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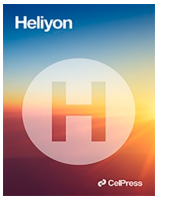

Research article

Pan-cancer analysis reveals the prognostic and immunologic roles of cereblon and its significance for PROTAC design

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ABSTRACT

Background: Cereblon (CRBN) has emerged as a vital E3 ubiquitin ligase for Proteolysis-targeting chimera (PROTAC) design. However, few studies focus on the physiological mechanism of CRBN, and more studies are needed to explore the influence of CRBN on tumorigenesis. This pan-cancer analysis aims to explore the prognostic and immunologic roles of CRBN, and provide new insight for CRBN into cancer treatment and PROTAC design.

Methods: The TCGA database, TIMER 2.0 database, and TISIDB database were used to analyze the role of CRBN in pan-cancer. Multiple bioinformatic methods (ssGSEA, Kaplan-Meier, univariate cox regression, ESTIMATE, CIBERSORT) were applied to investigate the CRBN expression status, gene activity, prognostic values, and its correlation with immune scores, immune infiltration, immune-related functions, HALLMARKs functions, and response to immunotherapy in pan-cancer.

Results: In most cancer types, the expression and activity of CRBN in tumor groups were lower compared with normal groups. Upregulated CRBN expression may indicate a better prognosis for cancer patients. The Immune score, stromal score, and tumor purity varied greatly among different cancer types. GSEA analysis showed that high CRBN expression was correlated with the downregulation of tumor-promoting signaling pathways. The level of CRBN was associated with Tumor mutation burden (TMB), Microsatellite instability (MSI), objective response rate (ORR), and immune cell infiltration in a few cancer types.

Conclusion: Pan-cancer analysis reveals the potential role of CRBN as a prognostic biomarker and versatile immunologic roles in different cancer types. Upregulated expression of CRBN may be beneficial to CRBN-related immunotherapy and PROTAC design.

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1. Introduction

Cereblon (CRBN) is a protein-encoding gene that was first identified and named in 2004 for its role in brain development [1]. It was regarded as a novel ATP-dependent Lon protease gene that encodes the protein CRBN [1]. CRBN is correlated with several disease processes and has diverse physiological effects. It decreases the excitability of neurons and causes autoimmune disorders. In addition, CRBN results in metabolism diseases through the inhibition of AMP-activated protein kinase (AMPK) activity [2]. For physiological functions, CRBN regulates ion transport, modulates the AMPK signaling pathway, involves in protein degradation, and influences cell proliferation, metabolism, and apoptosis [3]. Notably, CRBN is a component of the E3 ubiquitin ligase complex. It performs the function of substrate receptor and targets the C-terminal cyclic imide degron [4].

Currently, cancer is still the leading cause of death globally. Cancer immunotherapy has developed rapidly and has become a potent weapon that shifts the cancer treatment paradigm. Rather than killing cancer cells directly, immunotherapy drugs activate the immune system to attack cancer cells [5]. In recent years, targeted protein degradation (TPD) has become a novel therapeutic modality [6]. Proteolysis-targeting chimeras (PROTACs) is a drug design strategy. It is composed of a warhead (a ligand of protein of interest), a linker, and a ligand of E3 ubiquitin ligase [7]. PROTAC induces the degradation of disease-related protein via the ubiquitin-proteasome system (UPS) [8] and is used extensively in the treatment of various cancer types [9].

CRBN was identified as the primary target of immunomodulatory imide drugs (IMiDs) thalidomide [10]. More recently, C-terminal cyclic imides were identified by structural studies as the physiological degrons on substrates for CRBN, which is the foundation of the efficacy and broad applicability of IMiDs and PROTACs [11]. Based on the exploration of the mechanism of action of thalidomide and its analogues, CRBN appears to be a preferred E3 ligase for PROTAC drug design [12]. PROTACs using CRBN as the E3 ligase, such as ARV-110 and ARV-471, have entered phase II clinical trials [13,14]. Further research on CRBN is conducive to the design of more effective PROTACs.

In this study, we conducted a comprehensive analysis termed pan-cancer analysis [15] that focused on CRBN aberrations and functional roles across 33 cancer types based on diverse bioinformatic methods. The abbreviations and full names of the 33 cancers considered in this study are available in Table 1. The expression and gene activity profile of CRBN were evaluated, and the relationship between CRBN expression and prognostic value was comprehensively investigated. Meanwhile, vital immune-related factors were investigated, including immune score, stromal score, tumor purity, different cell types in immune infiltration, and immune modulators. Furthermore, the analyses of tumor mutational burden (TMB), microsatellite instability (MSI), and objective response rate (ORR) were conducted. Generally, this work shows valuable insight into the role of CRBN in pan-cancer and provides

Table 1
Abbreviations and details of the 33 cancer types used in this study.

Abbreviation	Detail
ACC	Adrenocortical Cancer
BLCA	Bladder Cancer
BRCA	Breast Cancer
CESC	Cervical Cancer
CHOL	Bile Duct Cancer
COAD	Colon Cancer
COADREAD	Colon and Rectal Cancer
DLBC	Large B-cell Lymphoma
ESCA	Esophageal Cancer
FPPP	FFPE Pilot Phase II
GBM	Glioblastoma
GBMLGG	lower grade glioma and glioblastoma
HNSC	Head and Neck Cancer
KICH	Kidney Chromophobe
KIRC	Kidney Clear Cell Carcinoma
KIRP	Kidney Papillary Cell Carcinoma
LAML	Acute Myeloid Leukemia
LGG	Lower Grade Glioma
LIHC	Liver Cancer
LUAD	Lung Adenocarcinoma
LUNG	Lung Cancer
LUSC	Lung Squamous Cell Carcinoma
MESO	Mesothelioma
OV	Ovarian Cancer
PAAD	Pancreatic Cancer
PANCAN	Pan-Cancer
PCPG	Pheochromocytoma & Paraganglioma
PRAD	Prostate Cancer
READ	Rectal Cancer
SARC	Sarcoma
SKCM	Melanoma
STAD	Stomach Cancer
TGCT	Testicular Cancer

immunotherapeutic evidence of CRBN, which may be beneficial to further functional experiments and CRBN-related drug design.

