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Research Article

A pan-cancer analysis revealed *SKP2* as an inhibitor of the tumor immune microenvironment and a promising therapeutic target for immunotherapy

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Abstract

Background: S-Phase kinase associated protein 2 (*SKP2*) is a key regulator of the cell cycle and proliferation linked to cancer development. Our recent study has revealed that knocking out *Skp2* in a mouse model significantly activates anti-tumor immunity. Although several studies have examined *SKP2* in relation to the tumor immune microenvironment using public datasets, a comprehensive pan-cancer evaluation that integrates multi-omics layers and *in vivo* validation has remained limited.

Methods: In this study, we integrated multi-omics data from diverse public datasets to comprehensively analyze *SKP2* expression characteristics and its relationship to tumor immunity across pan-cancer. A multiplex immunofluorescence assay was performed on tumors from *Skp2* knockout and *Skp2*-intact mouse models for validation.

Results: Our findings indicate that *SKP2* is overexpressed in various cancer types, leading to poor prognosis. Single-cell transcriptomic analyses further revealed that *SKP2* is predominantly expressed in malignant and immune cells. Notably, a multiplex immunofluorescence assay on tumors from *Skp2* knockout and *Skp2*-intact mouse models and pan-cancer data unveiled a correlation between *SKP2* and the "immune-cold" microenvironment, which, possibly linked to the weakened antigen presentation, reduced secretion of chemokines in *SKP2*-overexpressing cancers. Additionally, we observed that *SKP2* overexpression predicts worse immunotherapy efficacy.

Conclusion: Our findings provide novel insights into the role of *SKP2* in regulating the tumor immune microenvironment, suggesting targeting *SKP2* as a promising strategy to enhance immunotherapy efficacy in pan-cancer settings.

Keywords: *SKP2*; tumor immune microenvironment; pan-cancer; multi-omics; prognosis

Introduction

Cancer remains a leading cause of death globally, with an estimated 20 million new cases alongside 9.7 million deaths in 2022, imposing significant economic and social burdens worldwide and hindering improvements in human life expectancy [1]. While the count of cancer survivors is on the rise in developed nations, primarily due to advancements in early prevention and treatment methods, the mortality rate among cancer patients globally continues to exhibit a notable upward trend [2]. In recent years, there has been increasing recognition of the role of immunotherapy in cancer treatment [3, 4]. These therapies are gradually becoming established as a standard treatment modality, alongside conventional approaches like radiotherapy and chemotherapy [5]. However, despite the success of immune checkpoint inhibitors (ICIs) in treating certain cancer patients, only a small subset of individuals have benefited from immunotherapy thus far [6, 7]. Immunotherapy resistance poses a significant obstacle to the development and clinical application of these treatments [8]. Therefore, there is an urgent need to explore biomarkers associated with tumor prognosis and the efficacy of immunotherapy, as well as to develop sensitizers to enhance its effectiveness.

The *SKP2* gene encodes S-phase kinase associated protein 2 (SKP2), which functions as a substrate-recruiting subunit of the SCF–Skp2 E3 ligase complex, providing specificity for particular protein substrates [9, 10]. Previous research has indicated that *SKP2* primarily functions through the NUCKS1–SKP2–p27/p21 axis to regulate the cell cycle transition from G0/G1 phase to S phase [11]. In various types of cancer, *SKP2* overexpression leads to the degradation of p27/p21, resulting in cell cycle dysregulation and promoting cell proliferation [12, 13]. In addition, SKP2 targets various tumor suppressor proteins such as p21, FOXO1, and p130, as well as oncogenic proteins including Akt, YAP, and Twist [9, 14, 15]. By modulating the abundance or function of these proteins, SKP2 participates in multiple cellular processes, including apoptosis, DNA damage repair, and cell differentiation [10].

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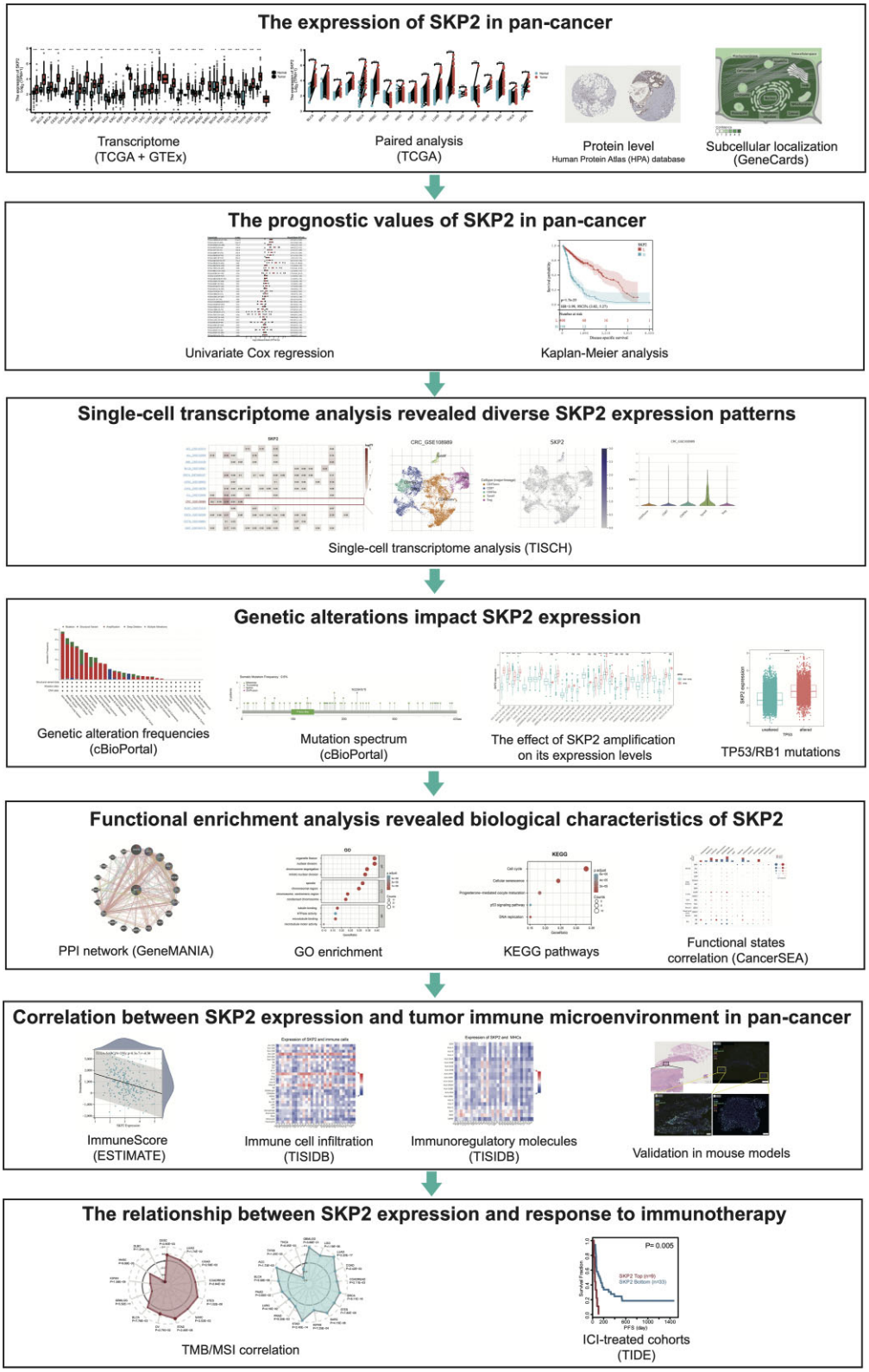


Figure 1. Flow chart summarizing the study design and principal results, integrating bulk and single-cell transcriptomics, genomic alterations, functional enrichment, TIME associations, and clinical immunotherapy outcomes, with *in vivo* validation.

While prior studies, including some of our own, have linked *SKP2* to immune regulation in selected contexts [16–18], an integrated pan-cancer analysis spanning bulk and single-cell transcriptomes, genomic alterations, cellular and acellular tumor immune microenvironment (TIME) components, and *in vivo* validation has been lacking. Here, we systematically address this gap and relate *SKP2* to immunotherapy outcomes across cohorts. Using an integrated multi-omics approach, we show that elevated *SKP2* is broadly associated with an immune-cold TIME across solid tumors, identify a negative association between *SKP2* expression and immunotherapy outcomes across multiple ICIs treatment cohorts, and validate the inhibitory effect of *Skp2* on the tumor immune microenvironment in *Skp2*-knockout osteosarcoma models via multiplex immunofluorescence (mIF) (Fig. 1).

Methods

Gene expression analysis

SKP2 mRNA expression profiles and clinical data from 33 cancer types and corresponding paracancer and normal samples were downloaded from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) databases. R software (v 4.0.2) was used for data analysis, and the R package "ggplot2" (v 3.3.3) was applied for visualization. Wilcoxon tests were used to analyze significant differences. The Human Protein Atlas (HPA) database (https://www.proteinatlas.org/) was used to evaluate the protein expression differences of SKP2. The subcellular locations of *SKP2* were visualized using GeneCards (https://www.genecards.org/).

Survival prognosis analysis

Cox's proportional hazards regression model and Kaplan–Meier analysis were employed to analyze the relationship between *SKP2* expression and prognosis by SangerBox [19]. The results of univariate Cox regression were visualized by "forest plot". R packages "survival" (v 3.2–7) and "survminer" (v 0.4.9) were used to draw the survival curve. R package "maxstat" (v 0.7–25) was utilized to locate the optimal cut-off point of *SKP2* expression levels.

Single-cell analysis of *SKP2*

The Tumor Immune Single-cell Hub (TISCH) database (http://tisch.comp-genomics.org/home/) was employed to quantify the expression level of *SKP2* in different cell types. The functional status of *SKP2* in several cancer types at the single cell level was evaluated by CancerSEA (http://biocc.hrbmu.edu.cn/CancerSEA/). It contains 14 tumor-related cellular functions of 41 900 single cancer cells from 25 cancers.

Genetic alteration analysis

cBioPortal (https://www.cbioportal.org/) is a website that can analyze tumor genomic characteristics [20]. The mutation type, alteration frequency, and copy number alteration of *SKP2* across all tumors were analyzed in "Cancer Types Summary". The mutated site information of *SKP2* was explored in "Mutations". Moreover, we obtained information from this website on whether *SKP2* was amplified and whether *TP53*/*RB1* was mutated for each sample, and further analyzed the differences in *SKP2* expression between subgroups.

Co-expressed genes and enrichment analysis of *SKP2*

GeneMANIA (http://genemania.org/) is a website that can explore gene interactions and functions and identify co-expressed genes [21]. A total of 20 genes co-expressed with *SKP2* were obtained through GeneMANIA. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment were performed using the "clusterProfiler" (v 3.14.3) and "org.Hs.eg.db" (v 3.10.0) package [22]. The results are presented using the "ggplot2" (v 3.3.3) R package. P value < 0.05 was considered to be statistically significant.

Relationship between *SKP2* expression and immunity

To evaluate the relationship between *SKP2* expression and TIME, the ImmuneScore of each patient in each tumor was calculated using the R package "ESTIMATE" (v 1.0.13) [23]. Further, Pearson's correlation coefficient of *SKP2* expression and ImmuneScore in each tumor was calculated using the corr.test function of the R package psych (v 2.1.6). The TISIDB database (http://cis.hku.hk/TISIDB/) is an online tool that can evaluate the interaction between cancer and the immune system [24]. The correlations between *SKP2* expression and molecular or immune subtypes of different cancer types were explored via the TISIDB database.

