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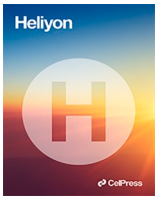

Research article

Potential immunologic and prognostic roles of CHRNA6 in SCLC and pan-cancer

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ABSTRACT

Background: Small cell lung cancer (SCLC) is considered the most malignant subtype of lung cancer, and it has a restricted range of therapeutic choices. The emergence of immunotherapy has offered new possibilities for patients with SCLC. However, the scarcity of clinical specimens has hampered the progress of clinical studies and we still face a shortage of dependable indicators to forecast the effectiveness of immunotherapy for SCLC.

Methods: In our study, we assessed the ImmuneScore and StromalScore of 81 SCLC samples obtained from the cBioPortal database. By comparing gene expression differences between the high and low immune scores groups, we identified 24 differentially expressed genes. Subsequently, an intersection was performed with genes that exhibited differential expression between normal and SCLC tissues, leading us to isolate the gene CHRNA6. To gain a deeper insight into the possible significance of CHRNA6 in SCLC, we singled out 50 genes that showed the most pronounced positive and negative associations with its expression. We then pinpointed hub genes for subsequent functional enrichment analyses by establishing a protein-protein interactions network. We additionally assessed the link between CHRNA6 expression in SCLC and characteristics of the immune microenvironment, along with the efficacy of immunotherapy, using the CIBERSORT, immunophenoscores (IPS), and tumor immune dysfunction and exclusion (TIDE) algorithms. Furthermore, we confirmed the prognostic impact of CHRNA6 expression in SCLC patients undergoing immunotherapy within a clinical cohort. Lastly, we obtained data from The Cancer Genome Atlas (TCGA) to investigate CHRNA6 expression in various tumors and its associations with genetic alterations, DNA methylation, copy number variation, clinicopathological characteristics, biological processes, immune microenvironment, prognosis, and drug sensitivity.

Results: In SCLC, we found that CHRNA6 function was associated with immune activation pathways such as antigen presentation processing and positive regulation of adaptive immune response, and that CHRNA6 demonstrated a strong correlation with immune cells infiltration. In addition, analysis of the clinical cohort revealed that patients with SCLC who exhibited elevated expression of CHRNA6 experienced better responses to immunotherapy. Our pan-cancer analysis disclosed that the expression of CHRNA6 is dysregulated in a multitude of cancers, potentially due to genetic mutations, copy number gains, and DNA demethylation. The gene set enrichment analysis (GSEA) outcomes indicated that CHRNA6 participates in immune responses and may play a positive immune regulatory role in most cancers. Furthermore, CHRNA6 has been observed

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to have a notable relationship with immune checkpoints, immunomodulators, immune cell infiltration, patient outcomes, and drug sensitivity across various cancers.
Conclusions: Our findings indicate that the CHRNA6 may act as a predictive indicator for SCLC patients receiving immunotherapy. The study also uncovers the aberrant expression of CHRNA6 in a range of human cancers and its potential roles in immunology and prognosis, offering novel perspectives for tailored cancer therapies.

1. Introduction

Globally, lung cancer is the primary reason of cancer deaths, with small cell lung cancer (SCLC) constitutes approximately 15 % of lung cancers [1]. SCLC is distinguished by its aggressive invasive properties, early dissemination and metastasis, and poor prognosis, and is the most malignant type of lung cancers, with a 5-year survival rate of <10 % [2,3]. SCLC typically responds well to chemotherapeutic agents, and in the past, patients with SCLC were mainly treated with chemotherapy [4,5].

More recently, the emergence of the immunotherapy has introduced innovative treatment to SCLC. Research such as IMpower133, CASPIAN, and CAPSTONE-1 indicates that the integration of immune checkpoint inhibitors with chemotherapy significantly enhances the survival in patients with extensive-stage SCLC, surpassing the results of chemotherapy alone, while also demonstrating a favorable safety profile [6–9]. Immunotherapy is also effective in patients with recurrent SCLC [10]. The integration of immunotherapy with chemotherapy has emerged as the new standard for the treatment in SCLC [3]. However, a limited number of patients can achieve lasting benefit from immunotherapy, and clarifying the benefit population and finding markers that can predict efficacy are key to studying SCLC.

Unlike non-small cell lung cancer (NSCLC), the molecular indicators that predict the effectiveness of immunotherapy for SCLC have not yet been pinpointed. Initially, SCLC was categorized into two types, classic and variant, and subsequently into neuroendocrine and non-neuroendocrine subtypes [11,12]. Recently, some studies have categorized SCLC into four distinct subtypes, distinguished by their transcription factors: ASCL1-defined (SCLC-A), NEUROD1-defined (SCLC-N), POU2F3-defined (SCLC-P), and YAP1-defined (SCLC-Y). Of these, the SCLC-Y subtype was considered a potential subtype that can benefit from immunotherapy [13,14]. Nevertheless, an exploratory analysis from the CheckMate 032 trial revealed that the SCLC-Y subgroup did not exhibit a substantial correlation with the therapeutic outcomes of SCLC immunotherapy. Furthermore, the study discovered that patients exhibiting elevated levels of expression for major histocompatibility complex class I (MHC-I) were more likely to experience improved survival following immunotherapy treatment [15]. In addition, one study found that YAP1 is also expressed in the SCLC-P subtype [16]. Therefore, YAP1 remains controversial as a marker for classifying SCLC subtypes and predicting the efficacy of SCLC immunotherapy. The latest study suggests a new classification system for SCLC, dividing it into four distinct categories: SCLC-N, SCLC-A, SCLC-P, and the newly identified SCLC-I. The SCLC-I subtype is characterized by an elevated expression of genes associated with antigen presentation and T cell gene expression profile linked to the 18-gene interferon-γ. Compared with the other subtypes, the SCLC-I subtype benefits most significantly from immunotherapy, but still requires to be validated by prospective studies with extensive participant numbers [17].

This study identified the gene CHRNA6, which is linked to the immune microenvironment of SCLC, and validated its impact on the prognosis of immunotherapy through clinical samples. This discovery offers a new perspective for identifying SCLC patient groups who may respond positively to immunotherapy, and guiding the clinical application of immunotherapy. Furthermore, using data from 33 different tumor types, our study uncovered the abnormal expression patterns of CHRNA6 across multiple cancers and confirmed its prospective significance in immunology and patient outcomes within pan-cancer, providing new ideas for personalized cancer therapy.

2. Materials and methods

2.1. Datasets

Small cell lung cancer transcriptome data from cBioPortal database and GEO dataset GSE60052. The cBioPortal database contains transcriptomic data for 81 SCLC samples as well as clinical data. Of these, 71 samples contain complete clinical information.

Transcriptional data, DNA methylation profiles, copy number variations and prognostic information of pan-cancer were retrieved from the UCSC Xena platform. Additionally, genetic mutation data from TCGA were accessed through the cBioPortal.

2.2. Calculation of ImmuneScore and StromalScore in SCLC

The ImmuneScore and StromalScore for each sample were determined through the application of the ESTIMATE method, leveraging molecular indicators to count immune and stromal cells. All SCLC samples were categorized into two distinct subgroups, determined by the median values of ImmuneScore and StromalScore. The identification of differentially expressed genes (DEGs) was performed by comparing groups with high and low scores using the library 'edgeR' (version: 3.40.2). The thresholds for statistical significance were set at a false discovery rate (FDR) below 0.05 and an absolute Fold Change (FC) exceeding 1. The visualization of heatmaps was constructed by the R package pheatmap, version 1.0.12.

2.3. Screening of differentially expressed genes in SCLC

Differentially expressed genes between SCLC and normal tissue were screened using GEO2R, with the criterion of an adjusted P value < 0.05. Subsequently, further manual screening was performed to select significant DEGs, which fulfilled the conditions of adjusted P-value less than 0.05 and absolute FC more than 4. We used a Venn diagram generated through the ggvenn library (version: 0.1.10), to illustrate the overlap of differential gene sets across the trio of groups.

2.4. Functional enrichment analysis of DEGs

The 'clusterProfiler' R package (version: 4.6.2) was utilized for performing Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses on the genes that exhibited differential expression. P value < 0.05 was considered to indicate statistical significance.

2.5. Construction and analysis of protein-protein interaction network

To further investigate the function of the key gene, protein-protein interaction (PPI) networks were developed based on the genes that had the strongest positive and negative correlations with the expression of the key gene in the cBioPortal database, respectively. The PPI networks were created with Cytoscape (version: 3.8.0) and STRING (version: 12.0) (https://stringdb.org) [18]. Following the construction of PPI networks, hub genes were screened through the cytoHubba plugin within Cytoscape [19]. The term "hub genes" refers to the top ten genes with the most interactions.

2.6. Immune cell proportion assessment in SCLC using the CIBERSORT method

CIBERSORT employs a computational method to ascertain the relative abundance of 22 immune cell types in tumors, relying on the LM22 gene expression signature [20]. Twenty-two immune cell ratios were calculated for 81 SCLC samples from the cBioPortal database using the package 'CIBERSORT' (version: 0.1.0) in R. Its parameter perm was set to 1000. To assess the link between key gene expression and immune cells, the samples were categorized into two cohorts at the median expression threshold of the gene, with subsequent comparison of the disparities between the groups.