2. Materials and methods

2.1. Data sources

The transcriptome data and corresponding clinical information of pan-cancer was obtained from the TCGA-Pancancer project in the UCSC-Xena database(http://xena.ucsc.edu/), the HALLMARK gene sets and GO gene sets were downloaded from the MSigdb (https://www.gsea-msigdb.org/gsea/msigdb), the tumor immune micro-environments data were retrieved and downloaded from the Tumor Immune Estimation Resource (TIMER) database for analysis of tumor infiltrating cells [16], and the database has been improved to TIMER 2.0 (http://timer.cistrome.org/) that uses six state-of-art algorithms [17]. The correlation between CRBN and immune stimulators or immune inhibitors or MHC molecules were retrieved from the Tumor–Immune System Interactions and Drug Bank (TISIDB) database(http://cis.hku.hk/TISIDB/) [18], and the objective remission rate (ORR) of 21 different cancer types in TCGA to anti-PD1/PD-L1 immunotherapy were obtained from the supplementary file of corresponding research [19]. CRBN protein expression in normal and tumor tissue of kidney and prostate detected by immunohistochemistry (IHC) was gained from the Human Protein Atlas (HPA) database(https://www.proteinatlas.org/).

2.2. Differential expression analysis of CRBN and CRBN activity in pan-cancer

We first searched TIMER 2.0 for the differential expression status of CRBN between normal tissues and tumor tissues in pan-cancer. Then we sorted the downloaded transcriptome data of pan-cancer by the median expression of CRBN in pan-cancers and merged the transcriptome data with the corresponding stage information to compare whether the expression of CRBN is associated with the pathological stage in different cancer types. Following this, we performed the correlation test between CRBN and other genes to identify the 100 most positively correlated genes of CRBN in pan-cancer, representing the activity of CRBN-related functions in some sense. Thus, we use a computational method termed single sample gene set enrichment analysis (ssGSEA) in the pan-cancer project to explore different CRBN-related biological processes by setting the 100 correlated genes as the CRBN-related function gene sets and acquired a CRBN activity of each sample in TCGA pan-cancer. The differences in CRBN activity between normal tissues and tumor tissues were also compared in pan-cancer.

2.3. Prognostic value of CRBN in pan-cancer

We extracted the expression of CRBN in pan-cancer. Then we merged the expression profiles with the survival information of each sample, including overall survival (OS) information, disease-free survival (DFS) information, disease-specific survival (DSS) information, and progression-free survival (PFS) information. Then we separately performed univariate Cox regression and log-rank tests in different cancer types to check the prognostic value of CRBN in pan-cancer. Kaplan-Meier (KM) survival analysis was conducted to evaluate the difference between the two groups with CRBN high and low expression. The univariate Cox regression was realized by the survival package in R to identify the noteworthy cancer types. Also, the forest plot and Kaplan-Meier method-based survival curve were plotted by Excel and R programs. Variations were considered significant if the p-value <0.05.

2.4. Association between CRBN expression and immune-related functions

We performed two different methods to quantify the micro-environments of each sample in pan-cancer: The Estimation of STromal and Immune cells in MAlignant Tumors using Expression data (ESTIMATE) and CIBERSORT. ESTIMATE is a method to infer the stromal and immune cells in tumor samples based on gene expression signatures. The stromal score and immune score represent the presence of stromal cell and the infiltration of immune cells in tumor tissue respectively. The estimate score is the combination of stromal and immune score and embodies the tumor purity [20]. CIBERSORT is a method to quantify cell fractions by calculate the data from bulk tissue gene expression profiles [21]. Having analyzed the detailed estimate score or cibersort ratio, we carried out the PEARSON correlation tests to investigate the correlation between CRBN and different immune cell types in pan-cancer. Besides, we searched the Timer 2.0 database for more details to reveal the correlation between CRBN expression and immune infiltration by following seven algorithms: QUANTISEQ, XCELL, CIBERSORT, CIBERSORT-ABS, EPIC, TIMER, and MCP-COUNTER. The correlation between CRBN and the expression of immune stimulators/inhibitors/MHC molecules was also evaluated to explore the association between CRBN expression and immune-related functions systematically.

2.5. Association between CRBN expression and hallmarks, TMB, MSI, and ORR

Samples in different cancer types were divided into high-/low- CRBN expression groups by the median expression value of CRBN in each cancer type, then we performed gene sets enrichment analysis (GSEA) to identify the differentially enhanced or weakened functions between high-/low- CRBN expression groups in different cancer types. Besides, we separately investigated the differentially activated HALLMARK gene sets in pan-cancer between high-/low- CRBN expression groups. Subsequently, we were interested in the association between CRBN and response to immunotherapy. Since TMB and MSI were predictors of response to immunotherapy, we performed the correlation test between CRBN expression and TMB or MSI in each cancer type. Finally, we directly performed the