Establishment of mouse models

Osx1-Cre, $Rb1^{lox/lox}$, $Trp53^{lox/lox}$, and $Skp2^{-/-}$ mice have been previously characterized [25–27]. All experimental mice were maintained on hybrid backgrounds, including FVB, C57BL6J, and 129Sv. Initially, $Rb1^{lox/lox}$ mice were bred with $Trp53^{lox/lox}$ mice to generate $Trp53^{lox/lox}$; $Rb1^{lox/lox}$ offspring, which were subsequently crossed with Osx1-Cre mice to produce Osx1-Cre; $Trp53^{lox/lox}$; $Rb1^{lox/lox}$ mice. $Skp2^{-/-}$ mice were then bred with Osx1-Cre; $Trp53^{lox/lox}$; $Rb1^{lox/lox}$ mice to generate Osx1-Cre; $Rb1^{lox/lox}$; $Trp53^{lox/lox}$; $Skp2^{-/-}$ progeny. All animals were housed under pathogen-free conditions at the Beijing Key Laboratory for Musculoskeletal Tumors animal facility. The experimental procedures were reviewed and approved by the Ethics Committee of Peking University People's Hosptial (2024PHB432-001), following established guidelines for ethical animal care. All methods are reported in accordance with Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. Tumor diameters were measured every 3 days using a caliper, and relative tumor volume was determined using the formula: (length × width^2) × 0.526. Tumors were excised once their volume reached ∼500 mm^3.

mIF

To validate the relationship between *SKP2* expression and immunity, an mIF staining assay was conducted on osteosarcoma tissues derived from three *Skp2* knockout and three control mouse models. The transgenic mouse models used in this study have been detailed in our earlier publication [16]. Multiplex immunofluorescent staining was performed utilizing the PANO 7-plex IHC kit (cat 0 004 100 100; Panovue, Beijing, China). The following antibodies were utilized: F4-80 (clone D2S9R, rabbit monoclonal antibody, 1:200, #70 076; CST), PDL1 (clone D5V3B, rabbit monoclonal antibody, 1:100, #64 988; CST), CD8A (clone D4W2Z, rabbit monoclonal antibody, 1:100, #98 941; CST), granzyme B (clone E5V2L, rabbit monoclonal antibody, 1:100, #44 153; CST), CD3 (clone SP162, rabbit monoclonal antibody, 1:100, ab135372; Abcam), and PD1 (clone D7D5W, rabbit monoclonal antibody, 1:100, #84 651; CST). These antibodies were applied sequentially,

followed by incubation with a horseradish peroxidase-conjugated secondary antibody and subsequent tyramine signal amplification. After each tyramide signal amplification (TSA) operation, the slides underwent microwave treatment. Subsequently, nuclei were stained with 4′-6′-diamidino-2-phenylindole (DAPI, Sigma-Aldrich) after all human antigens had been labeled. Whole-slide fluorescent imaging was conducted using an Olympus VS200 (Olympus Germany) with an Olympus UPLXAPO 20x objective lens. The resulting whole-slide fluorescence images were analyzed using QuPath software.

SKP2 expression and immunotherapy response

Tumor mutation burden (TMB) and microsatellite instability (MSI) were obtained from the TCGA database. Correlation analysis between *SKP2* expression and TMB or MSI was conducted using Spearman's method. The TIDE website (http://tide.dfci.harvard.edu) was used to analyze the prognostic KM curves of high- and low-expression groups of *SKP2* in different immunotherapy cohorts.

Results

Overexpression of *SKP2* in human pan-cancers

We initiated our analysis by examining *SKP2* mRNA expression in both tumor and normal tissues across 33 different cancer types. The abbreviations and full names of these cancer types are provided in the Abbreviations section below. Unpaired analysis was conducted by combining TCGA and GTEx transcriptome datasets. The results revealed a significant upregulation of *SKP2* expression in tumor tissues across the majority of cancers (26 out of 33), including ACC, BLCA, BRCA, CHOL, COAD, DLBC, ESCA, GBM, HNSC, KIRC, KIRP, LGG, LIHC, LUAD, LUSC, MESO, OV, PAAD, PCPG, READ, SARC, SKCM, STAD, TGCT, THYM, UCEG, and UCS. Conversely, *SKP2* exhibited a significant downregulation in a subset of cancers (4 out of 33), including KICH, LAML, PRAD, and THCA. Despite integrating data from both TCGA and GTEx databases, significance *P*-values could not be calculated for several cancer types, including MESO, SARC, and UVM, due to the limited number of normal samples available (Fig. 2A). Paired analysis using TCGA data yielded trends consistent with unpaired analysis, with the exception that the differences in PAAD and PRAD were no longer statistically significant (Fig. 2B).

Subsequently, we extended our analysis to the protein level by utilizing the HPA database to investigate the expression disparities of SKP2 between tumor and normal tissues across various cancer types. Immunohistochemistry results showed a higher staining intensity of SKP2 in tumor tissues compared to their corresponding normal tissues across many cancers, mainly including BRCA, COAD, UCEC, LIHC, PAAD, PRAD, STAD, THCA, lung cancer, and renal cancer (Fig. 2C). Finally, a cellular distribution diagram sourced from GeneCards revealed that *SKP2* is predominantly localized in the nucleus and cytosol, with minimal presence in organelles and the extracellular region (Fig. 2D). In summary, the collective findings indicate that *SKP2* is frequently overexpressed across cancers, highlighting its potential clinical significance and warranting further exploration.

Elevated expression of *SKP2* is associated with unfavorable prognosis

To elucidate the correlation between *SKP2* expression and clinical outcomes, we conducted comprehensive investigations into the prognostic significance of *SKP2* across various cancer types using univariate Cox regression and Kaplan–Meier analysis. Initially, our analysis focused on the association between *SKP2* expression and disease-specific survival (DSS), revealing a correlation between high *SKP2* expression and poor DSS in GBMLGG, LGG, KIPAN, KICH, ACC, KIRP, MESO, LIHC, SKCM, and PRAD (Fig. 3A). Subsequently, to further stratify patients based on *SKP2* expression levels, we categorized them into high- and low-expression groups using the optimal cut-off of *SKP2* expression. Kaplan–Meier analysis validated that higher *SKP2* expression was associated with poor DSS in GBMLGG, LGG, ACC, KIPAN, KICH, and KIRP (Fig. 3B).

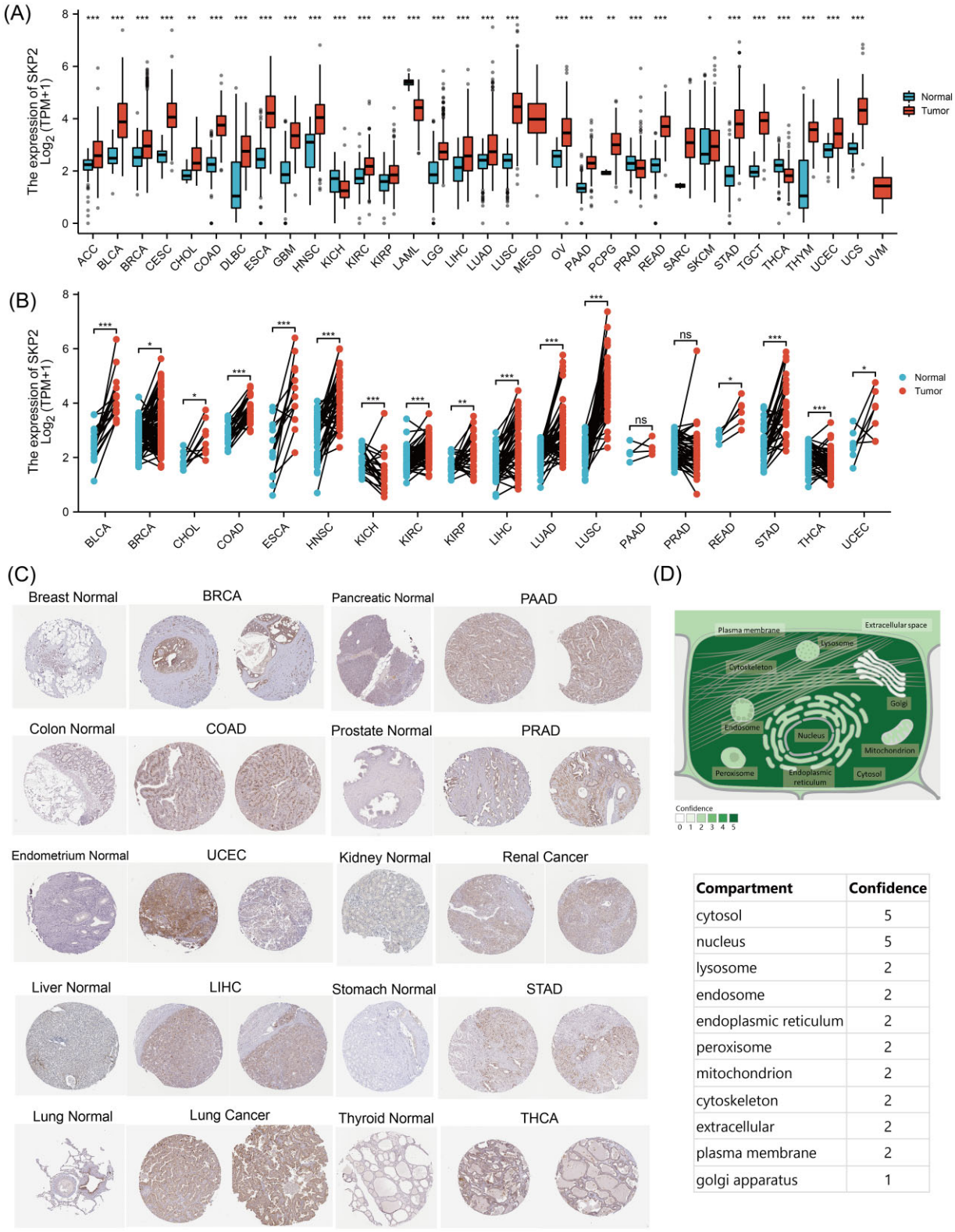
Regarding progression-free interval (PFI), our univariate Cox regression analysis demonstrated a significant correlation between elevated *SKP2* expression and poor PFI in GBMLGG, LGG, ACC, KIPAN, KICH, KIRP, LIHC, MESO, and UVM (Fig. 3C). The subsequent Kaplan–Meier analysis revealed that the *SKP2*-high group exhibited worse PFI in GBMLGG, LGG, ACC, KIPAN, KICH, and KIRP, aligning with the observed trends in DSS (Fig. 3D). Collectively, these findings suggest that elevated expression of *SKP2* is closely associated with an unfavorable prognosis, highlighting its potential significance in the occurrence and progression of various cancers.