2.7. Prediction of immunotherapy response in SCLC

The influence of gene expression on immunotherapy outcomes was evaluated through the calculation of the immunophenoscores (IPS) and tumor immune dysfunction and exclusion (TIDE) scores. IPS are calculated from the expression patterns of key genes or gene sets linked to MHC molecules, immune regulators, effector, and suppressor cells, offering a predictive measure for immunotherapy responsiveness [21]. IPS were computed from the package 'IOBR' (version: 0.99.9) in R [22]. The TIDE score can serve as an indicator of the effectiveness of immunotherapy by assessing the activity of tumor immune escape, and increased TIDE scores correlate with diminished effectiveness of immunotherapy (http://TIDE.dfci.harvard.edu) [23].

2.8. Patient cohorts

Paraffin-embedded tissue samples were collected from 55 individuals with SCLC between April 2019 and December 2023 at Zhongda Hospital, Southeast University. Among them, except for one patient who experienced recurrence after surgical resection and received immunotherapy, the rest of the patients had not undergone surgical treatment. The specific inclusion criteria of the patients were as follows: i) all patient specimens were confirmed to be small-cell lung cancer by pathological examination; ii) all patients were free of secondary tumors or other serious diseases and had not received preoperative chemotherapy or radiotherapy; iii) all patients had received at least two cycles of immunotherapy. Overall survival (OS) refers to the duration spanning from the initial diagnosis of a condition until the final follow-up or the occurrence of death due to any reason.

2.9. Immunohistochemistry

The immunohistochemistry (IHC) procedures for paraffin-embedded tissue microarrays (TMAs) were executed with precision following instructions. Initially, the paraffin-embedded sections underwent baking in a 62 °C environment for 60 min, then deparaffinized using xylene in two separate 20-min sessions (Sinopharm Chemical Reagent Co., Ltd., China). The sections were then rehydrated through a series of graded alcohol baths. Next, a single drop or 100 μl of 3 % hydrogen peroxide blocking solution was applied to the slide, and then incubated for 10 min. The slides were washed three times using phosphate-buffered saline (PBS), and then placed in a boiling antigen repair solution (Sigma, USA). The solution was brought to a rolling boil for 15 min, then held at that temperature for an additional 15 min. Afterward, the power was turned off, allowing the solution to cool naturally. The slides were washed again using the previously mentioned method. Subsequently, each slide received one drop or 100 μL of a 5 % BSA blocking solution (Sigma, USA), and was left to incubate at ambient temperature for a period of 20 min. Slides were then incubated with CHRNA6 antibody (1:200, Abcam, UK) overnight in a humidified room at 4 °C. After another round of washing in PBS three times, each slide received one drop or 50 μl of horseradish peroxidase labeled goat anti-rabbit IgG (Alkaline Tiangong Biotechnology Co., Ltd.,

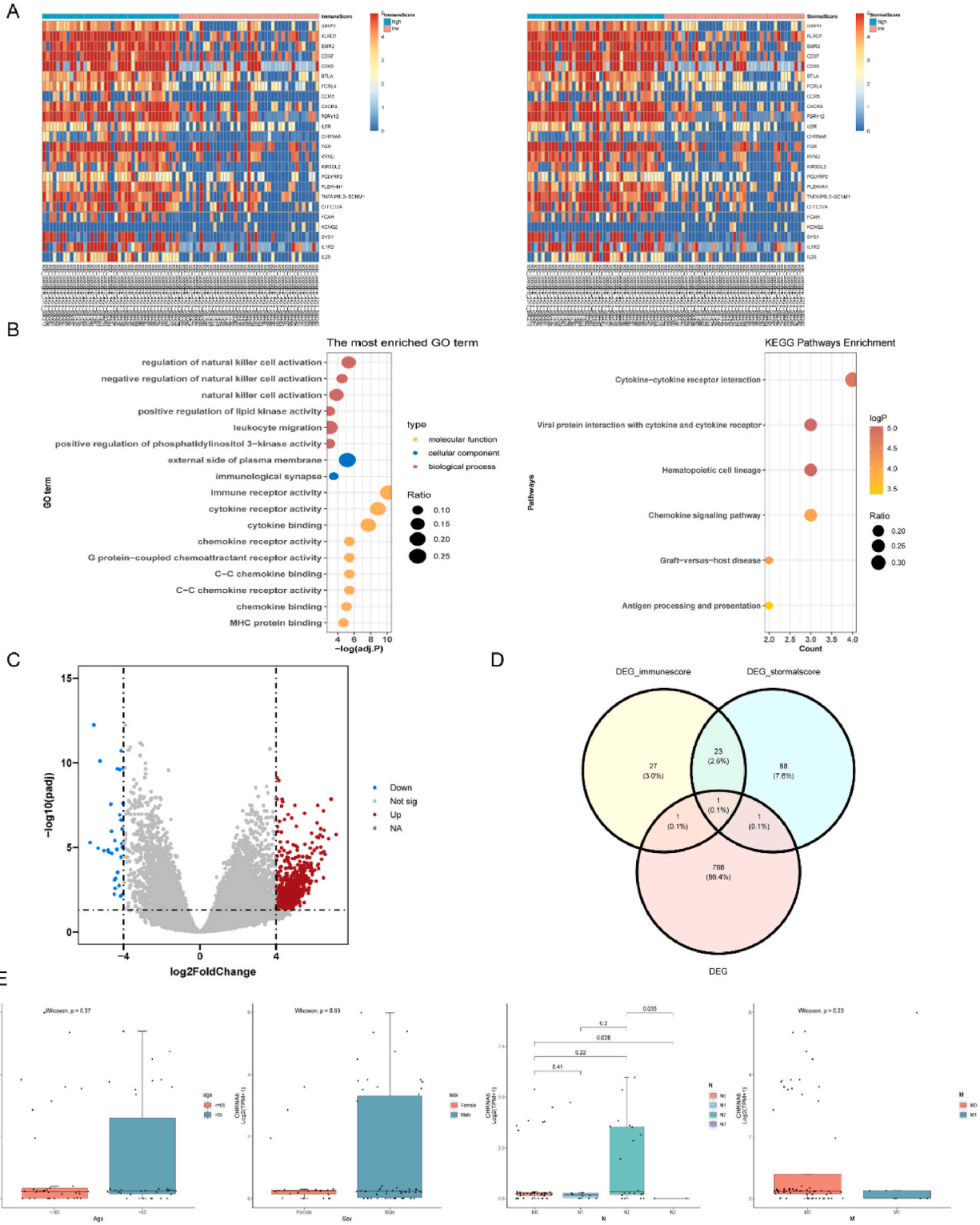

Fig. 1. Screening of key gene and its correlation with clinicopathologic features (A) The distribution of ImmuneScore and StromalScore of SCLC samples. (B) Functional enrichment analyses of DEGs. (C) The volcano plot of DEGs from dataset GSE60052. (D) Venn plot shows the number of immune-related differentially expressed genes crossed with genes expressed in SCLC. (E) Correlation of CHRNA6 expression with different ages, gender, N-stages and M-stages.

China) and was incubated for 30 min at 37 °C. Following three additional PBS washes, 3,3′diaminobenzidine (DAB) (Dako, Denmark) was applied to each slide for color development, and the staining levels were assessed using microscopic observation (Nikon, Japan). Next, the slides are placed into the hematoxylin staining solution for hydration treatment for 30–40 s. After being washed in PBS, the slides are successively rehydrated through a gradient of alcohol concentrations (from 70 % to 100 %) and xylene.

The slides were all scanned using a panoramic scanning device (Ningbo Jiangfeng Biological, China). Then, the area fraction of positive cells in a single field of view were measured by Fiji software.

2.10. Pan-cancer analysis

The analyses of the CHRNA6 expression in pan-cancer, along with its association with clinicopathological features, biological functions, immune scores, immune checkpoints, chemokines and their receptors, as well as molecules that either stimulate or suppress the immune response, and the immune cell infiltration, was conducted by the 'TCGAplot' package in R, version 7.0.1 [24].

The relationship between CHRNA6 expression levels and the prognosis of patients with various cancers was performed utilizing the GEPIA2 database, and presented the results with survival curves and log-rank P.

The Gene Set Cancer Analysis database (GSCA) was employed to assess the relationship between CHRNA6 expression and responses to drugs in the GDSC and CTRP databases (https://guolab.wchscu.cn/GSCA/).

2.11. Statistical analysis

The entire analytical process was performed by the R software. When it came to assessing differences between two groups, p-values were determined through the application of the Mann-Whitney *U* test. Spearman's rank correlation method was employed to determine the correlation. The R packages 'survival' (version: 3.5–7) and 'survminer' (version: 0.4.9) were utilized to conduct Cox analysis and produce Kaplan-Meier (KM) survival curves for examining the association between CHRNA6 expression and patient outcomes. The 'survivalROC' package in R (version: 1.0.3.1) was employed to craft receiver operating characteristic (ROC) curves, as well as to compute the area under the curve (AUC) values. The threshold for statistical significance was set at a p-value of below 0.05.

3. Results

3.1. Discovery of immune-associated genes in SCLC

To identify genes closely related to immunity, we calculated the ImmuneScore and StromalScore of 81 SCLC samples from the cBioPortal database, respectively. These samples were subsequently divided into two distinct groups: one with higher scores and another with lower scores, based on the median score value. Gene expression differences assessed comparatively between groups categorized by high and low ImmuneScore and StromalScore, respectively, and 24 differential genes were obtained after taking the intersection. The figures show the expression of these 24 differential genes in the ImmuneScore groups and the StromalScore groups, respectively (Fig. 1A).