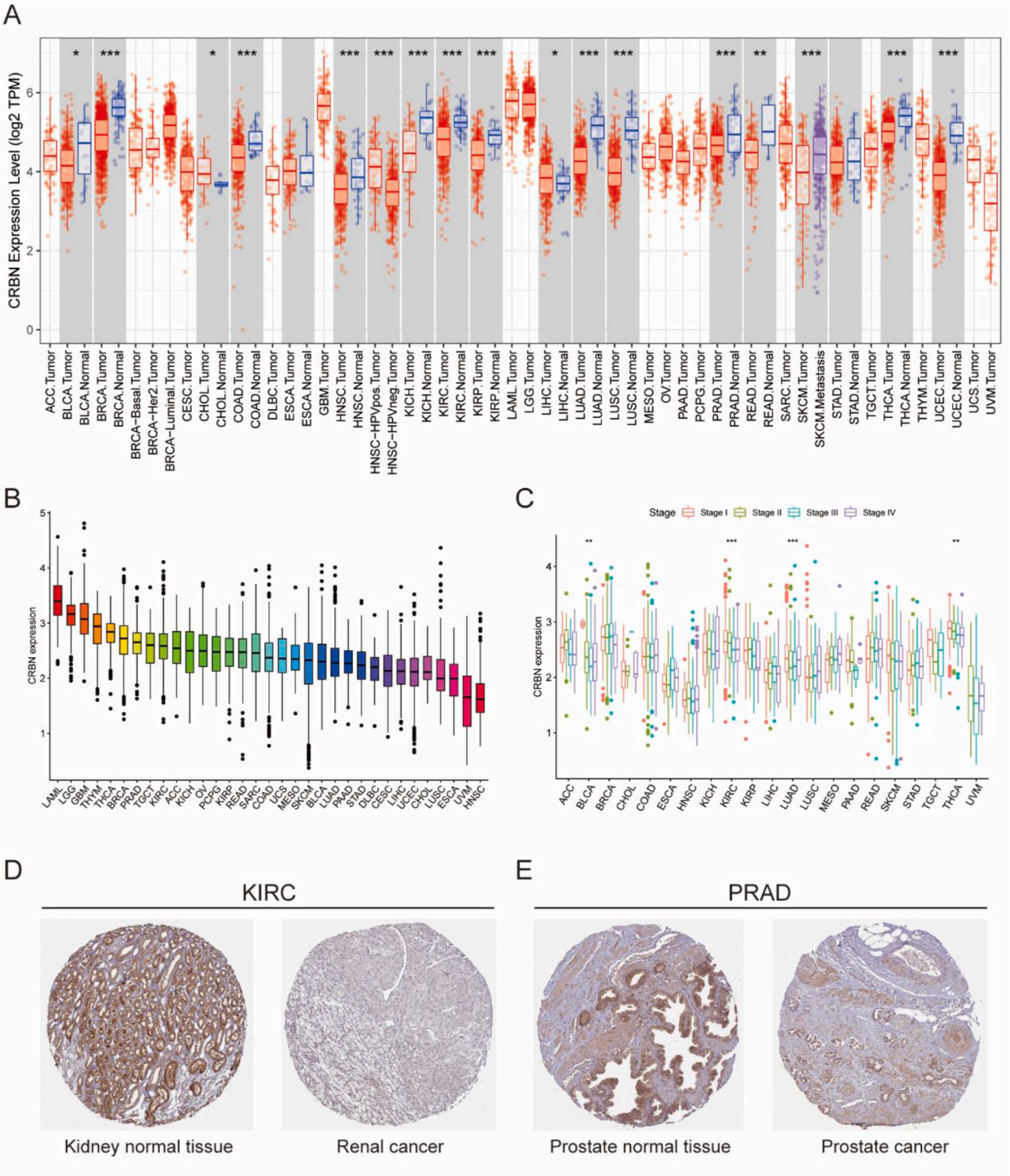

Fig. 1. CRBN expression in different cancers. (A) Differential expression of CRBN in various cancer types based on TGCA data in TIMER. (B) The level of CRBN in pan-cancer, LAML and LGG have the highest level. (C) The correlation between CRBN expression and tumor stage in pan-cancer. (D–E) Representative immunohistochemical images of CRBN expression in normal and tumor tissues of the kidney and prostate.

correlation test between the CRBN expression and ORR of each cancer in the TCGA database.

3. Results

3.1. CRBN expression profile in pan-cancer

The CRBN expression data was evaluated based on the TCGA databases. The result in Fig. 1 showed that the CRBN expression level was significantly downregulated in 11 types of cancer compared with normal tissue (i.e., BRCA, COAD, HNSC, KICH, KIRC, KIRP, LUAD, LUSC, PRAD, THCA, and UCEC; all $p < 0.001$). The upregulation of CRBN expression was observed in CHOL and LIHC ($p <$

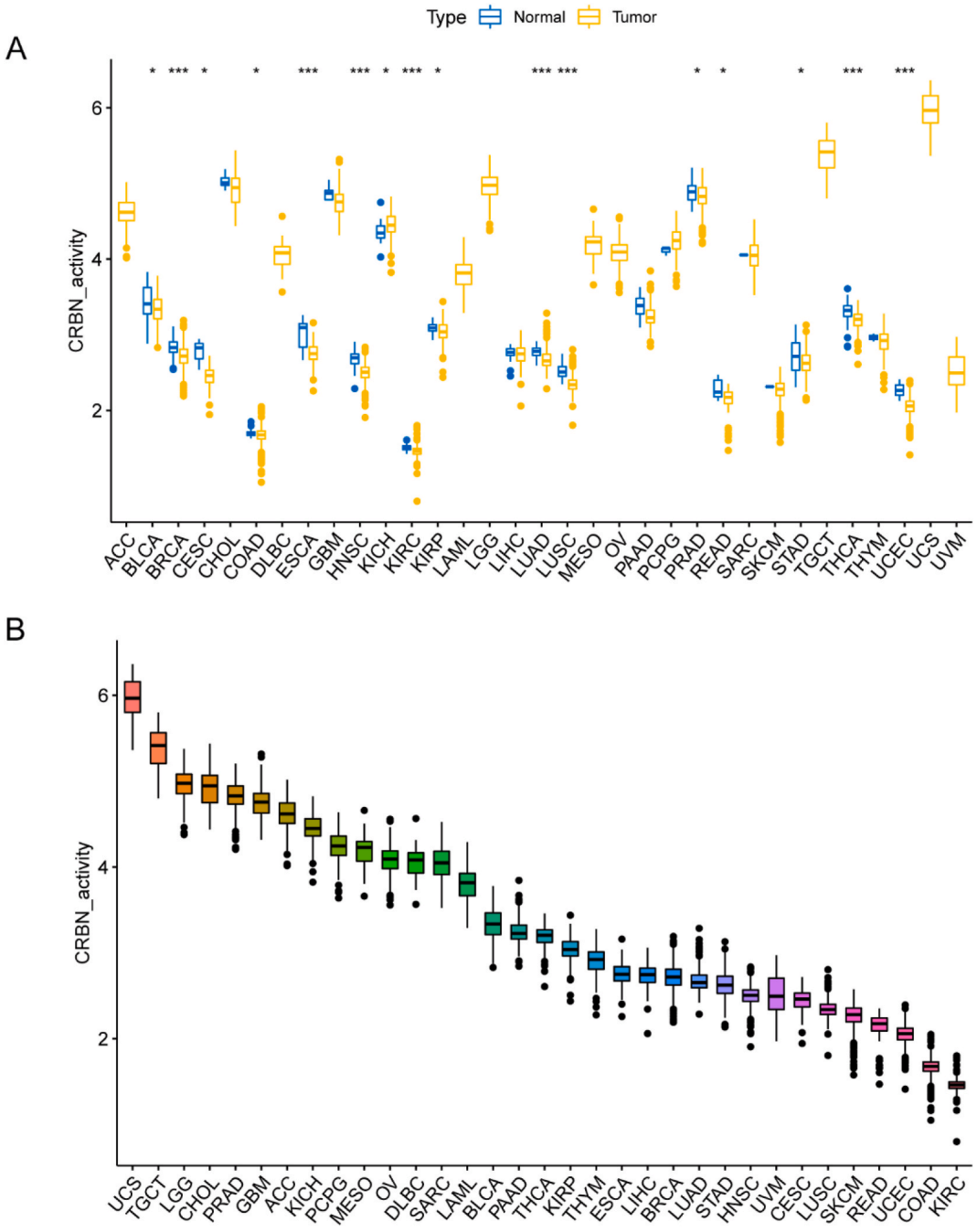

Fig. 2. Generation and investigation of CRBN activity. (A) shows the different activity analysis between tumor and normal groups of CRBN in 33 cancers; (B) shows the mean activity of CRBN in 33 cancers (from high to low). "*" indicates $p < 0.05$ and "***" indicates $p < 0.001$.

0.05). Particularly, remarkable CRBN expression upregulation was found in HPV-positive HNSC compared with HPV-negative HNSC and in metastasis SKCM compared with SKCM in situ (Fig. 1A). The highest expression level of CRBN was observed in LAML and the lowest in HNSC (Fig. 1B). Moreover, the correlation between CRBN expression and tumor stage were evaluated. The result showed that the expression level of CRBN decreased with the progression of the stage in KIRC, While the CRBN expression level remained high in stage IV LUAD (Fig. 1C). Furthermore, the comparison of the expression level of CRBN protein was detected by IHC. The IHC images demonstrated that the CRBN protein was mostly enriched and located in normal tissue, such as the cytoplasm or membrane of normal glomerular cells, tubular cells and glandular cells, while has low expression in tumor tissues (Fig. 1D–E).