Single-cell transcriptome analysis revealed diverse SKP2 expression patterns

For a comprehensive exploration of *SKP2* expression in diverse cell types, we conducted single-cell transcriptome analyses across multiple cancer datasets, investigating its levels in various cell subtypes. Generally, *SKP2* exhibited the highest expression in malignant cells; however, intriguingly, we observed moderate expression of *SKP2* in specific immune cell types, particularly in certain subtypes of T cells and monocytes/macrophages (Fig. 4A). Despite these commonalities, the expression patterns of *SKP2* still varied across different cancers (Fig. 4B–F). For example, in HNSC and NPC, *SKP2* was expressed at its highest levels in malignant cells. Additionally, monocytes/macrophages and certain T cell subtypes, such as proliferating T cells, CD8 T cells, and exhausted CD8 T cells, also exhibited moderate levels of *SKP2* expression. Similarly, in CRC and NSCLC, T cells, particularly proliferating T cells, were found to exhibit a moderate to high level of *SKP2* expression. However, in SCLC, while malignant cells displayed high levels of *SKP2* expression, monocytes/macrophages and T cells showed nearly undetectable levels of *SKP2* expression. These findings contribute further insights into the single-cell expression profile of *SKP2*, suggesting that beyond its role in tumorigenesis, *SKP2* may also be associated with tumor immunity.

Genetic alterations impact SKP2 expression

Given the significant impact of genetic variations on gene expression levels, we further explored the genetic alterations of *SKP2* across various cancers using TCGA datasets through the cBioPortal platform. Genetic alterations in *SKP2* were identified across multiple cancer types, with the highest frequencies observed in NSCLC, BLCA, and ESCA at 9.59%, 8.27%, and 7.56%, respectively. However, no *SKP2* mutations were observed in eight other cancer types, including LAML, CHOL, and THCA. Furthermore, among all types of genetic alterations, amplification was the most prevalent, followed by mutation and deep deletion for *SKP2* (Fig. 5A). Additionally, we delved into the somatic mutation landscape of *SKP2* across various cancers. The overall somatic mutation frequency of *SKP2* across various cancers is 0.6%, with a total of 59 mutation sites identified. Missense mutations are predominant, with



Compartment	Confidence
cytosol	5
nucleus	5
lysosome	2
endosome	2
endoplasmic reticulum	2
peroxisome	2
mitochondrion	2
cytoskeleton	2
extracellular	2
plasma membrane	2
golgi apparatus	1

Figure 2. *SKP2* expression analysis in human pan-cancer. (**A**) *SKP2* mRNA expression level in normal tissues and cancers from the TCGA and GTEx databases. (**B**) Paired analysis of *SKP2* mRNA expression in paracancerous tissues and cancers from the TCGA database. (**C**) Protein expression of SKP2 in human tumours and normal tissues from the HPA database. (**D**) *SKP2* expression mapping was obtained from the GeneCards database. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. ns, Not significant.

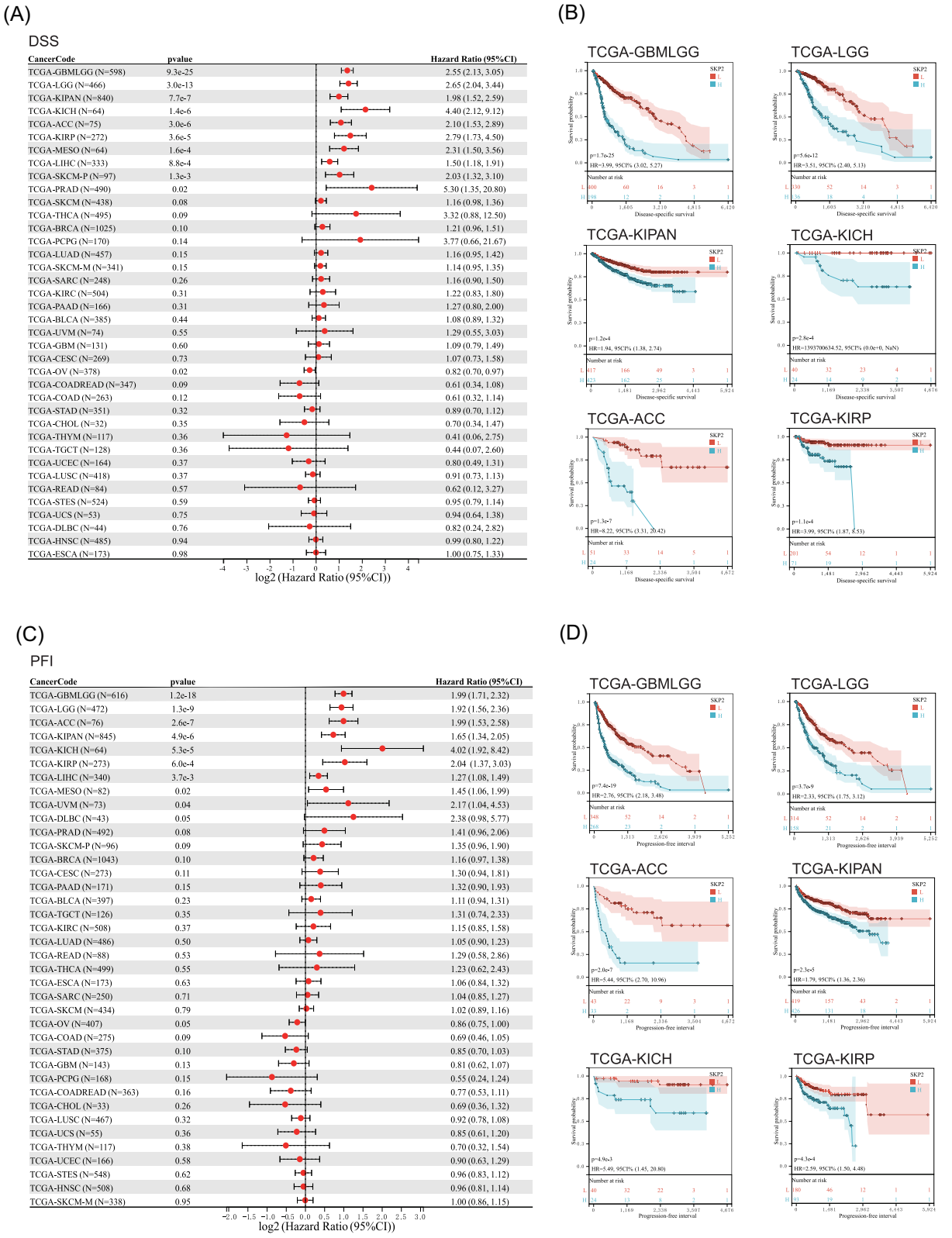


Figure 3. Prognostic significance of *SKP2* expression in pan-cancer. (**A**) Forest plots show the correlation between *SKP2* expression and DSS in different cancers. (**B**) Kaplan–Meier curves for a patient's DSS classified by different expression levels of *SKP2* in GBMLGG, LGG, KIPAN, KICH, ACC, and KIRP. (**C**) Forest plots show the correlation between *SKP2* expression and progression-free interval (PFI) in different cancers. (**D**) Kaplan–Meier curves for a patient's progression-free interval classified by different expression levels of *SKP2* in GBMLGG, LGG, KIPAN, KICH, ACC, and KIRP.

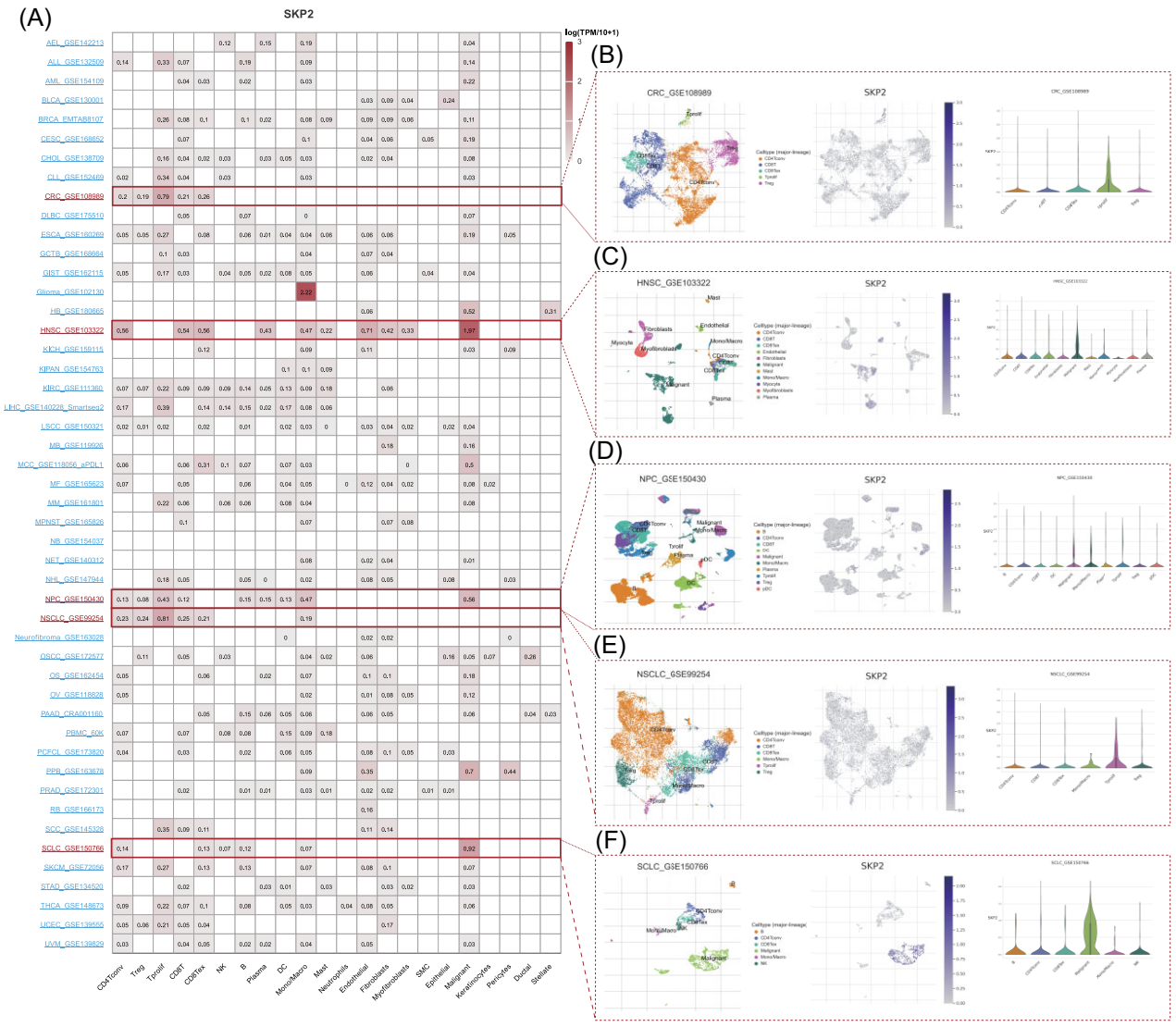


Figure 4. Single-cell expression of *SKP2* from the TISCH database. (**A**) *SKP2* expression of various cell types in different cancers. (**B–F**) Single-cell profile and *SKP2* expression in CRC, HNSC, NPC, NSCLC, and SCLC.

the mutations at the N229Kfs*8 and R182C sites exhibiting the highest frequencies (Fig. 5B).