3.2. Function enrichment analyses of DEGs

To further clarify the biological significance attributed to these 24 differential genes, we proceeded with enrichment analyses utilizing both GO and KEGG methods. The results revealed that a significant association existed between these genes and immune-associated functions. For example, the activation of natural killer cells, and interactions between cytokines and their receptors (Fig. 1B).

3.3. Identification of key gene

The dataset GSE60052 was utilized to pinpoint genes exhibiting differential expression between normal and SCLC tissues. A total of 771 genes displayed differential expression in SCLC, with 738 genes showing increased expression and 33 exhibiting decreased expression (Fig. 1C). The key gene CHRNA6 was obtained by taking the intersection with the previous 24 immune-related differential genes (Fig. 1D).

CHRNA6 plays an essential role in the cholinergic pathway and acts mainly by encoding the alpha subunit of the neuronal nicotinic acetylcholine receptor [25]. Recent studies suggested a strong link between the cholinergic pathway and cancer immunity. This pathway is capable of influencing the infiltration of immune cells through its regulatory effects on the microenvironment's components [26]. Refining the analysis of the correlation of ImmuneScore and StromalScore with CHRNA6, we discovered a positive correlation between CHRNA6 expression levels and both ImmuneScore and StromalScore (Figs. S1A and S1B).

3.4. Relationship between CHRNA6 expression and clinicopathologic characteristics

Wilcoxon rank-sum test was applied to assess potential disparities in CHRNA6 expression levels across subgroups categorized by various clinical and pathological features. The results revealed no significant variation in CHRNA6 expression across groups categorized by age, gender, and presence of distant metastasis, and CHRNA6 was mainly expressed in the group with fewer lymph node

metastases (Fig. 1E).

3.5. Identification of hub genes

To further investigate the function of CHRNA6, we identified the top 50 genes exhibiting the strongest positive and negative correlations to CHRNA6 expression in SCLC using the cBioPortal database and then constructed PPI networks, respectively (Fig. 2A–C). Subsequently, we utilized the Cytoscape to refine our analysis, determining ten hub genes from each group (Fig. 2D and E). To further elucidate the biological implications of these genes, we performed KEGG and GO enrichment assessments. The results of the GO enrichment analysis indicated that a significant association existed between the hub genes showing the strongest positive link to CHRNA6 expression, and immune activation processes. Specifically, these included processes such as antigen presentation processing, positive regulation of the adaptive immune response, and the enhancement of lymphocyte-mediated immunity (Fig. 2F). Concurrently, the KEGG enrichment analysis highlighted that the hub genes most strongly positively associated with CHRNA6 expression were primarily involved in critical biological processes like cytokine-cytokine receptor interactions and signaling pathways of the T cell receptor (Fig. 2F). On the other hand, the hub genes with the highest negative correlation with the expression of CHRNA6 were mainly associated with biological processes like chromosome segregation and the regulation of cell cycle phase transition (Fig. 2G).

3.6. Relationship between CHRNA6 and immune infiltration

Functional analyses of hub genes reveal a strong affiliation between CHRNA6 expression and the immune response. Consequently, we employed the CIBERSORT method to validate the link between CHRNA6 and immune constituents further. The findings indicated that the presence of dendritic cells was significantly higher within the group exhibiting elevated levels of CHRNA6 ($P < 0.05$) (Fig. 3A). The correlation study indicated a positive link between the quantities of memory B cells, dendritic cells, and follicular helper T cells and CHRNA6 expression (Fig. 3B).

3.7. Relationship between CHRNA6 and immunotherapy response

The efficacy of immunotherapy in groups exhibiting varying levels of CHRNA6 expression was evaluated using IPS and TIDE scores. IPS are composed of four main scores: MHC molecules scores, effector cells scores, suppressor cells scores, and immune checkpoints scores. Notably, prior research has indicated that the level of PDL1 expression does not correlate with the effectiveness of immunotherapy in SCLC [27]. Therefore, we only calculated the results for the three kinds of scores. There was no significant difference in MHC molecule scores between the high and low CHRNA6 expression subgroups; however, the scores for effector cells were elevated in the high CHRNA6 expression group, while the low CHRNA6 expression group exhibited higher suppressor cell scores (Fig. 3C). Correlation analysis did not uncover any substantial association between CHRNA6 expression levels and MHC molecule scores. However, CHRNA6 expression correlates positively with the scores of effector cells, while it exhibits a negative correlation with the scores of suppressor cells ($P < 0.05$) (Fig. 3D).

To enhance the understanding of CHRNA6's predictive capacity for immunotherapy outcomes, the TIDE score was utilized within the SCLC cohort. Our research revealed that individuals with elevated CHRNA6 expression levels had comparatively lower TIDE scores (Fig. 3E). To sum up, individuals exhibiting elevated CHRNA6 expression levels may derive greater benefits from immunotherapy treatments. Furthermore, an inverse relationship was identified between CHRNA6 expression and TIDE scores **(**Fig. 3F**)**.

3.8. Survival analysis of CHRNA6 expression for SCLC patients

Table 1 presents the basic details of all the patients involved in the study. The levels of CHRNA6 expression were analyzed using the fraction of positive cells in a single field of view observed in Image-FiJi after immunohistochemical staining. We collected a normal sample for immunohistochemical staining to verify the difference in CHRNA6 expression between normal and SCLC tissues (Fig. 4A). Patients were divided into two groups—those with high and those with low expression of CHRNA6—based on the median expression value of 0.0556 as a threshold value. The differential analysis indicated that there were no statistically significant disparities in clinicopathological features—including age, gender, and lymph node metastasis—between the groups with elevated and reduced CHRNA6 expression levels (Fig. S2).

Further survival analysis demonstrated that patients with tumors exhibiting low levels of CHRNA6 expression had a notably decreased OS compared to those with high CHRNA6 expression levels (Fig. 4B). The ROC curves revealed CHRNA6 as a good predictor of immunotherapy outcome, exhibiting AUC scores of 0.717, 0.805, and 0.749 at the 1st, 3rd, and 5th year marks. (Fig. 4C). Furthermore, univariate Cox analysis revealed that increasing age ($P < 0.05$; $HR = 1.072$) predicted an unfavorable prognosis, whereas higher levels of CHRNA6 expression are suggestive of more favorable prognoses ($P < 0.05$; $HR = 0.772$), as detailed in Fig. 4D. The multivariate Cox analysis showed that patients expressing higher amounts of CHRNA6 had a 0.786-fold lower risk of death (Fig. 4E). As such, CHRNA6 can be an independent prognostic indicator in SCLC.

3.9. Expression of CHRNA6 in pan-cancer

We assessed the CHRNA6 levels in 33 tumor categories from the TCGA database. The findings indicated that the expression of CHRNA6 was notably elevated in several cancers, including bladder uroepithelial carcinoma (BLCA), colorectal adenocarcinoma

