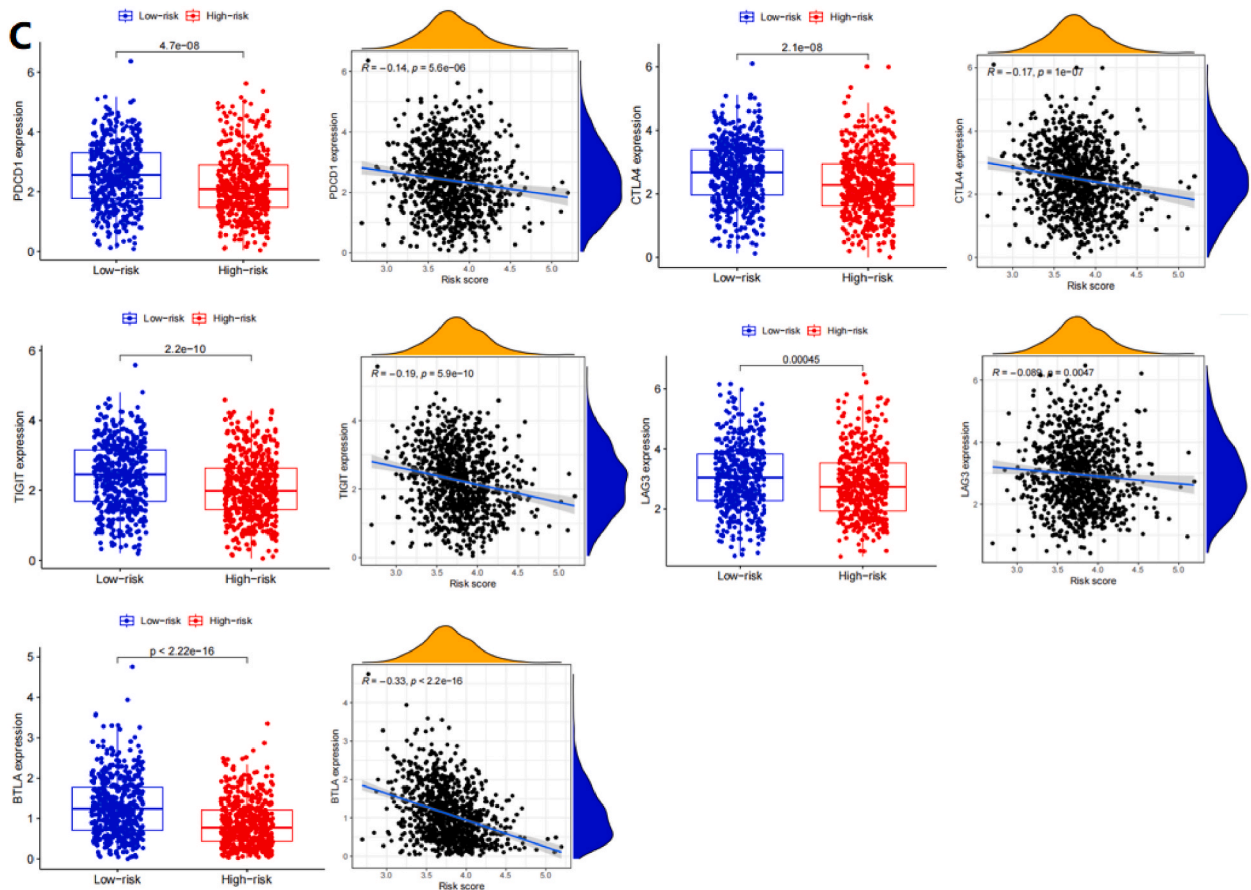
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3.4. Clinical characteristics in different IRGPRI subgroups

The heatmap and circular plots revealed notable differences in clinicopathological factors, such as gender, stage, T, and N, between the high-risk and low-risk groups (Fig. 3A and B). Univariate Cox regression analysis demonstrated that the risk score (HR = 3.422; 95%CI: 2.507–4.669), age (HR = 1.012; 95%CI: 1.001–1.024), and stage (HR = 1.452; 95%CI: 1.305–1.615) were statistically significant prognostic determinants for NSCLC patients ($P < 0.05$) (Fig. 3C). Furthermore, multivariate analysis indicated that the risk score remained an independent predictor of overall survival in NSCLC patients after adjusting for other clinicopathological factors (HR = 3.216; 95%CI:2.333–4.434) along with age (HR = 1.020; 95%CI:1 0.008–1.031) and stage (HR = l.402; 95%CI:l.257-l.564) with $P < 0.05$ (Fig. 3D).