Given that amplification is the predominant alteration type of *SKP2*, we conducted an analysis of the effect of *SKP2* amplification on its expression levels across various cancers. The findings indicated that *SKP2* amplification led to an elevation in the mRNA expression levels of *SKP2* (Fig. 5C). Furthermore, as common tumor suppressor genes, *TP53* and *RB1* are closely associated with the regulation of the cell cycle, and previous literature has reported their potential interaction with *SKP2* [10, 28, 29]. Our findings suggest that genetic alterations in *TP53* and *RB1* can also upregulate *SKP2* expression (Fig. 5D). Finally, to demonstrate the independent effect of *SKP2* amplification on enhancing *SKP2* expression, patients were grouped based on the presence or absence of genetic alterations in *SKP2* and *TP53/RB1*. The results consistently showed higher *SKP2* expression levels irrespective of *TP53/RB1* alteration status, indicating that *SKP2* amplification promoted *SKP2* expression independently of *TP53/RB1* status (Fig. 5E). In summary, the above findings indicate that amplification of *SKP2* is widely present across various cancers and independently affect its expression level.

Functional enrichment analysis revealed the biological characteristics of *SKP2*

Using GeneMANIA, we identified 20 *SKP2*-associated genes connected by physical interaction, co-expression, or co-localization, enriched for ubiquitination and cell-cycle functions (supplementary Fig. 1A, see online supplementary material). GO analysis highlighted mitotic processes (organelle fission, nuclear division, chromosome segregation), structures (spindle, chromosomal/centromeric regions, condensed chromosome), and activities (tubulin/microtubule binding, ATPase/motor activity). KEGG pathways included cell cycle, cellular senescence, progesterone-mediated oocyte maturation, p53 signaling, and DNA replication (supplementary Fig. 1B). Together, these data implicate *SKP2* in mitosis/proliferation and suggest roles in senescence and p53 signaling relevant to tumorigenesis.

At the single-cell level, correlating *SKP2* in malignant cells with 14 functional states across cancers showed positive associations with angiogenesis and proliferation, and negative associations with apoptosis, DNA damage, epithelial-mesenchymal transition (EMT), and invasion; no consistent links were seen with hypoxia,

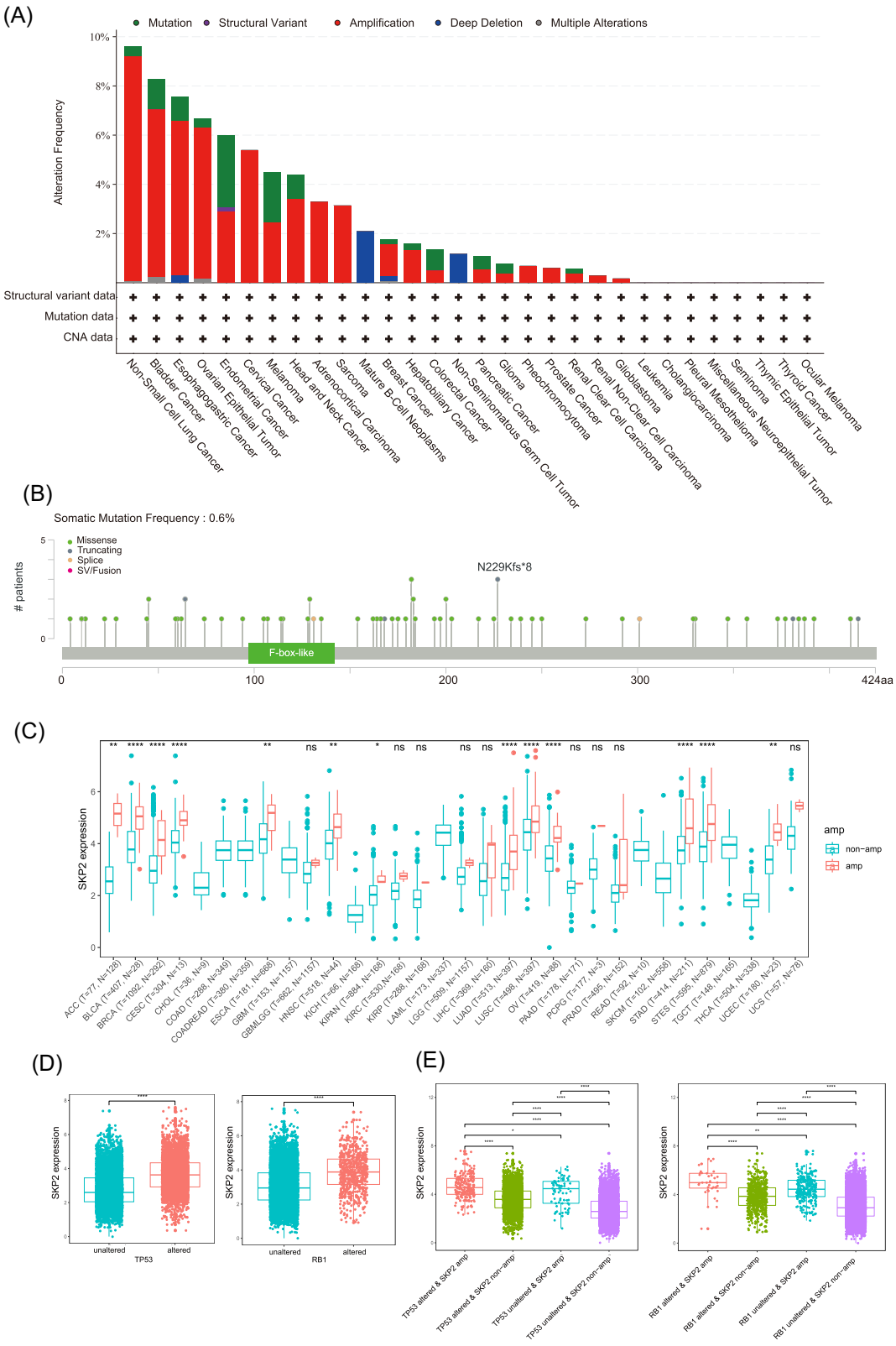


Figure 5. Effect of genetic alteration of *SKP2* on its expression level in pan-cancers. (**A**) Gene variation frequency of *SKP2* in pan-cancer was analyzed using the cBioPortal database. (**B**) Mutation type, number, and site of *SKP2* across protein domains. (**C**) *SKP2* mRNA expression level in groups with or without SKP2 amplification. (**D**) *SKP2* mRNA expression level in groups with or without TP53 alteration. (**E**) *SKP2* mRNA expression level in groups with or without *SKP2* amplification and *TP53*/*RB1* alteration.

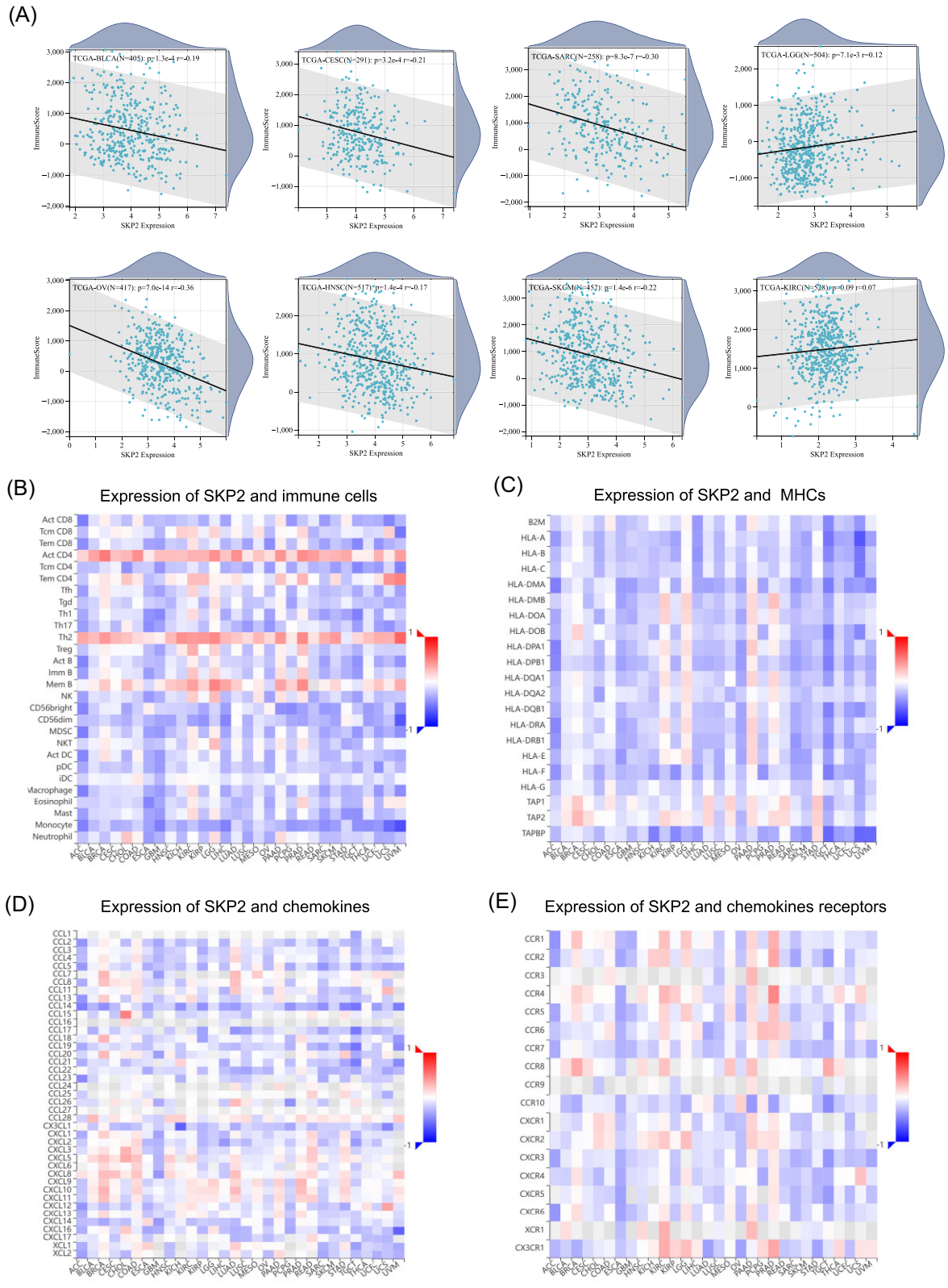


Figure 6. Correlation between *SKP2* expression and immune infiltration in pan-cancer. Correlation of *SKP2* expression with (**A**) ImmuneScore in BLCA, CESC, SARC, LGG, OV, and KIRP, (**B**) immune cell infiltration in cancers through TISIDB, (**C**) MHC genes in cancers through TISIDB, (**D**) chemokines in cancers through TISIDB, and (**E**) receptors in cancers through TISIDB.

metastasis, quiescence, or stemness. Associations with inflammation, cell cycle, DNA repair, and differentiation varied by tumor type—e.g. *SKP2* correlated positively with cell cycle/DNA repair and negatively with inflammation/differentiation in LUAD, whereas the opposite pattern appeared in RB (supplementary Fig. 1C). Overall, these findings reinforce *SKP2*'s connection to cell-cycle/mitotic programs and support its contribution to tumor initiation and progression through effects on proliferation, senescence/p53 signaling, inflammation, and DNA-damage repair.