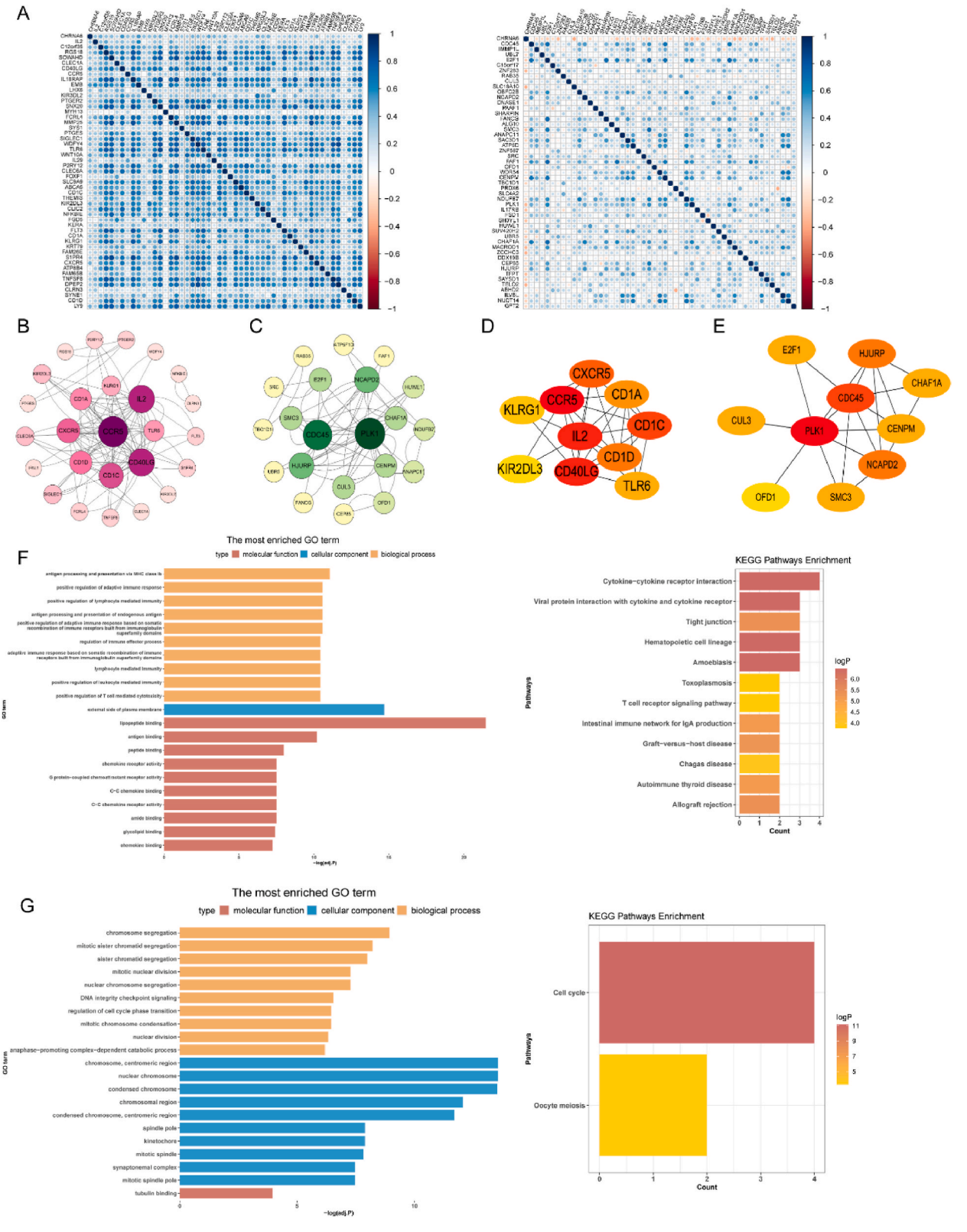

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Fig. 2. Building PPI networks and delineating the role of hub genes (A) The 50 most positively and negatively correlated genes to CHRNA6 expression. (B) Network of genes positively correlated to CHRNA6 expression. (C) Network of genes negatively correlated to CHRNA6 expression. (D) Hub genes highly positively associated with CHRNA6 expression. (E) Hub genes highly negatively associated with CHRNA6 expression. (F) GO and KEGG enrichment analyses of hub genes with the highest positive correlation with CHRNA6 expression. (G) GO and KEGG enrichment analyses of hub genes with the highest negative correlation with CHRNA6 expression.

(COAD), breast invasive carcinoma (BRCA), cholangiocarcinoma (CHOL), esophageal carcinoma (ESCA), kidney renal clear cell carcinoma (KIRC), head and neck squamous cell carcinoma (HNSC), kidney renal papillary cell carcinoma (KIRP), lung adenocarcinoma (LUAD), liver hepatocellular carcinoma (LIHC), rectum adenocarcinoma (READ), lung squamous cell carcinoma (LUSC), and stomach adenocarcinoma (STAD). Conversely, we observed significant reductions in CHRNA6 levels in thymoma (THYM) and pheochromocytoma and paraganglioma (PCPG), as compared to controls (Fig. 5A). The paired analysis obtained similar results, except that the result for uterine corpus endometrial carcinoma (UCEC) became significant (Fig. 5B).

3.10. Genetic alteration, DNA methylation and copy number variation analysis of CHRNA6 in pan-cancer

We investigated genetic alterations in CHRNA6 using the cBioPortal dataset and showed an overall mutation rate of 4 % for CHRNA6 (Fig. 5C). The highest frequency of CHRNA6 alterations appeared in UCEC (>10 % alteration frequency), BRCA, and BLCA (Fig. 5D). Genomic amplification is the predominant form of genetic alteration observed in the majority of tumors, followed by mutations and deep deletions (Fig. 5D). Epigenetic analyses revealed a large inverse correlation between methylation levels at multiple positions of the CHRNA6 gene and mRNA expression levels in various tumor types, including BRCA, CHOL, KIRC, mesothelioma (MESO), acute myeloid leukemia (LAML), adenocarcinoma (PRAD), brain lower grade glioma (LGG), thyroid carcinoma (THCA), prostate skin cutaneous melanoma (SKCM), and uveal melanoma (UVM) (Fig. 5E). Analysis of copy number variation showed that across the majority of tumor categories, an amplification of the CHRNA6 gene copy number was detected, whereas copy number deletions of the gene affected a significantly smaller fraction of the cases (Fig. 5F). The findings imply that the up-regulation of CHRNA6 in multiple tumors might be regulated by genetic alterations, amplification of DNA copy number, and changes in DNA methylation.

3.11. Association of CHRNA6 expression with clinicopathologic features in pan-cancer

We then analyzed the link between CHRNA6 expression and clinicopathological characteristics in pan-cancer. In ESCA, KIRP, ovarian serous cystadenocarcinoma (OV), STAD, and THCA, CHRNA6 expression was higher in patients ≤60 years of age, whereas in both types of tumors, BRCA and LGG, CHRNA6 expression was higher in patients older than 60 years of age (Fig. 6A). We also found that in LUAD and LGG, female patients exhibited notably higher levels of CHRNA6 expression compared to their male counterparts (Fig. 6A). When assessing CHRNA6 expression across diverse cancer stages, a trend was discovered where higher CHRNA6 expression correlated with earlier stages in cancers such as BRCA, kidney chromophobe (KICH), LUAD, and LUSC, whereas the opposite results were shown in ESCA, HNSC, pancreatic adenocarcinoma (PAAD), and READ (Fig. 6B). These results imply a strong connection between CHRNA6 expression levels and the clinical and pathological characteristics of the tumors.

3.12. Pan-cancer GSEA-GO and GESA-KEGG analysis

We conducted GESA-GO and GESA-KEGG analysis to explore the biological significance of CHRNA6 in pan-cancer. The GSEA-GO findings indicated a positive link between CHRNA6 expression levels and immune functions in most tumors, including antigen processing and presentation, and lymphocyte-mediated immunity, while was negatively correlated with antigen processing and presentation and major histocompatibility complex class II (MHC-II) protein complex assembly in LAML (Fig. 7A). GESA-KEGG analysis revealed a positive correlation between CHRNA6 and antigen processing and presentation in KIRC, LIHC, UVM and uterine carcinosarcoma (UCS), and an inverse relationship with oxidative phosphorylation was identified in BRCA, COAD, KIRP, and UCEC (Fig. 7B). These data suggest that, as in SCLC, CHRNA6 plays a positive immune regulatory role in most tumors and shows a strong correlation with antigen processing and presentation.

3.13. Association of CHRNA6 expression with immune microenvironment in pan-cancer

To delve into how CHRNA6 expression relates to the immune microenvironment, we first analyzed the relationship between CHRNA6 and immune scores, immune checkpoints, chemokines, chemokine receptors, immune stimulatory molecules, and immune suppressive molecules. The findings showed a positive link between CHRNA6 expression and immune scores in most cancers. However, there was no correlation between the two in adrenocortical carcinoma (ACC), CHOL, and LAML (Fig. 8A). CHRNA6 expression tends to positively correlate with immune checkpoints-associated genes in all tumors except LAML (Fig. 8B). More interestingly, we found that in LAML, CHRNA6 expression bore an inverse relationship with genes associated with chemokines and chemokine receptors, contrasting with a positive correlation observed in all other types of tumors examined (Fig. 8C and D). In addition, in the majority of cancers, CHRNA6 expression demonstrated a positive association with a wide range of immune-modulating genes, both inhibitory and stimulatory. In contrast, in LAML, the relationship with these genes exhibited a negative correlation (Fig. 8E and F).