3.2. CRBN activity analysis in pan-cancer

Fig. 2A illustrates the different CRBN activity across tumor and normal samples in 33 cancer types. There is a significant increase of CRBN activity in KICH ($p < 0.05$), and a significant decrease in 15 cancer types, including BLCA, BRCA, CESC, COAD, ESCA, HNSC, KIRC, KIRP, LUAD, LUSC, PRAD, READ, STAD, THCA and UCEC compared with normal groups. The boxplot (Fig. 2B) ranked the mean activity of CRBN activity in pan-cancer. Tumors of urogenital system were found to have high remarkable CRBN activity. For example, UCS and TGCT had the highest CRBN gene activity and PARD took the fifth place in the boxplot.

3.3. Prognostic value of CRBN in pan-cancer

The Cox regression and Kaplan-Meier survival analyses demonstrated that a high expression level of CRBN was associated with better overall survival (OS). The univariate Cox regression showed that upregulated CRBN expression was relevant to better OS in ACC ($p = 0.036$, HR = 0.38), BLCA ($p = 0.023$, HR = 0.68), KIRC ($p = 0.001$, HR = 0.52), LAML ($p = 0.009$, HR = 0.46), LGG ($p = 0.001$, HR = 0.41), LUAD ($p = 0.002$, HR = 0.54), SKCM ($p < 0.001$, HR = 0.62) (Fig. 3A). Kaplan-Meier survival analyses in KIRC, LUAD, LGG, and SKCM support the positive relationship between higher CRBN expression and better OS (Fig. 3B–E).

Another three clinical outcomes, survival Disease-free survival (DFS), disease-specific survival (DSS), and progression-free survival (PFS) were introduced to further analyze the prognostic value of CRBN. As Fig. 4A–C illustrated, high-expressed CRBN was correlated with worse DFS in STAD and OV based on Cox regression analysis, and in ESCA and OV based on Kaplan-Meier survival analysis. In terms of DSS, patients with CRBN overexpression had a significant longer DSS than patients with low expression of CRBN, covering BLCA, COAD, KIRP, LUAD, SKCM, KIRC, LGG and THYM (Fig. 4D–I). The results of the KIRC, LGG and THYM were consistent with Cox regression analysis, but differences existed between Cox regression and Kaplan–Meier survival analysis in KIRP and CHOL. At the same time, CRBN overexpression had a negative influence on DSS in DLBC, KICH, LIHC, PRAD, SARC, UCEC, UCS and significantly in PCPG. In addition, it turned out that PFS in patients with a high CRBN expression was shorter than those with low CRBN expression in 10 cancer types, including DLBC, ESCA, KICH, LIHC, LUAD, MESO, OV, PRAD and THCA (Fig. 4J-O). Significant longer PFS was shown in KIRC, SKCM ($p < 0.05$), KIRP, LGG and ($p < 0.001$), consistent with the Cox regression. However, difference existed between Cox

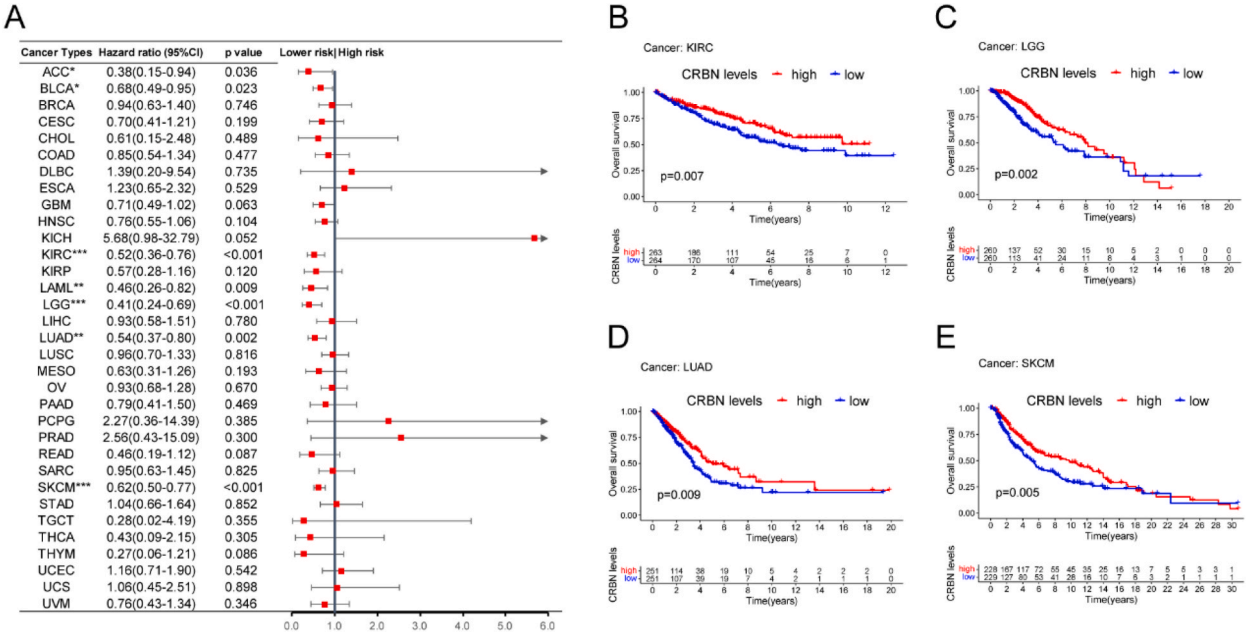

Fig. 3. Relationship between CRBN expression and patient overall survival (OS). (A) The forest plot of univariate Cox regression analyses of CRBN expression and OS in pan-cancer. (B–E) Kaplan-Meier analyses of the OS between high and low CRBN expression levels in four cancer types (KIRC, LUAD, LGG, SCKM). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