3.5. Correlation between molecular characteristics and risk signature

We conducted Gene set enrichment analysis (GSEA) to identify the gene sets that were enriched in different subgroups of IRGPRI within the TCGA cohort (Fig. 4A and B). The low-risk group exhibited significant enrichment of gene sets associated with the immune network involved in IgA production and immunodeficiency, while the high-risk group showed significant associations with cell cycle regulation, ECM receptor interaction, and pathways related to cancer. Additionally, we performed gene mutation analysis to investigate the immunological characteristics of IRGPRI subgroups within the TCGA cohort (Fig. 4C and D). The distinct IRGPRI subgroups displayed a prevalence of missense mutations followed by multi-hit mutations in the top 20 genes with the highest mutation rates. In comparison to the low-risk group, all genes exhibited elevated mutation rates in the high-risk group. Essentially, a greater burden of tumor mutations was observed in the high-risk group when compared to the low-risk group.

3.6. The infiltration and function score of immune cells in different IRGPRI subgroups

The infiltration of immune cells in patients with NSCLC was evaluated using the CIBERSORT algorithm.
The results indicated that the IRGPRI subgroups exhibited distinct proportions of infiltrating immune cells. Specifically, the low-risk group showed a higher prevalence of activated plasma cells, CTLs, T cells follicular helper, Tregs, and resting dendritic cells ($P <$

0.05), whereas the high-risk group demonstrated a greater prevalence of activated macrophages M0, macrophages M2, and neutrophils ($P < 0.05$) (Fig. 5A). Subsequently, ssGSEA analysis was conducted to explore differences in immune function among the different IRGPRI subgroups (Fig. 5B). The results demonstrated that compared to the high-risk group, the low-risk group exhibited higher immune function scores in B cells, CTLs, checkpoint signaling pathway activity related genes expression levels associated with T cell inhibition and stimulation ($P < 0.05$). Furthermore, an investigation into the expression patterns of well-studied classic checkpoint genes across distinct IRGPRI subgroups was performed to determine whether NSCLC patients could benefit from immunotherapy targeting these checkpoints (Fig. 5C). The findings revealed a potential association between increased risk scores and reduced levels of checkpoint gene expression, potentially indicating a diminished efficacy of immunotherapy.

3.7. Relationship between IRGPRI grouping and ICIs therapy

To investigate the predictive value of IRGPRI in relation to the administration of ICIs, an assessment was carried out on a group of individuals who received immunotherapy (IMvigor210). Our results revealed that patients categorized as low-risk demonstrated enhanced response to immunotherapy and attained superior outcomes in comparison to those classified as high-risk (Fig. 6A and B). As a result, an increased risk score was linked to a greater probability of immune evasion and diminished effectiveness of ICIs treatment. We conducted ROC analysis to assess the predictive capacity of IRGPRI (Fig. 6C) and found that it had an area under the curve of 0.706. These findings indicate that IRGPRI has promising potential as a prognostic tool for predicting response to checkpoint inhibition therapy.
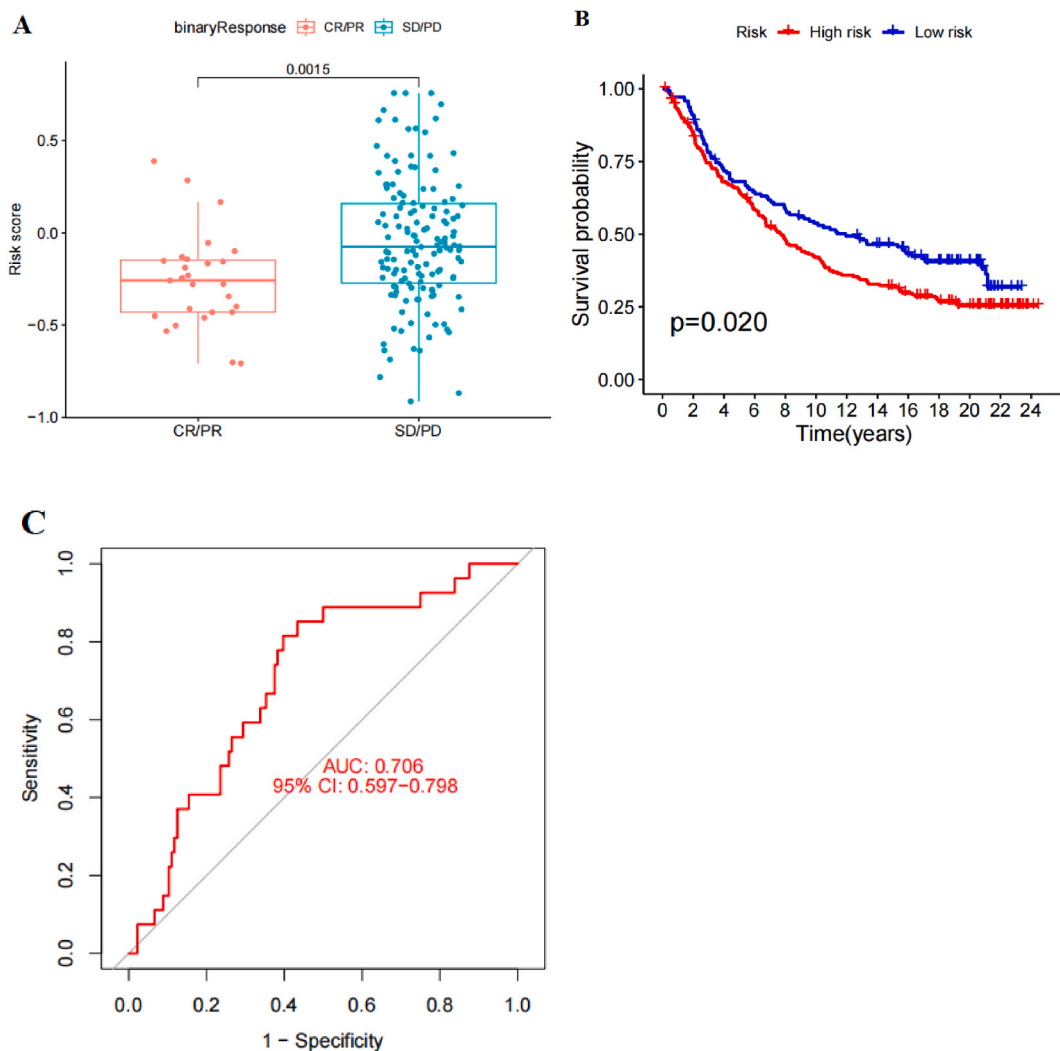