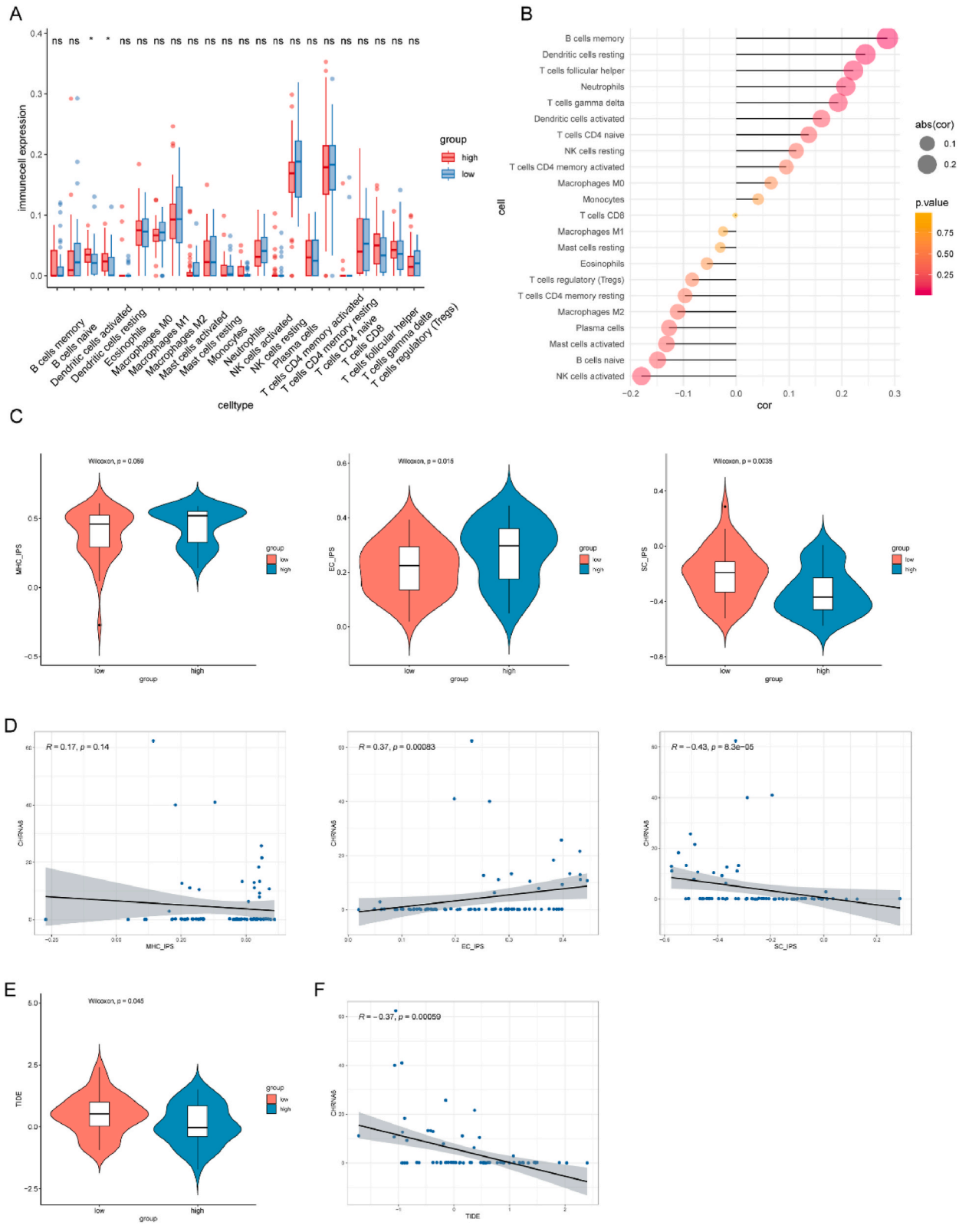

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Fig. 3. The correlation of CHRNA6 expression with immune infiltration, IPS and TIDE scores (A) Differences in the presence of immune cells between groups with elevated and reduced CHRNA6 expression levels. (B) Correlation of CHRNA6 expression with the level of immune cells infiltration. (C) Differences in IPS between groups exhibiting high and low levels of CHRNA6 expression. (D) Correlation of CHRNA6 expression with IPS. (E) Differences in TIDE scores groups exhibiting high and low levels of CHRNA6 expression. (F) Correlation of CHRNA6 expression with TIDE scores. *P < 0.05. ns meant no significant difference.

Table 1
Clinical and pathological features of SCLC patients.

Variables	N (%)
Age	
≥65	31(56.36 %)
<65	24(43.64 %)
Gender	
Male	45(81.82 %)
Female	10(18.18 %)
T	
T1	7(12.73 %)
T2	19(34.55 %)
T3	14(25.45 %)
T4	15(27.27 %)
N	
N0-N1	5(9.09 %)
N2	22(40.00 %)
N3	28(50.91 %)
M	
M0	20(36.36 %)
M1	35(63.64 %)

Secondly, we also analyzed the correlation of CHRNA6 expression with 22 types immune cells. The results indicated that in the majority of tumors, CHRNA6 exhibited a positive correlation with several immune cell types, including resting dendritic cells, resting CD4 memory T cells, naïve B cells, M1-type macrophages, regulatory T cells, and neutrophils. In contrast, an inverse association was observed between CHRNA6 expression and both natural killer (NK) cells and naïve CD4 T cells (Fig. 8G). These analyses suggest that CHRNA6 has a significant association with the immune microenvironment of most tumors and holds potential to be a prognostic indicator for predicting the efficacy of immunotherapy.

3.14. Pan-cancer prognosis and drug sensitivity analyses of CHRNA6

Survival analysis indicated that individuals with high CHRNA6 expression in ACC, HNSC, LUAD, and SKCM generally had longer survival times, while those with high CHRNA6 expression in UVM had a poorer prognosis (Fig. 9A). Cox regression analysis revealed that elevated levels of CHRNA6 expression were linked to improved OS in LUAD, LAML, KIRC, and ACC, while it was linked to poorer OS in LGG (Fig. 9B). Similarly, high CHRNA6 expression was identified as a protective factor for disease-specific survival (DSS) in ACC, KIRP, and LUAD, but a risk factor for DSS in LGG (Fig. 9C).

Subsequent study of the relationship between CHRNA6 expression and drug sensitivity in the CTRP and GDSC databases showed a negative correlation between CHRNA6 expression and the drug's half-maximal inhibitory concentration (IC50) for the majority of pharmaceuticals (Fig. 9D and E). This implies that the levels of CHRNA6 expression could potentially act as an indicator to predict the responsiveness of cancer patients to specific drugs.

4. Disscusion

While the advent of immunotherapy improves the prognosis of patients with SCLC, only a small number of patients experience long-lasting benefits. PD-L1 is widely recognized as a valid biomarker for measuring the effectiveness of immunotherapy in NSCLC, however, unlike NSCLC, its value in forecasting the effectiveness of immunotherapy in SCLC patients is very limited. Studies have found that PDL1 is poorly expressed in SCLC and is not associated with immunotherapy efficacy [27]. The search for biomarkers that predict the effectiveness of immunotherapy is necessary to better predict efficacy and to ensure that different patients with SCLC achieve maximum benefit from immunotherapy.

The tumor microenvironment, comprising immune cells, cancer-associated fibroblasts (CAFs), and other elements, significantly influences tumor progression and patient outcomes [28]. Therefore, in this study, we initially screened the immune-related gene CHRNA6 in SCLC using ImmuneScore and StromalScore. CHRNA6 is one of the key genes in the cholinergic system, which is closely related to tumor growth and metastasis. However, its impact on the tumor immune microenvironment has been little studied [29,30].

Previous studies have suggested that the poor response to SCLC immunotherapy is primarily associated with the majority of SCLC having low or absent MHC-I [15,31]. SCLC exhibiting low or absent MHC-I features may result in an inhibitory immune microenvironment that prevents SCLC patients from benefiting from immunotherapy [32]. In this study, we found that the function of CHRNA6

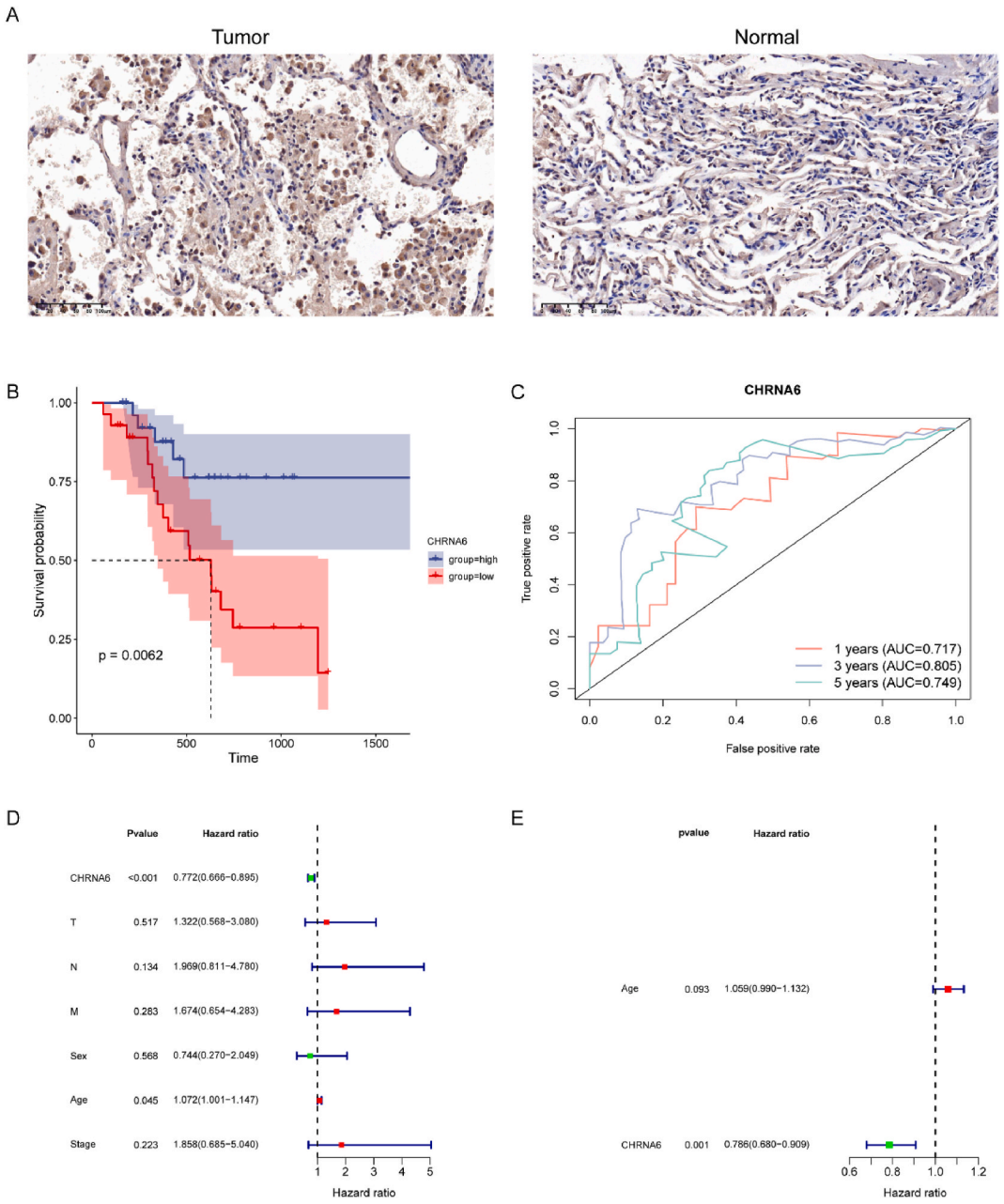

Fig. 4. Prognostic analyses in the clinical cohort (A) Representative immunohistochemical images of CHRNA6 expression in SCLC tissue and normal tissue. (B)KM curves of 55 SCLC patients. (C) Time-dependent ROC curves for CHRNA6 at 1, 3, and 5-Year Markers. (D–E) Univariate and multivariate COX regression forest plots.

in SCLC is predominantly associated with immune mechanisms, including antigen presentation processing and positive regulation of adaptive immune response. Moreover, correlation analysis involving 22 types of immune cells revealed a positive association between CHRNA6 expression and dendritic cells and B cells in SCLC. Dendritic cells are a type of specialized antigen-presenting cell with an abundance of antigen-presenting molecules (MHC-I and MHC-II) on their surfaces [33]. B-cells also function as vital components in antigen presentation throughout the body and are linked to the effectiveness of immunotherapy for a multitude of cancers [34,35].