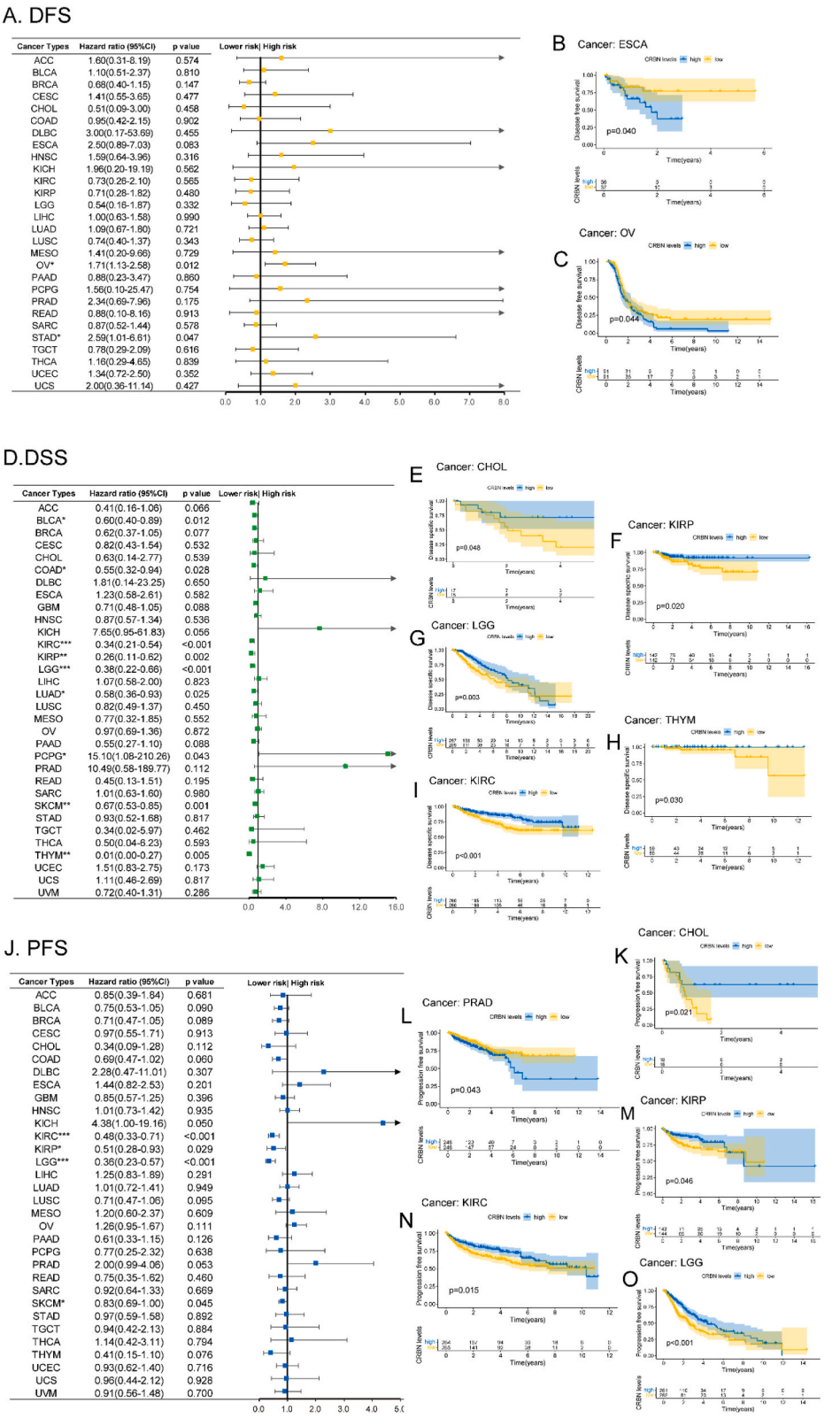

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Fig. 4. Kaplan–Meier survival and Cox regression analyses of the prognostic value of CRBN expression level in clinical survival outcomes. (A–C) Forest plot and Kaplan-Meier survival analyses in ESCA and OV for patient disease free survival (DFS); (D–I) Forest plot and Kaplan-Meier survival analyses in CHOL, KIRP, LGG, THYM, and KIRC for patient disease specific survival (DSS); (J–O) Forest plot and Kaplan-Meier survival analyses in CHOL, PRAD, KIRP, KIRC and LGG for patient progression free survival (PFS). *p < 0.05, **p < 0.01, ***p < 0.001.

regression and Kaplan–Meier survival analysis in PRAD and CHOL. Anyway, the results of OS, DSS, and PFS indicated that high expression of CRBN in most tumors may be a prognostic factor of a longer survival in patients, but the divergent relation in DFS is intriguing.

3.4. Correlation of CRBN with tumor immune microenvironment

To assess the relationship between CRBN and tumor immune microenvironment, we calculated the immune score, stromal score and tumor purity. CRBN expression was positively associated with immune cell abundance in LUSC, ESCA, HNSC, LUAD and PAAD, while negatively in BLCA, KIRP, LGG and SARC, in which CRBN expression was negatively associated with stromal cell content (Fig. 5A–K). The ESTIMATE score, immune score, stromal Score and Tumor purity were summarized in Fig. 5O ($p < 0.05$).

As demonstrated in Fig. 6, the effect of CRBN expression on the infiltration of immune cells was significantly different in cancers. CRBN expression was positively correlated with B cell infiltration in ESCA and TGCT. In contrast, for T regulatory cells, CRBN expression was positively associated with infiltration in ESCA, but negatively in KIRC. Intriguingly, the two relations between CRBN expression and the activation status of $CD4^+$ memory T cell were opposite and both significant, which strongly suggest the positive influence of $CD4^+$ memory T cell' s activation on the CRBN expression (Fig. 6A-P). In general, the effect of CRBN on immune infiltration was heterogeneous among different cancer types (Fig. 6Q).

The relationship between immune modulators and CRBN expression was summarized in Fig. 7. Take three modulators in BLCA and PRAD as examples, the analysis of 45 immune stimulators illustrated that CRBN expression was negatively associated with CD276 (Fig. 7A). For 24 immune inhibitors, upregulated CRBN expression indicated lower PDCD1 expression level in BLCA and PRAD (Fig. 7B). Moreover, negative association between CRBN expression and B2M were found in BLCA and PRAD. (Fig. 7C).

3.5. Gene set enrichment analysis in pan-cancer

According to the Gene set enrichment analysis (GSEA), the results of GSEA with regard to CRBN high and low expression group in pan-cancer were shown in Fig. 8A, and the GSEA analysis between CRBN high and low expression group in THCA and LUSC were provided in Fig. 8B–C. In most cancer types, most pathways tended to be low expressed in high-expressed CRBN patients, especially in BLCA, KIRP and THCA, indicating a possible positive role of CRBN expression with regard to the inhibition of development and progression of malignant tumors. For example, tumorigenesis-positive related pathways, such as TNFA signaling via the NF-κB pathway, were widely high expressed in low level CRBN patients of most cancer types. High expression of negative regulation of cell cycle G1/S phase translation pathways in high-expressed CRBN patients in THCA supported the idea. What's more, CRBN high-expressed patients in LUAD, LUSC and CHOL represented high expression of JAK/STAT1 signaling pathways, which are associated with the inhibition of tumor occurrence as well as the tumor immunologic environment [22].