SKP2 expression correlates with the TIME

Previous studies have confirmed the significant role of the TIME in tumor initiation, growth, invasion, and metastasis. However, the associations between *SKP2* and specific TIME features have not been systematically characterized across cancers. In the aforementioned functional enrichment analysis, *SKP2* was found to be associated with ubiquitination, cell proliferation, signaling pathway regulation, and anti-apoptosis, which has been reported in previous studies. Interestingly, our analysis revealed a negative correlation between *SKP2* and inflammation across various cancer types, suggesting its potential role in suppressing the TIME (supplementary Fig. 1C).

To comprehensively elucidate the relationship between *SKP2* expression and the cellular and acellular components of the TIME, we assessed the correlation between *SKP2* expression and ImmuneScore calculated by ESTIMATE across various cancer types. The results revealed a negative correlation between *SKP2* expression levels and ImmuneScore in most tumors, including BLCA, CESC, SARC, OV, HNSC, and SKCM, whereas only a minority of tumors, such as KIRC and LGG, showed a positive correlation (Fig. 6A). Subsequently, we further analyzed the correlation between *SKP2* expression and the infiltration levels of various immune cell subsets in the TIME. The results revealed a negative correlation between *SKP2* expression and the infiltration levels of most immune cells, including CD8+ T cells, natural killer (NK) cells, natural killer T (NKT) cells, and gamma delta (Tgd) T cells, which play crucial roles in tumor immune surveillance and cytotoxicity. Conversely, *SKP2* expression exhibited a significant positive correlation with activated CD4+ T cells and Th2 cells, which mediate processes involved in tumor immune evasion (Fig. 6B). Finally, we investigated the relationship between *SKP2* expression and the acellular components in the TIME, including MHC molecules, chemokines, and their receptors. The results indicated that *SKP2* expression levels were negatively correlated with the expression levels of major histocompatibility complex (MHC) molecules and chemokines across various cancer types (Fig. 6C and D). However, there was some heterogeneity observed in the correlation between *SKP2* expression and the expression levels of chemokine receptors across different tumor types. For instance, negative correlations were found between *SKP2* expression and the expression levels of chemokine receptors in ACC, ESCA, LUSC, READ, SARC, and STAD, while low to moderate positive correlations were observed in KIRC, PAAD, and PRAD (Fig. 6E).

To validate the effect of *SKP2* on the TIME, immunofluorescence staining assay of multiple immune markers was conducted on osteosarcoma tissues derived from three *Skp2* knockout ("TKO": *Osx1-Cre*; $Rb1^{lox/lox}$; $p53^{lox/lox}$; *Skp2-/-*) and three control transgenic ("DKO": *Osx1-Cre*; $Rb1^{lox/lox}$; $p53^{lox/lox}$) mouse models. The transgenic mouse models used in this study have been detailed in our earlier publication [16]. As showed in Fig. 7, the osteosarcoma tissue-derived *Skp2*-intact mouse models showed an "immune-cold" microenvironment, while the *Skp2* knockout osteosarcoma showed increasing immune cell infiltration, including macrophages (F4-80+), T cells (CD3+), CD8+ T cells (CD8A+), cytotoxic T cells (CD8A+, granzyme B+), NK cells (CD8A-, granzyme B+), and exhausted T cells (CD8A+, PD-1+) (Fig. 7A–C). Taken together, these data support a pan-cancer association between higher *SKP2* expression and immune-cold features of the TIME, while *in vivo* validation was performed in an osteosarcoma model; tumor-type-specific exceptions are noted (e.g. KIRC and LGG). Overall, the pattern is consistent with the hypothesis that *SKP2* inhibition could enhance responses to immunotherapy in selected contexts.

SKP2 overexpression predicted poor response to immunotherapy

Given the potential immunomodulatory role of *SKP2*, we conducted further analyses to explore the relationship between *SKP2* expression levels and the efficacy of immunotherapy. Previous research suggests that TMB and MSI can predict the efficacy of immunotherapy. Therefore, we initially analyzed the correlation between *SKP2* expression levels and TMB as well as MSI. We observed a positive correlation between *SKP2* expression and MSI in CESC, LUAD, COAD, COADREAD, STES, SARC, STAD, OV, and BLCA. Conversely, a negative correlation was found in DLBC, HNSC, KIPAN, and GBMLGG (Fig. 8A, left panel). Regarding TMB, positive correlations between *SKP2* expression and TMB were observed in several cancer types, including GBMLGG, LGG, LUAD, COAD, COADREAD, BRCA, STES, SARC, KIPAN, STAD, PRAD, LUSC, PAAD, BLCA, and ACC. Conversely, negative correlations were found only in THYM and THCA (Fig. 8A, right panel).

Furthermore, we utilized Kaplan–Meier analysis to explore the direct relationship between *SKP2* expression levels and immunotherapeutic responses across eight datasets based on immune checkpoint inhibitors. Based on the findings, elevated levels of *SKP2* were significantly correlated with poor immunotherapeutic responses in three melanoma datasets; while in three other melanoma datasets, patients with higher *SKP2* expression also exhibited a trend towards worse overall survival, albeit without statistical significance. On the other hand, in two renal cancer datasets, elevated expression of *SKP2* appears to be associated with better clinical outcomes. This aligns precisely with our earlier finding that *SKP2* correlates positively with the immune infiltration level in a subset of tumors represented by KIRC (Fig. 8B).

In summary, these results not only suggest that high expression of *SKP2* is associated with immunotherapy resistance across most cancers but also indicate that *SKP2* can be used as a biomarker to predict the efficacy of immunotherapy. This once again underscores the potential of *SKP2* inhibitors to enhance the effectiveness of immunotherapy.

Discussion

In this study, we integrated multi-omics data from several large public databases to comprehensively investigate *SKP2* at the pan-cancer level. We aimed to evaluate the role of *SKP2* in tumorigenesis and its prognostic significance, particularly its relationship with the immune microenvironment and immunotherapeutic efficacy. We observed that *SKP2* is overexpressed in the majority of cancer types, and its increased expression is closely associated with poor clinical outcomes. We further discovered that the elevated expression of *SKP2* can be attributed to both the amplification of the *SKP2* gene and genomic alterations involving tumor suppressor genes *TP53/RB1*. Notably, our pan-cancer analysis re-

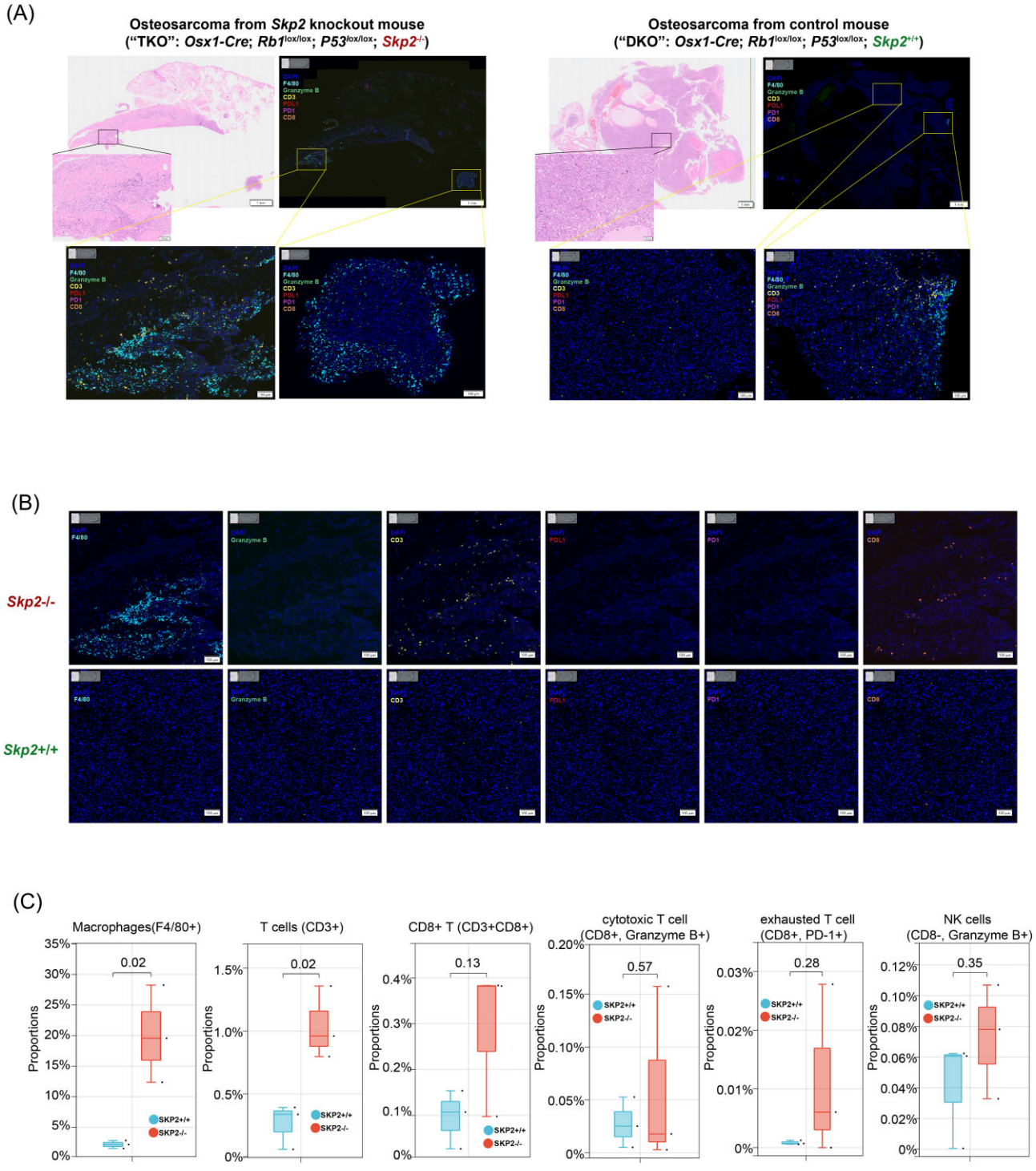


Figure 7. mIF of osteosarcoma tissues derived from *Skp2* knockout and control transgenic mouse models. (**A**) Example of whole sections output from *Skp2* knockout osteosarcoma (left) and control osteosarcoma (right) using mIF staining technology. DAPI, F4-80, granzyme B, CD3, PD-L1, PD-1, and CD8 staining, with the colors indicated, are shown on a whole section, with magnified insets from a representative donor. Scale bars, 1 mm and 100 μm. (**B**) Multiplex immunofluorescence staining showing F4-80 (celestial blue), granzyme B (green), CD3 (yellow) PD-L1 (red), PD-1 (purple), and CD8 (orange) of osteosarcoma sections from representative *Skp2* knockout and control transgenic mouse models as indicated. Scale bars, 100 μm. (**C**) Summary plot of various immune cell abundances from *Skp2* knockout ($n = 3$) and control transgenic ($n = 3$) mouse models. *P*-values < 0.05 are considered to be statistically significant.