Subsequently, we also performed the prediction of immunotherapy efficacy by IPS and TIDE scores and found that SCLC patients exhibiting elevated CHRNA6 levels had a higher probability of responding positively to immunotherapy treatments. Therefore, we posit that CHRNA6 has the potential to be a marker for forecasting the efficacy of immunotherapy in SCLC. We also used immunohistochemical staining of clinical samples to verify the impact of CHRNA6 expression on the survival outcomes of SCLC patients receiving immunotherapy, and consistent with the above analyses, it was observed that patients with elevated CHRNA6 levels

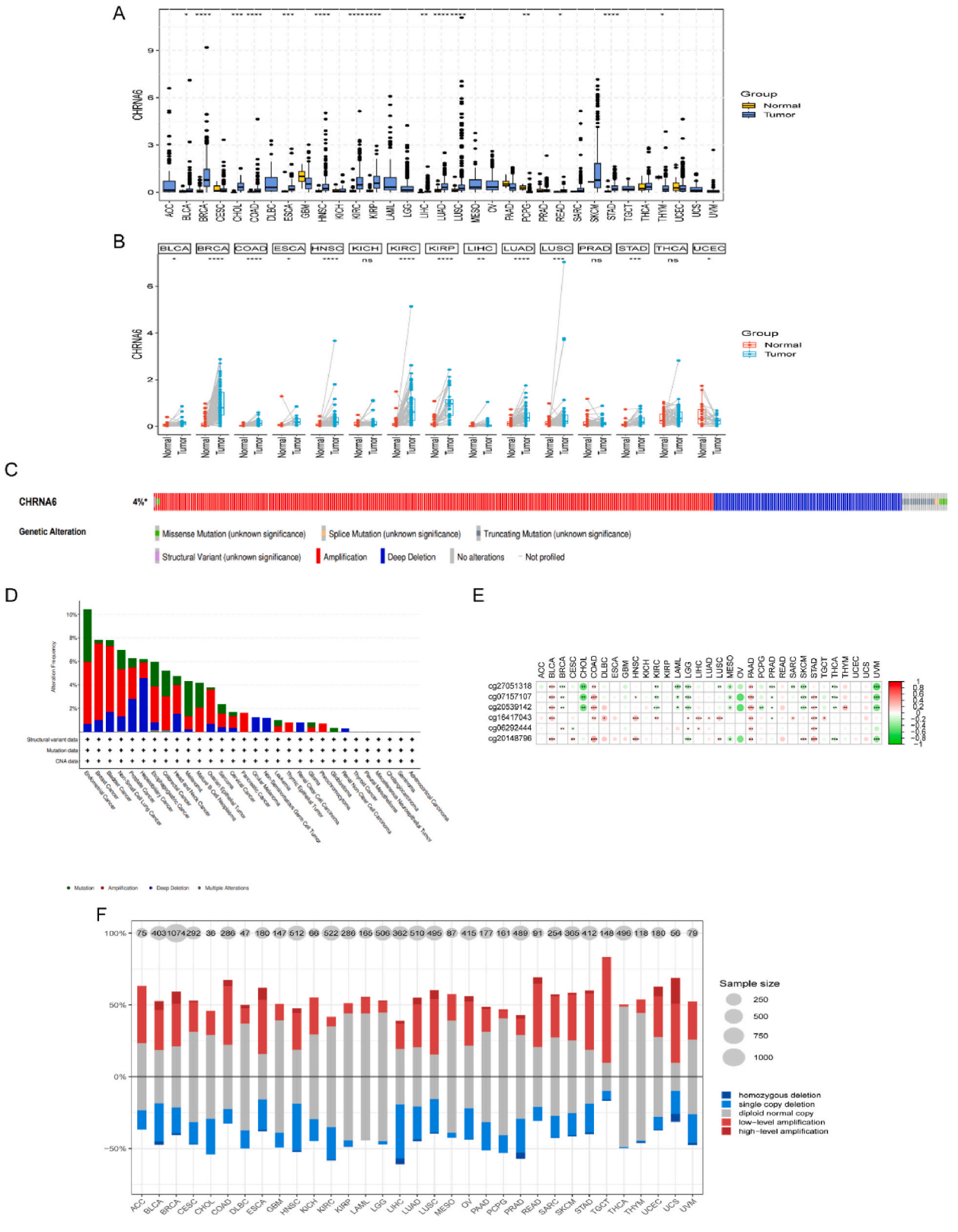

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Fig. 5. CHRNA6 expression across various cancer types (A) CHRNA6 expression in tumor and normal samples for all tumor categories from TCGA. (B) CHRNA6 expression between matched tumors and their corresponding normal samples for 15 tumor categories. (C) Mutation frequency of CHRNA6. (D) The CHRNA6 gene alteration status in cancers based on cBioPortal database. (E) Correlation of CHRNA6 expression with DNA methylation in pan-cancer. (F) DNA copy number variation analysis of 33 tumors. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. "ns" indicates no significant difference.

experienced longer OS. Multivariate Cox analysis further confirmed that CHRNA6 emerged as an independent prognostic indicator for SCLC patients undergoing immunotherapy.

For the pan-cancer bioinformatics study, we performed an exhaustive analysis of CHRNA6 at multiple levels, encompassing its tumoral expression, genetic alterations, epigenetic alterations, copy number variation, biological function, tumor microenvironment, prognosis, and drug responsiveness. We discovered that CHRNA6 is highly expressed in tumors and that this may result from genetic alterations, DNA methylation and copy number variations.

The levels of CHRNA6 expression correlate with clinicopathological characteristics in various tumors. CHRNA6 was found to have higher expression levels in patients under 60 years of age and those with early stages tumors. Functional analysis of CHRNA6 in pan-cancer showed that it is mainly linked to the antigen processing and presentation, aligning with the analysis results in SCLC. Besides, the results indicated that CHRNA6 expression were positively linked to immune scores, immune checkpoints, chemokines, chemokine receptors, immune stimulatory molecules, immune suppressive molecules, and immune cell infiltration in most tumors. These findings imply that CHRNA6 could be correlated with better tumor staging and tumor microenvironment. Survival analyses indicated a positive correlation between CHRNA6 expression and better prognosis in several cancers, and further COX regression analyses identified CHRNA6 as a protective factor for ACC and LUAD.

We also performed drug sensitivity analysis and discovered an inverse relationship between CHRNA6 expression and the IC50 of many drugs from the CTRP and GDSC databases. The discoveries imply that CHRNA6 may be an indicator for forecasting the efficacy of these drugs. However, the mechanisms are not clear and further studies are still needed.

In conclusion, our study provides an extensive analysis of CHRNA6 in SCLC and pan-cancer, revealing its value in tumor immunity and prognosis. The results showed that CHRNA6 exhibited significant up-regulation in the majority cancer tissues when contrasted with normal tissues. In many cancers, CHRNA6 was closely related to the immune microenvironment and may play a positive immune regulatory role. In addition, the expression levels of CHRNA6 were found to be significantly correlated with clinicopathological features and outcomes of tumors. Therefore, CHRNA6 may serve as an indicator for forecasting patient outcomes and evaluating the effectiveness of cancer immunotherapies.

5. Limitations

There are several limitations to our study. Primarily, it is a retrospective analysis conducted at a single center with limited participant numbers, and thus, corroboration through larger, multicenter trials is warranted. Second, the immunomodulatory mechanism of CHRNA6 in tumors is unclear and remains to be further investigated by fundamental experiments. Finally, the pan-cancer study relied primarily on public databases, which may inevitably introduce systematic bias.

Ethics approval and consent to participate

This study was approved by the Medical Ethics Committee of Zhongda Hospital, Southeast University, China (Approval number: 2022ZDKYSB350).

Consent for publication

Not applicable.

Availability of data and materials

The datasets underpinning the findings of this article can be accessed openly from cBioPortal database (https://www.cbioportal.org/), GEO dataset GSE60052 (https://www.ncbi.nlm.nih.gov/geo/), GEPIA2 database (http://gepia2.cancer-pku.cn) and UCSC Xena database (https://xenabrowser.net/). All the other data of this study can be accessed from the article, or by reaching out to the corresponding authors with a reasonable inquiry.

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CRediT authorship contribution statement

Qingqing Zhao: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis.