3.6. Association between CRBN expression and TMB, MSI, ORR and detail immune infiltration

Tumor mutation burden (TMB) is a vital but imperfect response biomarker, which reflects cancer mutation quantity [23]. Microsatellite instability (MSI) represents the patient response to immunotherapy [24]. We evaluated the correlation between CRBN expression and TMB and MSI. The CRBN expression was negatively associated with TMB in BRCA, KIRC, LGG, LUAD, while positively correlated with TMB in LAML ($p < 0.001$; Fig. 9A). In terms of MSI, significant negative association was observed in DLBC, LUAD, LUSC, whereas no positive association existed ($p < 0.001$; Fig. 9B). ORR is commonly used to evaluate the efficacy of antitumor therapy. With the increase of CRBN expression, ORR was observed to descend in cancers (Fig. 9C). Different immune cell infiltration may require distinct immunotherapeutic interventions [25]. We further analyzed the relationship between CRBN and immune cell infiltration. We discovered that the CRBN expression in pan-cancer was positively correlated with $CD4^+$ T cell, B cell, common lymphoid progenitor and $CD8^+$ T cell, whereas negatively correlated with CD4 + Th1 T cell, and MDSC (Fig. 9D).

4. Discussion

In order to better understand the physiological role of CRBN, great efforts have been made on the mechanism of action of thalidomide and its derivatives. Despite the side effect of human teratogen, thalidomide has been found to have therapeutic effect on inflammatory diseases and cancers [26]. However, the identification of cyclic imides as the physiological degrons on substrates recognized by CRBN represents a deeper understanding of the interaction between substrates and CRBN and close the cycle between IMiDs and fundamental CRBN research [27]. With the exploration of the molecular mechanism of action of CRBN, a breakthrough discovery reveals CRBN as a component of E3 ubiquitin ligase complex and a substrate recognition receptor, which promotes the development of targeted protein degradation [4,28].

In this study, several databases were chosen to analyze the role of CRBN in pan-cancer, including TCGA and GEO. Compared to

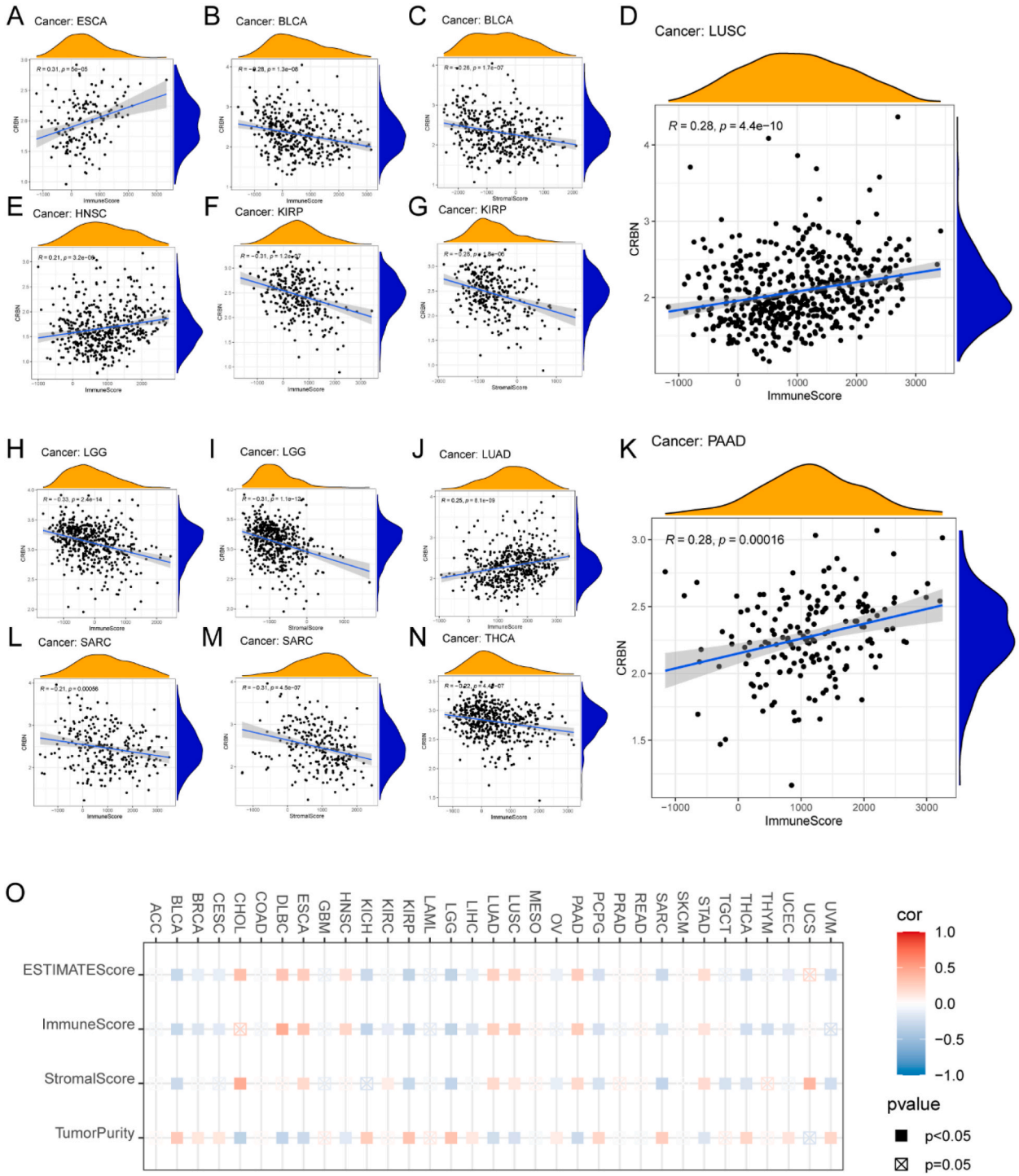

Fig. 5. Correlation between CRBN expression and both the ESTIMATE score and immune cell infiltration. The ESTIMATE score includes the stromal score (indicates the presence of stromal cells in tumor tissues), the immune score (represents the infiltration of immune cells in tumor tissues), and tumor purity. (A–K) The correlation plots in ESCA, BLCA, HNSC, KIRP, LUSC, LGG, LUAD, SARC, THCA and PAAD; (O) Correlation heatmap of CRBN and ESTIMATE score in pan-cancer.

normal groups, the expression and activity of CRBN in tumor groups were widely found to show decrease in pan-cancer. On one hand, it suggests an expectable role of CRBN expression in tumor prognosis, recognizing the significant relation between CRBN' s expression level and tumor grade in BLCA, KIRC, LUAD and THCA, as well as the fact that high expression of CRBN refers to a longer OS, DSS, and