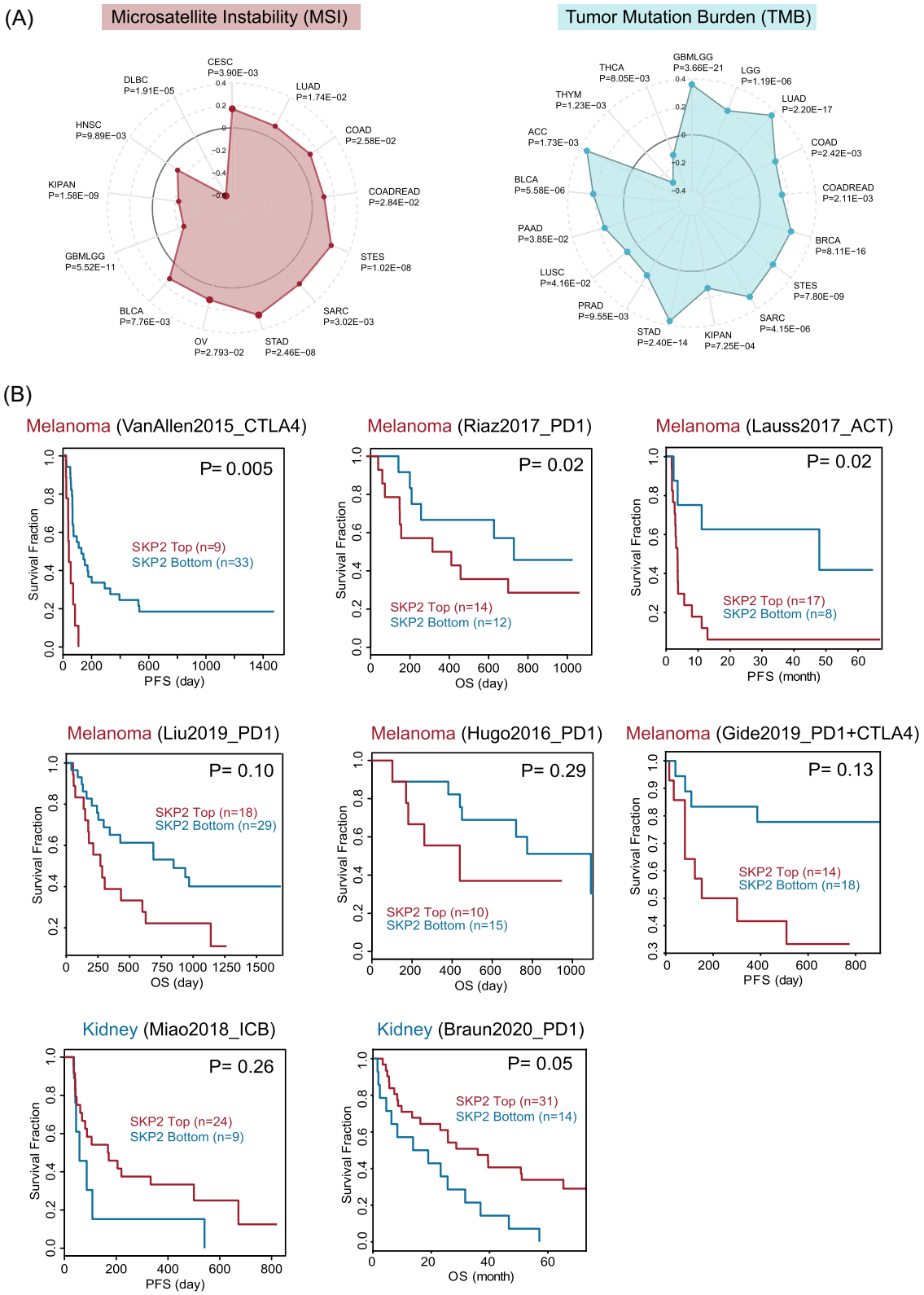


Figure 8. *SKP2* expression and immunotherapy response. (**A**) Correlation of *SKP2* expression with MSI and TMB. (**B**) Kaplan–Meier survival curves for SKP2 high- and low-expression groups across multiple immune checkpoint inhibitor (ICI) treatment cohorts.

vealed an inhibitory role of *SKP2* in the TIME, further supported by increased immune cell infiltration observed in *Skp2* knockout transgenic mouse models using mIF staining. In addition, we identified a negative correlation between *SKP2* expression and the efficacy of immunotherapy. These results demonstrate the broad oncogenic roles of *SKP2* in pan-cancer, revealing its association with the TIME and immunotherapy responses, and suggesting its potential as both a prognostic biomarker and a therapeutic target for immunotherapy.

The overexpression of *SKP2* and its correlation with clinical outcomes have been reported in numerous studies across various cancer types, including breast cancer [30, 31], esophageal cancer [32], gastric cancer [33], non-small cell lung carcinoma [34, 35], lymphoma [36], melanoma [37], oral squamous carcinoma [13], and prostate cancer [38]. However, most of these early studies drew conclusions using only a single modality of data, typically mRNA or protein-level data, with relatively small sample sizes and significant heterogeneity across different studies. Furthermore, these studies focused on *SKP2* expression patterns in a restricted number of cancer types, whereas systematic pan-cancer investigations integrating multi-omics data remain limited. In this study, we integrated multi-omics datasets from several large public resources to comprehensively investigate the expression of *SKP2* and its correlation with prognosis in pan-cancer at both mRNA and protein levels, utilizing a large sample size. Our findings revealed that *SKP2* is overexpressed in the majority of cancer types, consistent with previous reports. Additionally, elevated *SKP2* expression was found to be negatively correlated with the prognosis of various cancers. In summary, our findings extend previous research conclusions, confirming the oncogenic characteristics of *SKP2* and elucidating its broad roles in tumorigenesis across various tissue types.

SKP2 is overexpressed in the majority of cancer types, and its increased expression is closely associated with poor clinical outcomes. We further explored the underlying causes of *SKP2* overexpression in tumors. Several previous studies have indicated that certain transcription factors, oncogenic pathways, or transcriptional repressors may regulate the expression of *SKP2* [39–41]. In contrast to these studies, our research delves into the potential reasons for *SKP2* overexpression in tumors across various cancer types from a genomic perspective. Firstly, we observed that amplification is the predominant type of genomic alteration affecting *SKP2*, and this amplification significantly elevates the expression level of *SKP2*. Additionally, given the close association between the common tumor suppressor genes *TP53* and *RB1* and cell cycle regulation [10, 28, 29], we further investigated the regulatory impact of genomic alterations in *TP53* and *RB1* on *SKP2* expression. The findings revealed that genomic alterations in *TP53* and *RB1* can also contribute to the overexpression of *SKP2*. Lastly, to mitigate potential confounding factors, we stratified the study population and found that *SKP2* amplification could independently increase *SKP2* expression regardless of *TP53*/*RB1* mutation status. These findings not only enrich the spectrum of oncogenic variants of *SKP2* but also offer novel insights for further investigating the mechanisms by which *SKP2* mediates tumorigenesis.

In recent years, TIME has emerged as a crucial determinant influencing carcinogenesis, cancer progression, and treatment responses across numerous studies [42, 43]. However, despite some prior studies suggesting potential links between *SKP2* and the TIME, its role has not yet been systematically characterized [44–46]. Our recent study performed bulk RNA-seq on tumors from *Skp2* knockout mice compared to *Skp2*-intact controls, and reported significantly higher infiltration of immune cells, revealing the inhibitory role of *Skp2* in the TIME of osteosarcoma for the first time [16]. In this study, we systematically investigated the relationship between *SKP2* expression and the TIME, as well as the efficacy of immunotherapy, across various cancer types. Initially, when analyzing the expression distribution of *SKP2* at the single-cell level, we found *SKP2* expression not only in tumor cells but also in certain immune cell subtypes such as macrophages and proliferating T cells. Furthermore, the results from the functional enrichment analysis of *SKP2*-related genes suggested that *SKP2* might be associated with the inflammatory state of cancer, in addition to previously reported pathways such as ubiquitination and cell proliferation. The above evidence strongly suggests that *SKP2* may play a role in regulating the TIME. Therefore, we proceeded to investigate the correlation between *SKP2* and the cellular and acellular components of the TIME across various cancer types. The results showed a significant negative correlation between *SKP2* expression and immune infiltration, revealing a potential but extensive inhibitory role of *SKP2* in the TIME. This phenomenon was validated by the increasing tumor immune cell infiltration in *Skp2* knockout transgenic mouse models based on mIF staining. Finally, we observed a significant negative correlation between *SKP2* expression levels and the efficacy of immunotherapy, further highlighting its value as a biomarker for immunotherapeutic effectiveness.

Mechanistically, it is widely recognized that dysregulation of the cell cycle and increased proliferation may lead to a higher accumulation of somatic mutations and microsatellite destabilization [47–50]. In our study, the positive correlation between *SKP2* expression and MSI as well as TMB in most cancer types verified this hypothesis, which is also supported by similar results in breast cancer and melanoma [51, 52]. Drawing upon previous studies, tumors with higher TMB or MSI are associated with an increased presence of tumor neoantigens [53]. Upon presentation and recognition, these novel antigens can trigger higher immune infiltration levels and anti-tumor immune responses [54]. Surprisingly, we discovered a negative correlation between *SKP2* expression and immune infiltration levels. Our results suggest that elevated *SKP2* levels may induce widespread downregulation of MHC and chemoattractant cytokine ligand (CCL) molecules, along with their receptors, inhibiting antigen presentation and impeding the activation of immune cells responsible for tumor killing [55, 56]. Additionally, *SKP2* can activate certain immunosuppressive cells in cancer, such as activated CD4+ T cells and Th2 cells, ultimately leading to the formation of an immune-cold TIME [57]. Thus, by upregulating cell proliferation and mediating the formation of an immune-cold TIME, *SKP2* overexpression promotes the malignant phenotypes of cancers and drives immune evasion, ultimately resulting in a worse prognosis and reduced efficacy of immunotherapy. Last but not least, building upon our previous research and the findings presented above, we reasonably speculate that inhibiting *SKP2* could potentially transform "immune-cold" tumors into "immune-hot" tumors, rendering them more susceptible to concurrent or subsequent immunotherapy treatments (e.g. immune checkpoint blockade (ICB)), thereby eliciting objective antitumor responses [58–60]. Indeed, small-molecule inhibitors of SKP2, such as C1, SMIP001, and SMIP004, have been discovered to elevate p27 expression, induce cell cycle arrest, hinder colony formation in soft agar, and exhibit selective cytotoxicity in cancer cells [61–64]. However, whether these inhibitors can serve as sensitizers for immunotherapy requires further investigation.

Despite the general trend of *SKP2* overexpression correlating with immune-cold TIME and poor prognosis across most cancers, exceptions were noted in KIRC and LGG. In these cancers,

SKP2 overexpression correlated with enhanced immune infiltration and potentially more favorable clinical outcomes. This suggests that the relationship between *SKP2* and the TIME can be modulated by cancer-specific genetic and immune landscape factors. In KIRC, *SKP2* overexpression correlates with increased immune infiltration, possibly due to the unique molecular features of KIRC, including frequent mutations in the Von Hippel-Lindau (*VHL*) gene, which lead to hypoxia-inducible-factor (HIF) accumulation and the activation of immune pathways [65, 66]. These factors likely promote immune cell recruitment, including CD8+ T cells and NK cells, and may enhance immune surveillance in tumors with high *SKP2* expression. Similarly, in LGG, *SKP2* overexpression was associated with immune cell infiltration, likely reflecting the immune-active nature of low-grade gliomas in their early-stage tumors; *SKP2* might facilitate immune responses by modulating immune cell interactions, a role that is distinct from the immune-suppressive actions observed in higher-grade tumors [67]. These exceptions in KIRC and LGG underscore the complexity of *SKP2*'s role in regulating the TIME. Future studies should investigate how *SKP2* interacts with other molecular and immune factors in these cancers, as these findings may inform therapeutic strategies targeting *SKP2* to enhance immune responses.