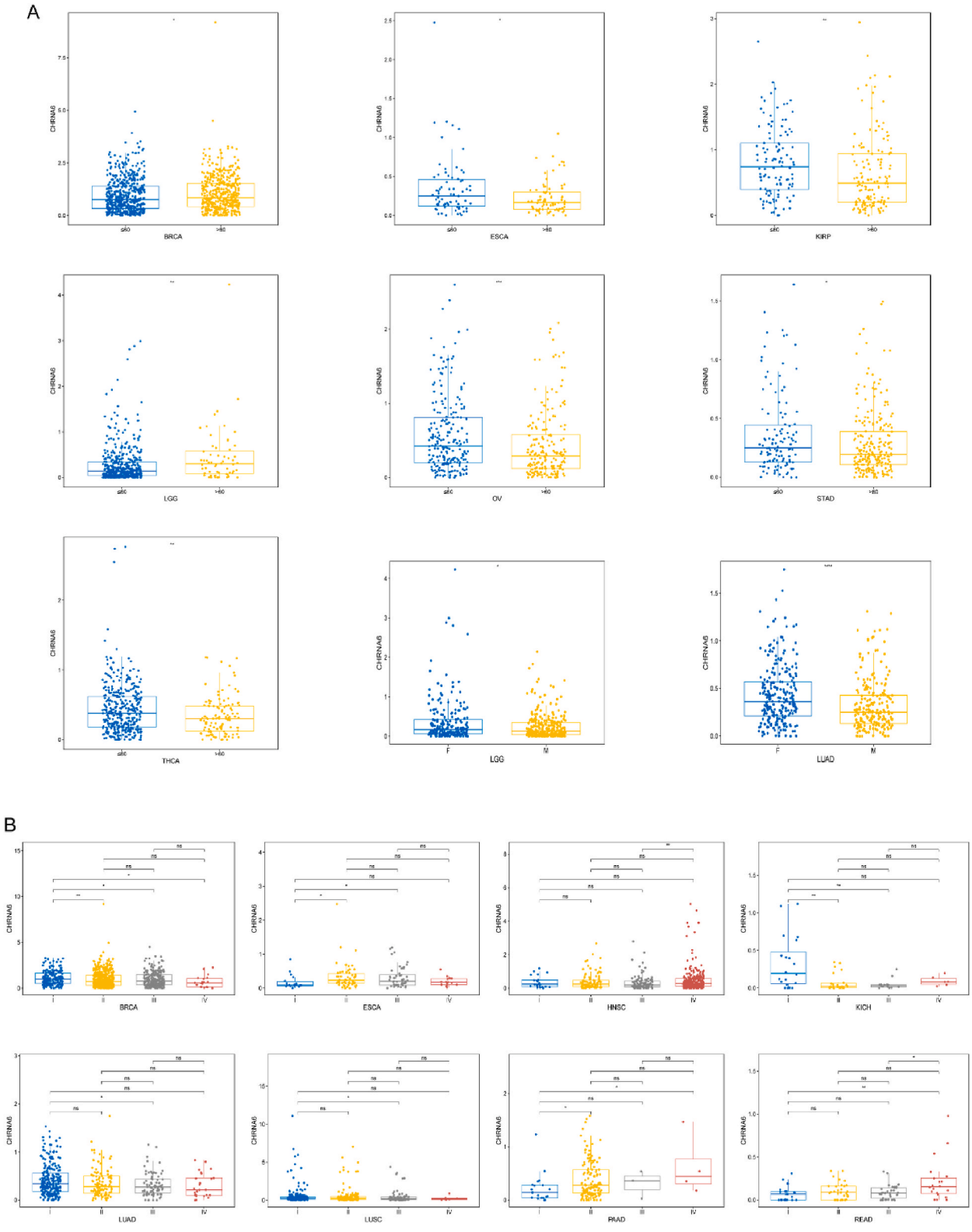


Fig. 6. Association of CHRNA6 expression with clinicopathologic features (A) Correlation of CHRNA6 expression with different ages and gender. (B) Correlation of CHRNA6 expression with different stages.

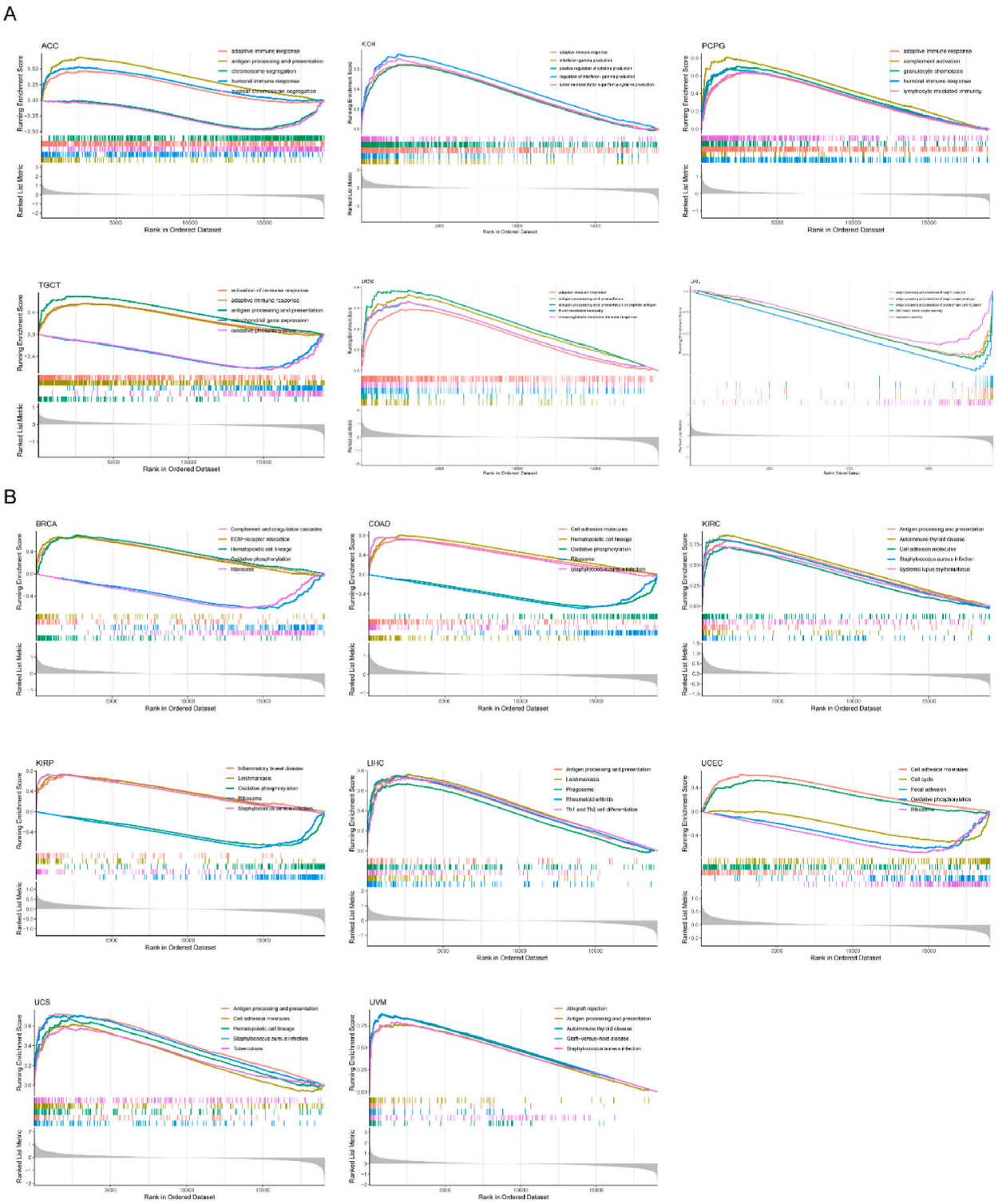


Fig. 7. GSEA analysis in pan-cancer (A) GO-based GSEA analysis. (B) KEGG-based GSEA analysis.

Cong Wang: Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Wucui Huang:** Writing – review & editing, Writing – original draft, Resources, Formal analysis, Data curation, Conceptualization. **Zhongquan Song:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Conceptualization. **Yang Lang:** Writing – review & editing, Writing – original draft, Resources, Data curation, Conceptualization. **Xiaoli Zhu:** Writing – review & editing,