Fig. 6. Prognostic value of IRGPRI in ICIs therapy. (A) The low-risk group exhibited a significantly elevated rate of immune response in ICIs therapy. (B) The low-risk group exhibited a more favorable outcome compared to the high-risk group. (C) ROC analysis of IRGPRI in IMvigor210 cohort.

4. Discussion

NSCLC plays a significant role in the worldwide mortality caused by cancer [19]. Despite the advent of ICIs therapy, the response rate remains suboptimal, limiting its efficacy to a minority of NSCLC patients [20]. Hence, there exists an imperative need to identify a robust biomarker capable of discerning patients likely to derive benefits from ICIs therapy. Cancer immunotherapy is a therapeutic strategy that utilizes the cytotoxic potential of the human immune system, specifically tumor-specific cytotoxic T cells, to combat malignancies [21]. Tumors utilize distinct strategies to interfere with T cell reactions, resulting in a decrease in the immunogenicity of the majority of tumors and establishing a suppressive TME that impedes the function of developing cytotoxic T cells targeting the tumor [22]. Therefore, the current focus in cancer immunotherapy lies in enhancing T cell-mediated immune responses, aiming to convert cancer cells' resistance into a state that can be targeted by chemotherapy or radiotherapy [23]. CTLs are considered as the optimal immune cells for effectively eradicating cancer cells [23]. CD8 coreceptor is expressed by CTLs, which serve as a primary barrier against the advancement of cancer [24,25]. The significance of these cells in efficiently eradicating cancer cells is considered greater than that of CD4 T cells [26].

In this investigation, scRNA-seq data from 44 individuals with NSCLC were examined to identify 209 DEGs between CTLs and other cell types. This led to the identification of 109 genes related to the immune system. Analyses using GO and KEGG revealed an enrichment in processes associated with immunity and antigen processing/presentation. Regression analysis highlighted 11 immune-related genes that could potentially serve as prognostic factors. Among these genes, *TRBC1*, *HLA-DMA*, *CTSH*, *RAC1*, *CTSL*, *ANXA2*, and *CEBPB* were utilized to construct a novel biomarker called IRGPRI. By utilizing the median IRGPRI score as a threshold value, patients from both TCGA cohort and GEO Cohort were categorized into low-risk or high-risk groups for further analysis. It should be emphasized that our study has certain limitations as it includes NSCLC patients who were not specifically administered immuno-therapy treatment in both cohorts (TCGA and GEO), potentially affecting the applicability of our findings concerning the outcomes of immunotherapy. Nevertheless, we found that IRGPRI was effective in predicting OS among NSCLC patients. The independent GEO cohort also validated the prognostic importance of IRGPRI, as better survival outcomes were observed in individuals with lower IRGPRI scores, while those with higher scores had a poorer prognosis. These findings indicate that IRGPRI shows potential as a biomarker associated with the immune system for prognostic prediction in NSCLC patients. Furthermore, our study revealed a robust association between IRGPRI and clinicopathological factors that have been shown to significantly impact OS in individuals with NSCLC.