There are several limitations to the study. First, most associations reported here are correlational; although the *Skp2* knockout osteosarcoma model provides supportive evidence, causality across tumor types remains to be established. To address this, we propose *in vitro* studies perturbing *SKP2* in human tumor models to assess effects on immune-cell abundance, activation, and function, and to elucidate underlying mechanisms, as well as *in vivo* studies testing whether *SKP2* inhibition, alone or in combination with immune checkpoint inhibitors, improves antitumor efficacy in solid tumors. Second, despite multi-database integration, certain tumor types (e.g. MESO, SARC, UVM) remain underpowered and will benefit from larger cohorts. Third, reliance on public datasets can introduce systematic biases (batch effects, cohort selection, treatment heterogeneity), despite harmonization. Finally, the clinical utility of *SKP2* as an immunotherapy biomarker—and the therapeutic potential of *SKP2*-targeting strategies—requires prospective validation in biomarker-driven trials.

Across cancers, *SKP2* is frequently overexpressed and is associated with copy-number gain and the *TP53/RB1* context. Its expression links cell-cycle activation to an immune-cold TIME and is broadly consistent with poorer prognosis, while recognizing that effects may vary by tumor type. These findings support *SKP2* as a biomarker to guide risk stratification and immunotherapy decisions and as a therapeutic target, either alone or in combination with checkpoint blockade in biomarker-selected patients. Future work should establish whether *SKP2* causally induces an immune-cold microenvironment in experimental models, clarify the underlying mechanisms, and evaluate *SKP2*-directed strategies in well-designed clinical trials.

Abbreviations

ACC:	Adrenocortical carcinoma
ARRIVE:	Animal Research: Reporting of In Vivo Experiments
BLCA:	Bladder urothelial carcinoma
BRCA:	Breast invasive carcinoma
CCL:	Chemoattractant cytokine ligand
CESC:	Cervical squamous cell carcinoma and endocervical adenocarcinoma
CHOL:	Cholangiocarcinoma
COAD:	Colon adenocarcinoma
CRC:	Colorectal carcinoma
DLBC:	Lymphoid neoplasm diffuse large B-cell lymphoma
DSS:	Disease-specific survival
EMT:	Epithelial-mesenchymal transition
ESCA:	Esophageal carcinoma
GBM:	Glioblastoma multiforme
GTEx:	Genotype-tissue expression
HIF:	Hypoxia-inducible-factor
HNSC:	Head and neck squamous cell carcinoma
HPA:	Human protein atlas
ICB:	Immune checkpoint blockade
ICIs:	Immune checkpoint inhibitors
KICH:	Kidney chromophobe
KIPAN:	Pan-kidney cohort (KICH+KIRC+KIRP)
KIRC:	Kidney renal clear cell carcinoma
KIRP:	Kidney renal papillary cell carcinoma
LAML:	Acute myeloid leukemia
LGG:	Brain lower grade glioma
LIHC:	Liver hepatocellular carcinoma
LUAD:	Lung adenocarcinoma
LUSC:	Lung squamous cell carcinoma
MESO:	Mesothelioma
MHC:	Major histocompatibility complex
mIF:	Multiplex immunofluorescence
MSI:	Microsatellite instability
NK cells:	Natural killer cells
NKT cells:	Natural killer T cells
NPC:	Nasopharyngeal carcinoma
NSCLC:	Non–small cell lung cancer
OV:	Ovarian serous cystadenocarcinoma
PAAD:	Pancreatic adenocarcinoma
PCPG:	Pheochromocytoma and paraganglioma
PFI:	Progression-free Interval
PRAD:	Prostate adenocarcinoma
RB:	Retinoblastoma
READ:	Rectum adenocarcinoma
SARC:	Sarcoma
SCLC:	Small cell lung cancer
SKCM:	Skin cutaneous melanoma
SKP2:	S-Phase kinase associated protein 2
STAD:	Stomach adenocarcinoma
STES:	Stomach and esophageal carcinoma
TCGA:	The Cancer Genome Atlas
TGCT:	Testicular germ cell tumors
Tgd T cells:	Gamma delta T cells
THCA:	Thyroid carcinoma
THYM:	Thymoma
TIME:	Tumor immune microenvironment
TISCH:	Tumor Immune Single-cell Hub
TMB:	Tumor mutation burden
TSA:	Tyramide signal amplification
UCEC:	Uterine corpus endometrial carcinoma
UCS:	Uterine carcinosarcoma
UVM:	Uveal melanoma.
VHL:	Von Hippel-Lindau

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Author contributions

Xingyu Liao (Writing – original draft), Yaxin Zhang (Visualization), Xue Yu (Data curation, Resources, Software), Wei Du (Resources, Software), Linxi Chen (Resources, Software), Zhiqing Zhao (Resources, Software), Haijie Liang (Resources, Software), Xingyu Liu (Resources, Software), Feiyang Qi (Resources, Software), David S. Geller (Resources, Software), Rui Yang (Resources, Software), Bang H. Hoang (Resources, Software), Li Hu (Supervision, Writing – original draft, Writing – review & editing), and Jichuan Wang (Resources, Supervision, Writing – review & editing).

Supplementary data

Supplementary data is available at *PCMEDI Journal* online.

Conflict of interest

None declared.

Ethics statement

All animals were housed under pathogen-free conditions at the Beijing Key Laboratory for Musculoskeletal Tumors animal facility. The experimental procedures were reviewed and approved by the Ethics Committee of Peking University People's Hosptial (2024PHB432-001), following established guidelines for ethical animal care. All methods are reported in accordance with Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

Data availability

Transcriptome and genomic data of 33 cancers and corresponding normal samples were obtained from TCGA and GTEx. Protein expression data of *SKP2* in various cancers were obtained from the Human Protein Atlas (HPA) database. scRNA-seq data were obtained from The Tumor Immune Single-cell Hub (TISCH) database and CancerSEA. Any additional information required is available from the corresponding author upon reasonable request.

References

1. Bray F, Laversanne M, Sung H *et al.* Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2024;**74**:229–63. https://doi.org/10.3322/caac.21834
2. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. *CA Cancer J Clin* 2024;**74**:12–49. https://doi.org/10.3322/caac.21820
3. Topalian SL. Targeting immune checkpoints in cancer therapy. *JAMA* 2017;**318**:1647–8. https://doi.org/10.1001/jama.2017.14155
4. Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: A common denominator approach to cancer therapy. *Cancer Cell* 2015;**27**:450–61. https://doi.org/10.1016/j.ccell.2015.03.001
5. Tang T, Huang X, Zhang G *et al.* Advantages of targeting the tumor immune microenvironment over blocking immune checkpoint in cancer immunotherapy. *Sig Transduct Target Ther* 2021;**6**:1–13. https://doi.org/10.1038/s41392-020-00449-4
6. Postow MA, Callahan MK, Wolchok JD. Immune checkpoint blockade in cancer therapy. *J Clin Oncol* 2015;**33**:1974–82. https://doi.org/10.1200/JCO.2014.59.4358
7. Davoli T, Uno H, Wooten EC *et al.* Tumor aneuploidy correlates with markers of immune evasion and with reduced response to immunotherapy. *Science* 2017;**355**:eaaf8399. https://doi.org/10.1126/science.aaf8399
8. Sharma P, Hu-Lieskovan S, Wargo JA *et al.* Primary, adaptive, and acquired resistance to cancer immunotherapy. *Cell* 2017;**168**:707–23. https://doi.org/10.1016/j.cell.2017.01.017
9. Cai Z, Moten A, Peng D *et al.* The Skp2 pathway: A critical target for cancer therapy. *Semin Cancer Biol* 2020;**67**:16–33. https://doi.org/10.1016/j.semcancer.2020.01.013
10. Gupta P, Zhao H, Hoang B *et al.* Targeting the untargetable: RB1-deficient tumours are vulnerable to Skp2 ubiquitin ligase inhibition. *Br J Cancer* 2022;**127**:969–75. https://doi.org/10.1038/s41416-022-01898-0
11. Hume S, Grou CP, Lascaux P *et al.* The NUCKS1-SKP2-p21/p27 axis controls S phase entry. *Nat Commun* 2021;**12**:6959. https://doi.org/10.1038/s41467-021-27124-8
12. Bloom J, Pagano M. Deregulated degradation of the cdk inhibitor p27 and malignant transformation. *Semin Cancer Biol* 2003;**13**:41–7. https://doi.org/10.1016/s1044-579x(02)00098-6
13. Asmamaw MD, Liu Y, Zheng Y-C *et al.* Skp2 in the ubiquitin-proteasome system: A comprehensive review. *Med Res Rev* 2020;**40**:1920–49. https://doi.org/10.1002/med.21675
14. Bornstein G, Bloom J, Sitry-Shevah D *et al.* Role of the SCFSkp2 ubiquitin ligase in the degradation of p21Cip1 in S phase∗. *J Biol Chem* 2003;**278**:25752–7. https://doi.org/10.1074/jbc.M301774200
15. Chan C-H, Li C-F, Yang W-L *et al.* The Skp2-SCF E3 ligase regulates akt ubiquitination, glycolysis, herceptin sensitivity, and tumorigenesis. *Cell* 2012;**149**:1098–111. https://doi.org/10.1016/j.cell.2012.02.065
16. Ferrena A, Wang J, Zhang R *et al.* SKP2 Knockout in Rb1/p53–Deficient mouse models of osteosarcoma induces immune infiltration and drives a transcriptional program with a favorable prognosis. *Mol Cancer Ther* 2023;**23**:OF1–12. https://doi.org/10.1158/1535-7163.MCT-23-0173
17. Peng Y, Qi X, Ding L *et al.* SKP2 inhibition activates tumor cell-intrinsic immunity by inducing DNA replication stress and genomic instability. *Br J Cancer* 2025;**132**:81–92. https://doi.org/10.1038/s41416-024-02909-y
18. Hu K, Zhao L, Miao P *et al.* Inhibition of Skp2 enhances anti-tumor immunity of macrophages by regulating CD47 in gastric cancer. *Int Immunopharmacol* 2025;**163**:115262. https://doi.org/10.1016/j.intimp.2025.115262
19. Shen W, Song Z, Zhong X *et al.* Sangerbox: A comprehensive, interaction-friendly clinical bioinformatics analysis platform. *iMeta* 2022;**1**:e36. https://doi.org/10.1002/imt2.36
20. Gao J, Aksoy BA, Dogrusoz U *et al.* Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal* 2013;**6**:pl1. https://doi.org/10.1126/scisignal.2004088
21. Franz M, Rodriguez H, Lopes C *et al.* GeneMANIA update 2018. *Nucleic Acids Res* 2018;**46**:W60–4. https://doi.org/10.1093/nar/gky311
22. Kanehisa M, Furumichi M, Sato Y *et al.* KEGG for taxonomy-based analysis of pathways and genomes. *Nucleic Acids Res* 2023;**51**:D587–92. https://doi.org/10.1093/nar/gkac963
23. Yoshihara K, Shahmoradgoli M, Martínez E *et al.* Inferring tumour purity and stromal and immune cell admixture from ex-