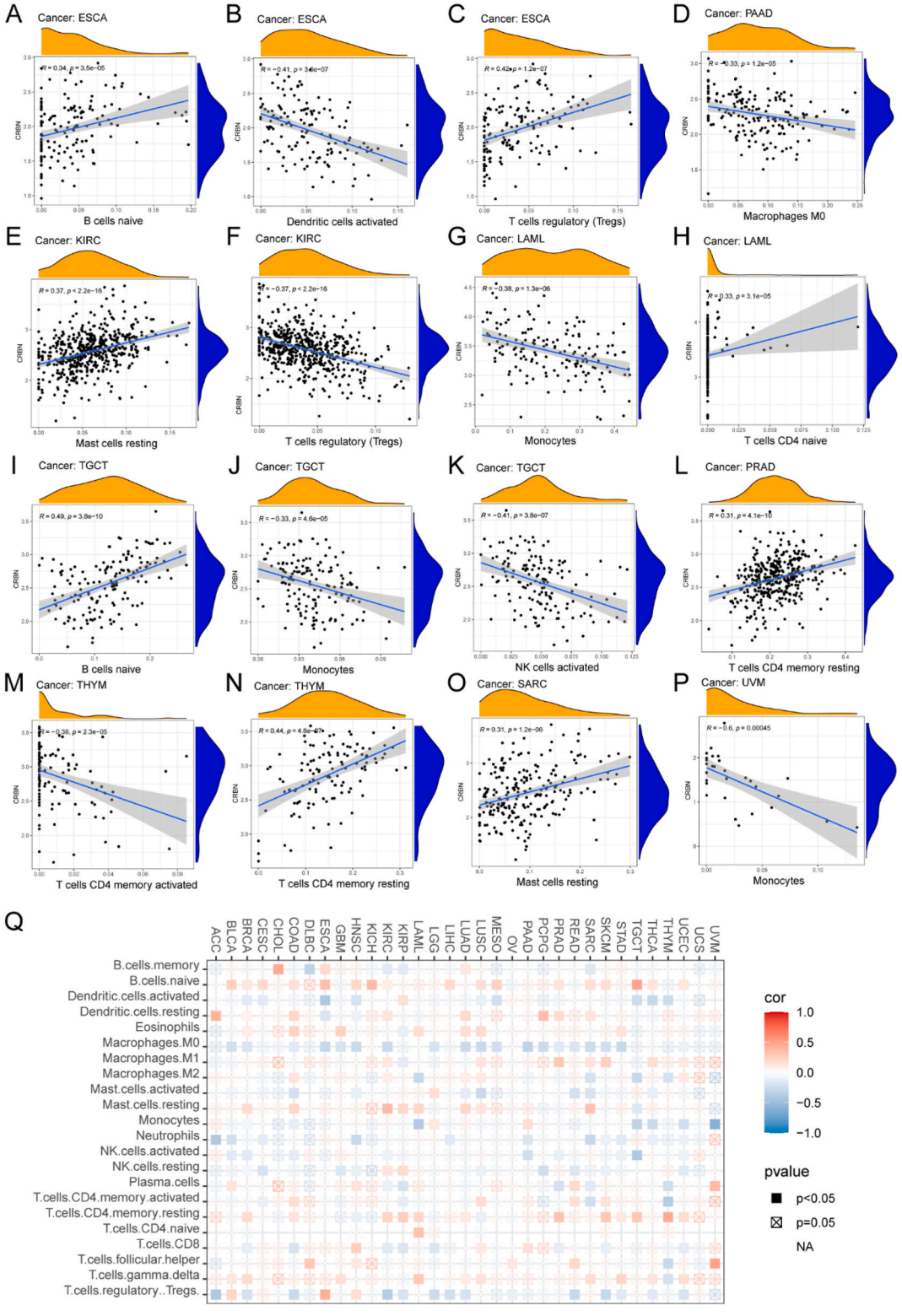

Fig. 6. | Correlation between CRBN expression and immune cell infiltration. (A–P) The correlation plots with $p < 0.05$; (Q) Correlation heatmap of CRBN and immune cell infiltration in pan-cancer.