In order to enhance our comprehension of the immunological characteristics linked with each subgroup of IRGPRI, we conducted an investigation into the molecular attributes and genetic mutations. Our research revealed that individuals in the high-risk group had a greater occurrence of pathways linked to cancer, whereas those in the low-risk group showed an increase in gene sets related to immune response pathways, including IgA production and immunodeficiency. Although T-cell activation is widely acknowledged as a primary therapeutic approach in cancer immunotherapy, recent research has underscored the vital roles played by tumor-associated B cells and antibodies in regulating immune responses within the TME and in cancer immunotherapy [27,28]. As serum's second most abundant antibody, IgA has demonstrated significant potential for recruiting and activating neutrophils via FcαRI, thereby leading to eradication of tumors. This characteristic renders it a promising target for monoclonal antibody-based anti-tumor therapy [29]. Previous studies have established a correlation between polyclonal and antigen-specific IgA antibodies and favorable prognoses in ovarian cancer, nasopharyngeal carcinoma, and endometrial cancer [28,30,31]. Henceforth, we hypothesize that IgA may play a pivotal role in immunotherapeutic interventions for NSCLC patients with low IRGPRI scores by potentially contributing to improved survival outcomes. The examination of genetic mutations indicated that the group at high risk showed a significantly increased mutation rate, with TP53 gene exhibiting the highest occurrence of mutations, primarily in the form of missense mutations. TP53 gene, which was the first tumor suppressor gene to be discovered, is responsible for around 50 % of NSCLC mutations and up to 80 % of squamous-cell carcinoma mutations [32]. Changes in metabolism caused by TP53 mutation and hypoxia may lead to the acidification of the microenvironment, influencing its immune characteristics to support the advancement of cancer [33]. Previous studies have suggested that NSCLC patients with TP53 mutations experience more aggressive tumor growth and higher resistance to chemotherapy compared to those with TP53 wild-type [34,35]. Additionally, patients harboring TP53 missense mutations have an unfavorable prognosis, particularly in cases of adenocarcinoma [34–41]. Our survival analysis results are consistent with these studies, indicating that individuals in the high-risk group with increased TP53 mutations have a less favorable prognosis compared to those in the low-risk group who have fewer instances of TP53 mutations.

The correlation between the immune infiltrates in the TME and patient prognosis has been increasingly acknowledged in NSCLC and other types of cancer. The Immunoscore, initially developed as a tool to assess the risk of colon cancer, highlights the importance of evaluating immune infiltration in tumors to guide clinical decision-making [42–46]. The aim of this research was to examine the prevalence and functional scores of 22 distinct types of immune cells in NSCLC patients categorized into low-risk and high-risk groups. Additionally, our objective was to investigate the relative proportions of these immune cells within each group. The low-risk group demonstrated higher immune fraction and function scores for B cells, CTLs, checkpoint molecules, T cell inhibition, and T cell stimulation. Conversely, the high-risk group exhibited increased immune fraction and function scores for M0 and M2 macrophages. The presence of activated memory B cells, cytotoxic T cells, and CTLs is linked to a more favorable prognosis in cancer patients, indicating an enhanced immune response within the low-risk subgroup. These specific immune cells have a critical function in identifying and eliminating cancerous cells from the body. M0 macrophages are often regarded as unpolarized macrophages that remain inactive until they undergo polarization. Recent studies have provided evidence linking M0 macrophages to high-grade tumors and poor prognosis in glioma and hepatocellular carcinoma, suggesting their potential involvement in promoting tumorigenesis [47, 48]. Multiple pathways have been identified through which M2 macrophages contribute to tumor progression. For example,