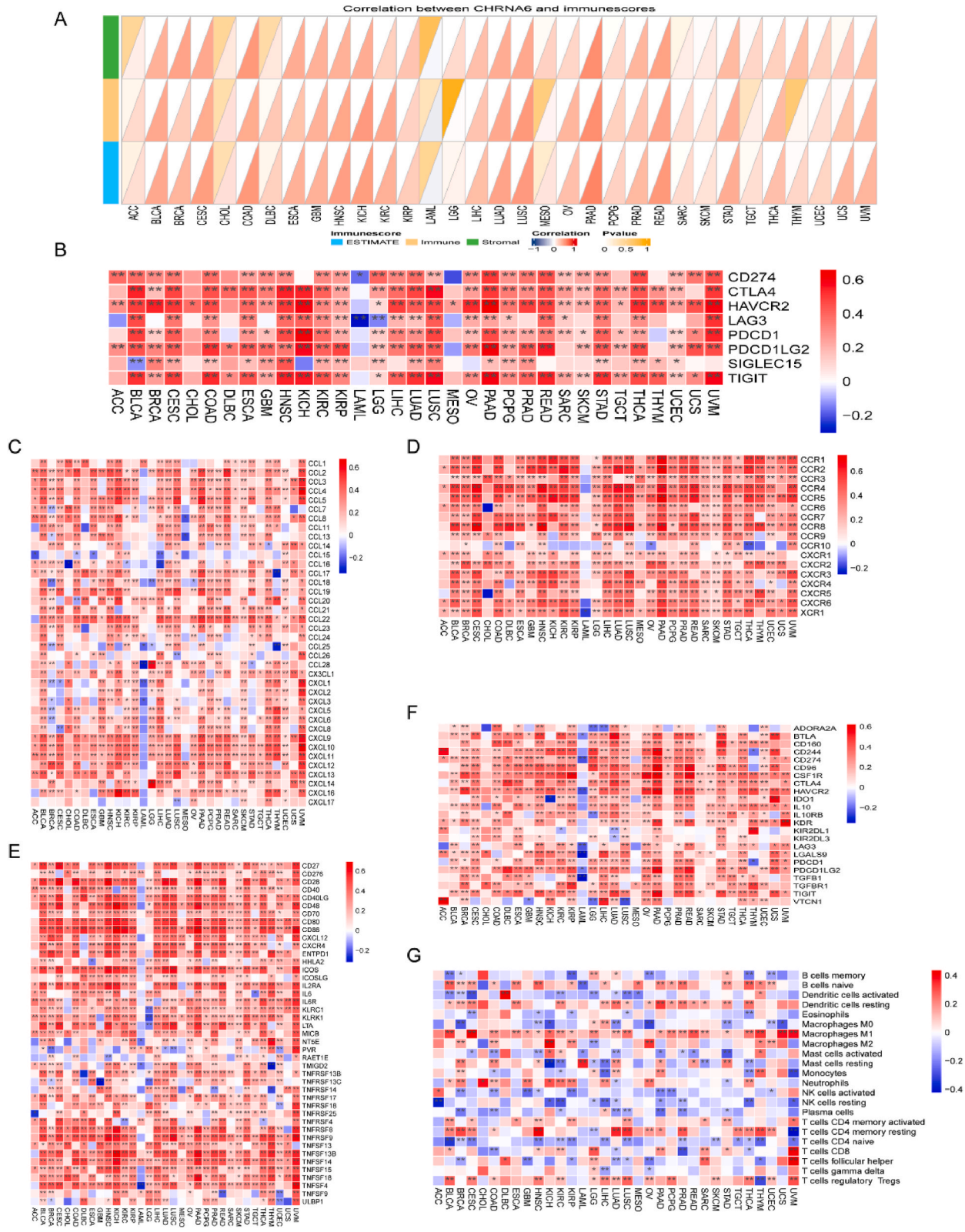
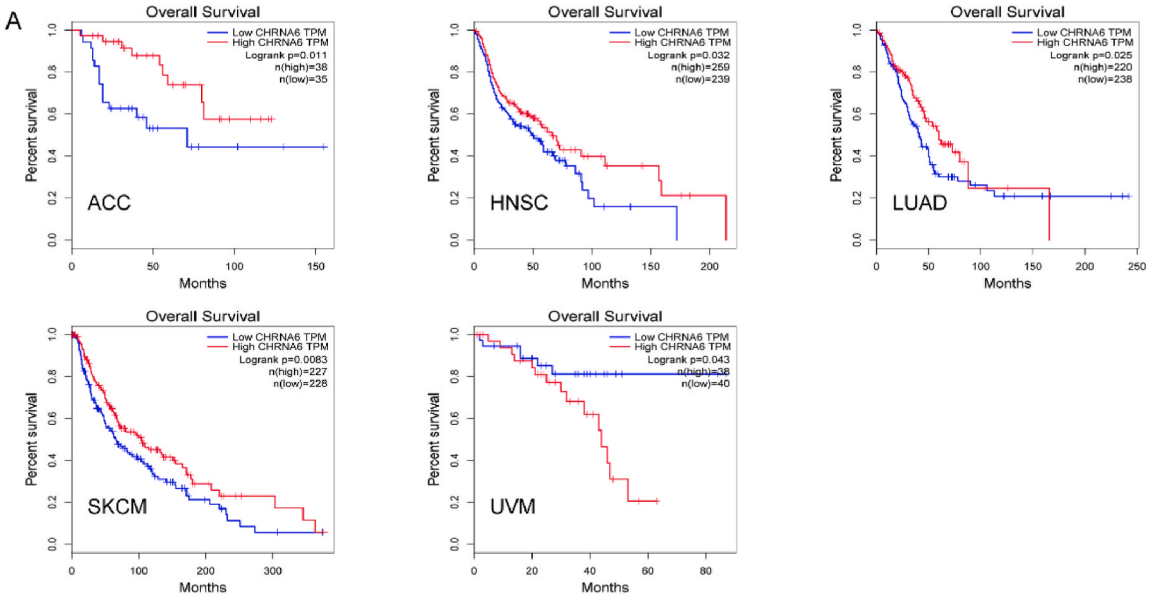
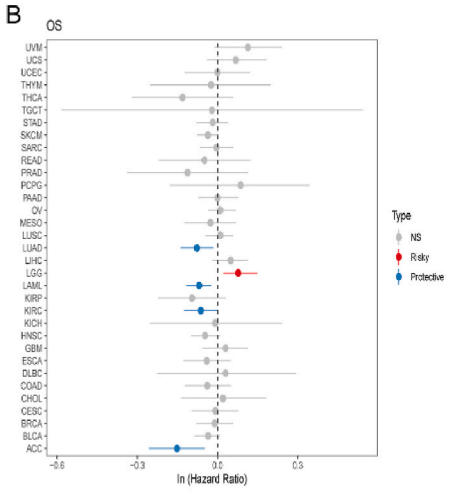
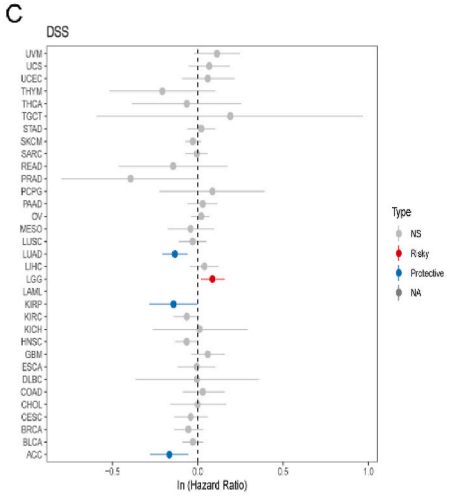
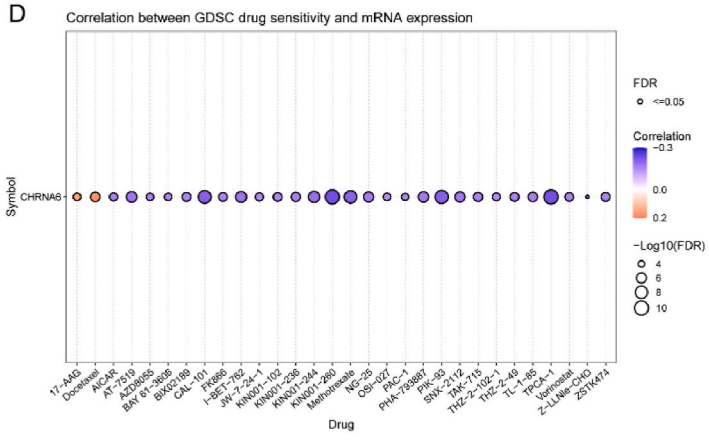


Fig. 8. Correlation of CHRNA6 with immune microenvironment in pan-cancer (A–G) Correlation between CHRNA6 expression and immune scores, immune checkpoints, chemokines, chemokine receptors, immune stimulatory molecules, immune suppressive molecules, and immune infiltration.









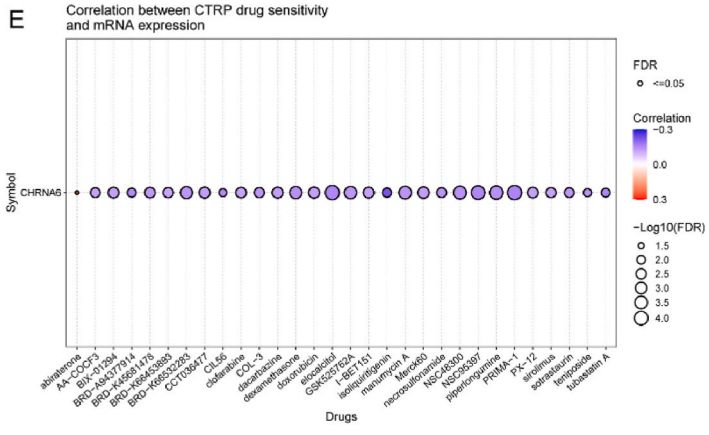


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Fig. 9. Correlation of CHRNA6 expression with prognosis and drug sensitivity in pan-caner (A) Representative survival curves for prognostic analysis comparing patients with CHRNA6-high and CHRNA6-low in ACC, HNSC, LUAD, SKCM, and UVM. (B–C) Univariable Cox regression analysis on the Impact of CHRNA6 Expression on OS and DSS in pan-cancer. (D) Association of CHRNA6 with top 30 GDSC drug sensitivity. (E) Association of CHRNA6 with top 30 CTRP drug sensitivity.

Writing – original draft, Supervision, Resources, Methodology.

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.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e38572.

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