pression data. *Nat Commun* 2013;**4**:2612. https://doi.org/10.1038/ncomms3612
24. Ru B, Wong CN, Tong Y *et al.* TISIDB: an integrated repository portal for tumor–immune system interactions. *Bioinformatics* 2019;**35**:4200–2. https://doi.org/10.1093/bioinformatics/btz210
25. Walkley CR, Qudsi R, Sankaran VG *et al.* Conditional mouse osteosarcoma, dependent on p53 loss and potentiated by loss of Rb, mimics the human disease. *Genes Dev* 2008;**22**:1662–76. https://doi.org/10.1101/gad.1656808
26. Wang J, Aldahamsheh O, Ferrena A *et al.* The interaction of SKP2 with p27 enhances the progression and stemness of osteosarcoma. *Ann NY Acad Sci* 2021;**1490**:90–104. https://doi.org/10.1111/nyas.14578
27. Zhao H, Wang H, Bauzon F *et al.* Deletions of retinoblastoma 1 (Rb1) and its repressing target S phase kinase-associated protein 2 (Skp2) are synthetic lethal in mouse embryogenesis. *J Biol Chem* 2016;**291**:10201–9. https://doi.org/10.1074/jbc.M116.718049
28. Kitagawa M, Lee SH, McCormick F. Skp2 Suppresses p53-dependent apoptosis by inhibiting p300. *Mol Cell* 2008;**29**:217–31. https://doi.org/10.1016/j.molcel.2007.11.036
29. Dulić V, Kaufmann WK, Wilson SJ *et al.* p53-dependent inhibition of cyclin-dependent kinase activities in human fibroblasts during radiation-induced G1 arrest. *Cell* 1994;**76**:1013–23. https://doi.org/10.1016/0092-8674(94)90379-4
30. Yang C, Nan H, Ma J *et al.* High Skp2/low p57(Kip2) expression is associated with poor prognosis in Human breast carcinoma. *Breast Cancer (Auckl)* 2015;**9**:13–21. https://doi.org/10.4137/BCBCR.S30101
31. Liu J, Wei X-L, Huang W-H *et al.* Cytoplasmic Skp2 expression is associated with p-Akt1 and predicts poor prognosis in human breast carcinomas. *PLoS One* 2012;**7**:e52675. https://doi.org/10.1371/journal.pone.0052675
32. Wang X-C, Wu Y-P, Ye B *et al.* Suppression of anoikis by SKP2 amplification and overexpression promotes metastasis of esophageal squamous cell carcinoma. *Mol Cancer Res* 2009;**7**:12–22. https://doi.org/10.1158/1541-7786.MCR-08-0092
33. Masuda T, Inoue H, Sonoda H *et al.* Clinical and biological significance of S-phase kinase-associated protein 2 (Skp2) gene expression in gastric carcinoma: modulation of malignant phenotype by Skp2 overexpression, possibly via p27 proteolysis. *Cancer Res* 2002;**62**:3819–25.
34. Osoegawa A, Yoshino I, Tanaka S *et al.* Regulation of p27 by S-phase kinase-associated protein 2 is associated with aggressiveness in non-small-cell lung cancer. *J Clin Oncol* 2004;**22**:4165–73. https://doi.org/10.1200/JCO.2004.01.035
35. Zhu CQ, Blackhall FH, Pintilie M *et al.* Skp2 gene copy number aberrations are common in non-small cell lung carcinoma, and its overexpression in tumors with ras mutation is a poor prognostic marker. *Clin Cancer Res* 2004;**10**:1984–91. https://doi.org/10.1158/1078-0432.ccr-03-0470
36. Lim MS, Adamson A, Lin Z *et al.* Expression of Skp2, a p27(Kip1) ubiquitin ligase, in malignant lymphoma: correlation with p27(Kip1) and proliferation index. *Blood* 2002;**100**:2950–6. https://doi.org/10.1182/blood.V100.8.2950
37. Rose AE, Wang G, Hanniford D *et al.* Clinical relevance of SKP2 alterations in metastatic melanoma. *Pigment Cell Melanoma Res* 2011;**24**:197–206. https://doi.org/10.1111/j.1755-148X.2010.00784.x
38. Yang G, Ayala G, De Marzo A *et al.* Elevated Skp2 protein expression in human prostate cancer: association with loss of the cyclin-dependent kinase inhibitor p27 and PTEN and with reduced recurrence-free survival. *Clin Cancer Res* 2002;**8**:3419–26.
39. Zhang L, Wang C. F-box protein Skp2: a novel transcriptional target of E2F. *Oncogene* 2006;**25**:2615–27. https://doi.org/10.1038/sj.onc.1209286
40. Mamillapalli R, Gavrilova N, Mihaylova VT *et al.* PTEN regulates the ubiquitin-dependent degradation of the CDK inhibitor p27KIP1 through the ubiquitin E3 ligase SCFSKP2. *Curr Biol* 2001;**11**:263–7. https://doi.org/10.1016/S0960-9822(01)00065-3
41. Martin F, Ladoire S, Mignot G *et al.* Human FOXP3 and cancer. *Oncogene* 2010;**29**:4121–9. https://doi.org/10.1038/onc.2010.174
42. Hiam-Galvez KJ, Allen BM, Spitzer MH. Systemic immunity in cancer. *Nat Rev Cancer* 2021;**21**:345–59. https://doi.org/10.1038/s41568-021-00347-z
43. Hu A, Sun L, Lin H *et al.* Harnessing innate immune pathways for therapeutic advancement in cancer. *Sig Transduct Target Ther* 2024;**9**:1–59. https://doi.org/10.1038/s41392-024-01765-9
44. Lwin T, Hazlehurst LA, Dessureault S *et al.* Cell adhesion induces p27Kip1-associated cell-cycle arrest through down-regulation of the SCFSkp2 ubiquitin ligase pathway in mantle-cell and other non-Hodgkin B-cell lymphomas. *Blood* 2007;**110**:1631–8. https://doi.org/10.1182/blood-2006-11-060350
45. Pernicová Z, Slabáková E, Kharaishvili G *et al.* Androgen depletion induces senescence in prostate cancer cells through down-regulation of Skp2. *Neoplasia* 2011;**13**:526–36. https://doi.org/10.1593/neo.11182
46. Lin H-K, Chen Z, Wang G *et al.* Skp2 targeting suppresses tumorigenesis by arf-p53-independent cellular senescence. *Nature* 2010;**464**:374–9. https://doi.org/10.1038/nature08815
47. Feitelson MA, Arzumanyan A, Kulathinal RJ *et al.* Sustained proliferation in cancer: mechanisms and novel therapeutic targets. *Semin Cancer Biol* 2015;**35**:S25–54. https://doi.org/10.1016/j.semcancer.2015.02.006
48. Matthews HK, Bertoli C, de Bruin RAM. Cell cycle control in cancer. *Nat Rev Mol Cell Biol* 2022;**23**:74–88. https://doi.org/10.1038/s41580-021-00404-3
49. Taylor EM, Lindsay HD. DNA replication stress and cancer: cause or cure? *Future Oncol* 2016;**12**:221–37. https://doi.org/10.2217/fon.15.292
50. Matsuno Y, Atsumi Y, Shimizu A *et al.* Replication stress triggers microsatellite destabilization and hypermutation leading to clonal expansion in vitro. *Nat Commun* 2019;**10**:3925. https://doi.org/10.1038/s41467-019-11760-2
51. Takahashi H, Asaoka M, Yan L *et al.* Biologically aggressive phenotype and anti-cancer immunity counterbalance in breast cancer with high mutation rate. *Sci Rep* 2020;**10**:1852. https://doi.org/10.1038/s41598-020-58995-4
52. Grzywa TM, Koppolu AA, Paskal W *et al.* Higher mutation burden in high proliferation compartments of heterogeneous melanoma tumors. *Int J Mol Sci* 2021;**22**:3886. https://doi.org/10.3390/ijms22083886
53. Xie N, Shen G, Gao W *et al.* Neoantigens: promising targets for cancer therapy. *Sig Transduct Target Ther* 2023;**8**:1–38. https://doi.org/10.1038/s41392-022-01270-x
54. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. *Science* 2015;**348**:69–74. https://doi.org/10.1126/science.aaa4971
55. Jhunjhunwala S, Hammer C, Delamarre L. Antigen presentation in cancer: insights into tumour immunogenicity and immune evasion. *Nat Rev Cancer* 2021;**21**:298–312. https://doi.org/10.1038/s41568-021-00339-z
56. Kallingal A, Olszewski M, Maciejewska N *et al.* Cancer immune escape: the role of antigen presentation machinery. *J Cancer Res Clin Oncol* 2023;**149**:8131–41. https://doi.org/10.1007/s00432-023-04737-8

57. Tie Y, Tang F, Wei Y *et al.* Immunosuppressive cells in cancer: mechanisms and potential therapeutic targets. *J Hematol Oncol* 2022;**15**:61. https://doi.org/10.1186/s13045-022-01282-8
58. Duan Q, Zhang H, Zheng J *et al.* Turning cold into hot: firing up the tumor microenvironment. *Trends Cancer* 2020;**6**:605–18. https://doi.org/10.1016/j.trecan.2020.02.022
59. Zhang J, Huang D, Saw PE *et al.* Turning cold tumors hot: from molecular mechanisms to clinical applications. *Trends Immunol* 2022;**43**:523–45. https://doi.org/10.1016/j.it.2022.04.010
60. Khosravi G-R, Mostafavi S, Bastan S *et al.* Immunologic tumor microenvironment modulators for turning cold tumors hot. *Cancer Commun (Lond)* 2024;**44**:521–53. https://doi.org/10.1002/cac2.12539
61. Lough L, Sherman D, Ni E *et al.* Chemical probes of Skp2-mediated p27 ubiquitylation and degradation. *Medchemcomm* 2018;**9**:1093–104. https://doi.org/10.1039/c8md00140e
62. Wu L, Grigoryan AV, Li Y *et al.* Specific small molecule inhibitors of Skp2-mediated p27 degradation. *Chem Biol* 2012;**19**:1515–24. https://doi.org/10.1016/j.chembiol.2012.09.015
63. Zhao H, Iqbal NJ, Sukrithan V *et al.* Targeted inhibition of the E3 ligase SCFSkp2/Cks1 has antitumor activity in RB1-deficient Human and mouse small-cell lung cancer. *Cancer Res* 2020;**80**:2355–67. https://doi.org/10.1158/0008-5472.CAN-19-2400
64. Rico-Bautista E, Yang C-C, Lu L *et al.* Chemical genetics approach to restoring p27Kip1 reveals novel compounds with antiproliferative activity in prostate cancer cells. *BMC Biol* 2010;**8**:153. https://doi.org/10.1186/1741-7007-8-153
65. Wolf MM, Madden MZ, Arner EN *et al.* *VHL* loss reprograms the immune landscape to promote an inflammatory myeloid microenvironment in renal tumorigenesis. *J Clin Invest* 2024;**134**:e173934. https://doi.org/10.1172/JCI173934
66. Xu Z, Liu L, Jiang W *et al.* VHL missense mutation delineate aggressive clear cell renal cell carcinoma subtype with favorable immunotherapeutic response. *J Immunother Cancer* 2024;**12**:e009963. https://doi.org/10.1136/jitc-2024-009963
67. Lin W-W, Ou G-Y, Zhao W-J. Mutational profiling of low-grade gliomas identifies prognosis and immunotherapy-related biomarkers and tumour immune microenvironment characteristics. *J Cell Mol Med* 2021;**25**:10111–25. https://doi.org/10.1111/jcmm.16947

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