researchers such as Yulei Chen et al. observed that CHI3L1 secretion by M2 macrophages triggers activation of the MAPK signaling pathway, thereby facilitating metastasis in gastric and breast cancer cells [49]. Exosomes released by lung tumor cells play a role in both immune suppression and tumor development within the TME by inducing M2 polarization and regulating mitochondrial metabolism [50]. In the context of chronic inflammation, particularly in advanced stages of cancer, myeloid cells such as macrophages, dendritic cells (DCs), and granulocytes have been observed to exert suppressive effects on immune function. These specific cell types are commonly referred to as myeloid-derived suppressor cells (MDSCs) [51]. Numerous studies have presented evidence suggesting that a decrease in the quantity of MDSCs is linked to a favorable response to ICIs, thus serving as a prognostic indicator for improved patient survival. Consequently, it can be inferred that MDSCs significantly influence the development of initial resistance [52,53]. The immunosuppressive capabilities of MDSCs may be augmented by upregulating the expression of additional immune checkpoint molecules on activated T cells [54]. Clinical investigations have revealed that administering anti-PD-1 antibodies triggers a signaling pathway involving PD-L1-NLRP3 inflammasome activation in cancerous cells, leading to an increased recruitment of poly-morphonuclear MDSCs (PMN-MDSC) into the TME. This process serves as an acquired resistance mechanism [55]. Our research results provide additional evidence to support these conclusions, suggesting that individuals in the low-risk subgroup demonstrate a greater likelihood of having tumor immunity, whereas those classified in the high-risk group exhibit traits linked to immune suppression.

We conducted an analysis to investigated the association between IRGPRI and well-known genes that act as checkpoints in the immune system, such as PDCD1, CTLA4, TIGIT, LAG3, and BTLA. These checkpoint genes hinder the activation and proliferation of T cells through various signaling pathways, thereby suppressing the immune response. It has been noted that tumors expressing these checkpoint genes are more responsive to ICIs therapy compared to those lacking their expression. As of May 2023, the FDA has approved five monoclonal antibodies (pembrolizumab [56], nivolumab [57], atezolizumab [58], durvalumab [59], and avelumab [60]) specifically targeting PD-1 or PD-L1 for treating NSCLC. However, there is currently no FDA-approved monoclonal antibody targeting CTLA-4 for NSCLC treatment despite its approval for melanoma and other cancer types. Limited research exists on investigating LAG3 and BTLA's potential use in NSCLC treatment; thus far, their therapeutic efficacy remains unestablished. In our investigation, we noticed an inverse relationship between IRGPRI scores and immune checkpoint gene expression. This suggests that individuals classified as low-risk may have a higher chance of benefiting from ICIs. To assess IRGPRI's predictive capacity in patient response to immunotherapy, we conducted an analysis on the IMvigor210 immunotherapy cohort. The findings suggested that the low-risk group exhibited a more positive reaction to immunotherapy, leading to better overall results in comparison to the high-risk group. Therefore, it is plausible to consider that involvement of IRGPRI may contribute to the determination of the effectiveness of cancer immunotherapy.

5. Conclusion

The construction of the model involved utilizing data acquired from the TCGA database, which was subsequently verified using the GEO dataset. IRGPRI has potential as a prognostic biomarker for guiding personalized clinical decision-making and identifying patients with NSCLC who could potentially experience positive outcomes from immunotherapy. However, it is crucial to note that this analysis is limited due to the inability of the TCGA database and GEO dataset to exclude NSCLC patients who did not receive immunotherapy. Future research should focus on validating the predictive role of IRGPRI in prognosis and ICIs therapy for NSCLC patients through laboratory experiments.

Declaration of figures authenticity

All figures provided have been exclusively generated by the authors, ensuring their originality and absence of any duplication or prior publication in whole or in part.

Funding

Ethics approval and consent to participate

Not applicable.

Consent for publication

All study subjects signed written informed consent.

Data availability statement

The present study utilized publicly available datasets, which can be accessed through the following sources: TCGA (https://portal.gdc.cancer.gov), GEO (https://www.ncbi.nlm.nih.gov/geo/), ImmPort (https://www.immport.org/shared/home), InnateDB (https://www.innateDBdb.com), and TISCH 2 (http://tisch.comp-genomics.org).

CRediT authorship contribution statement

Shenjing Cui: Writing – review & editing, Writing – original draft. **Yikun Yang:** Writing – review & editing, Writing – original draft. **Shuang Lou:** Software, Data curation. **Rong Huang:** Software, Formal analysis. **Jing Wang:** Software, Formal analysis. **Zhongbiao Chen:** Software, Formal analysis. **Jingjing Xie:** Software, Formal analysis.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Yikun Yang reports article publishing charges was provided by Shenzhen Key Medical Discipline Construction Fund. Shengjin Cui reports article publishing charges was provided by Basic Medical and Health Research Project of Baoan District. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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