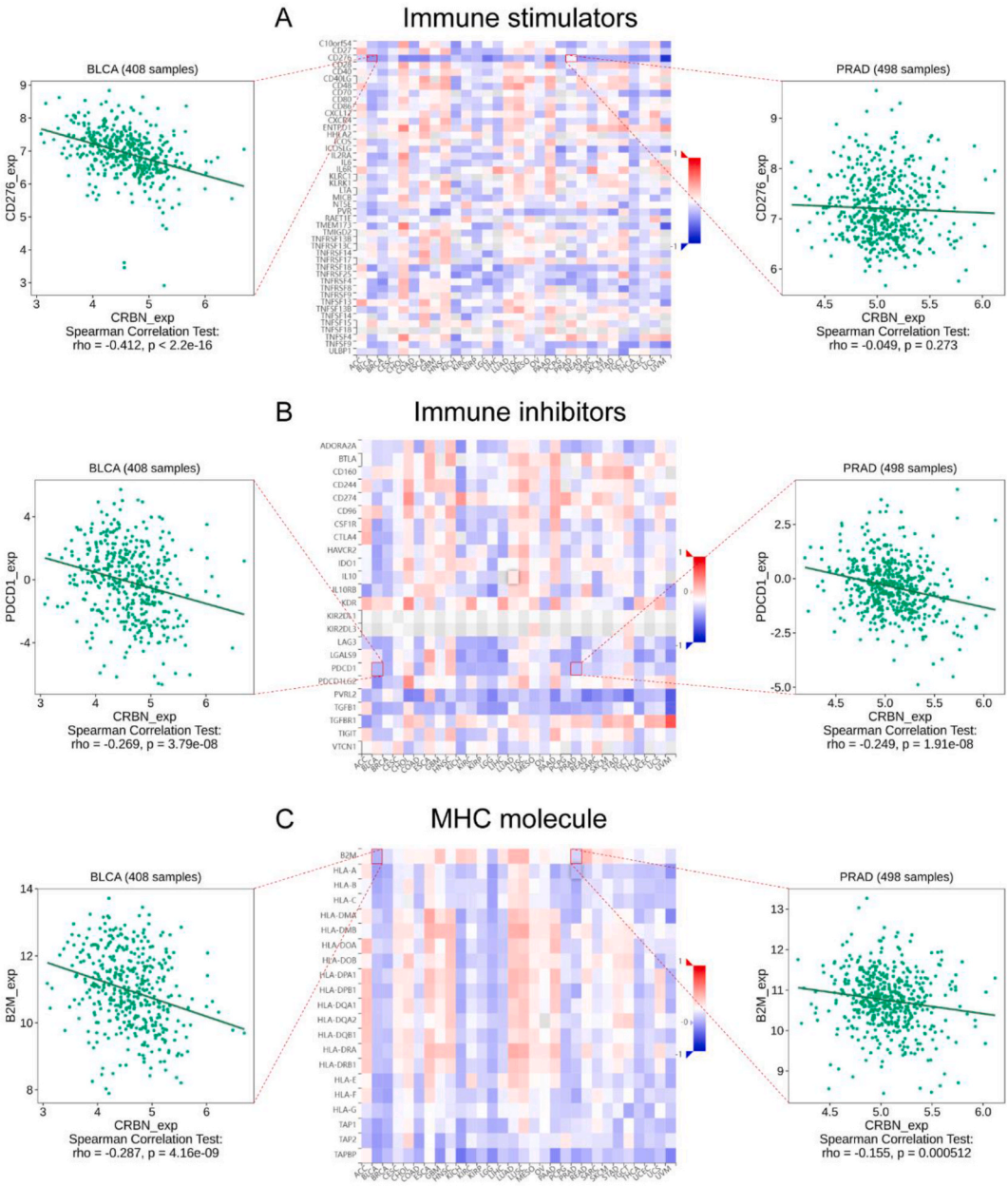

Fig. 7. | Relationship between three kinds of immunomodulators and CRBN expression according to the TISIDB database. Expression correlations between CRBN and (A)immune stimulators; (B)immune inhibitors; and (C)MHC molecules. Red and blue represent positive and negative correlations, respectively. The correlation plots in BLCA and PARD were shown in the dot plot. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

PFS in most cancer types except DLBC, ESCA, KICH, PRAD. Notably, the results of DFS evaluation showed a negative influence of higher-expressed CRBN on DFS. The diversity may be related to the nonuniform definition of 'disease recurrence' and the fact that the protocol defined assessment techniques and intervals time to this event significantly affect the time to event [29]. For example, the recurrence of tumor is measured by sensitive blood tests in early stage, while the cancer grows relatively slowly in the process of recurrence, it may take several years for the patient to develop symptoms. In this situation, the selection of DFS in the evaluation of patient's conditions should be treated. On the other hand, the lower expression and activity of CRBN in tumor groups may become a disadvantageous factor for the application of CRBN-based medicine. It implies a beneficial role of the up-regulation of CRBN expression acting as an auxiliary regulation in concert with CRBN-based medicine and as a direct inhibition for tumorigenesis, which is supported by the functional enrichment analysis based on GSEA.

To further investigate the immunologic role of CRBN, the relationship between CRBN expression and immune score, stromal score,

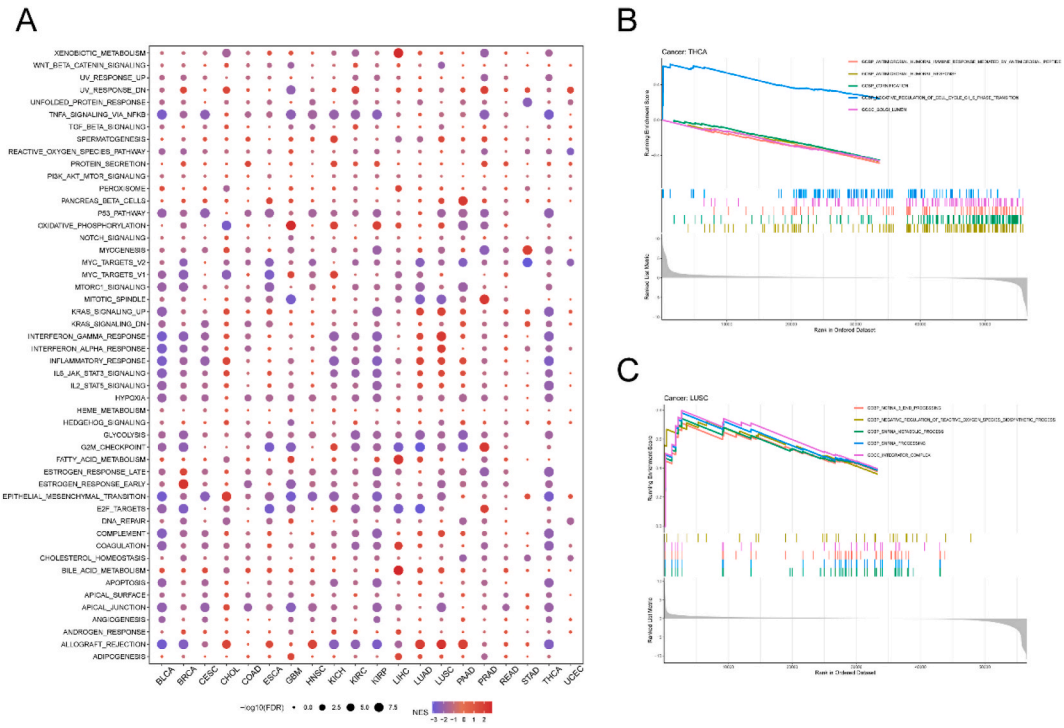

Fig. 8. | Results of gene set enrichment analysis (GSEA). (A) Results of GSEA regarding CRBN high and low expression group in pan-cancer. (B–C) The GSEA analysis between CRBN high and low expression group in THCA and LUSC.

tumor purity, immune cell infiltration and immunomodulators was explored. Increased expression of CRBN was positively correlated with the immune infiltration of naive B cells and plasma cells, and naive $CD4^+$ T cells and negatively associated with $CD8^+$ T cells and M0 Macrophages. A previous study elucidated that lenalidomide modulates the CRBN-CRL4 E3 ligase to realize the degradation of the transcription factor zinc finger 1 (IKZF1), which promotes T-cell stimulatory properties in M2-like myeloma-associated macrophages [30]. Lenalidomide redirects CRBN to degrade IKZF1 and IKZF3, unleashes paracrine interleukin-2 (IL-2), and activates intra-tumoral $CD8^+$ T cell through Notch signaling [31]. These results on the infiltration of T cells are consistent with the conclusion of our study. In terms of B cell infiltration, CRBN-related immunomodulatory drugs are currently used in the treatment of multiple myeloma [32]. CRBN acts as a co-chaperone of HSP90 and determines HSP90 activity towards transmembrane proteins in multiple myeloma. Immunomodulatory drugs targeting CRBN have achieved the curative effect by disrupting the interaction between HSP90 and CRBN [33]. Since the pathogenesis of multiple myeloma is the aberrant proliferation of plasma cells, we hypothesized that CRBN-related B cell infiltration was correlated with the curative effect of immunomodulatory drugs. More fundamental research is needed to prove the point.

Notably, 22 splice variants of CRBN mRNA was found in MM cell lines and primary $CD138^+$ tumor cells and only 4 of these variants encode proteins [34]. The full length of CRBN mRNA is capable of encoding functional CRBN, while variants of CRBN mRNA with the deletion of the binding region of DNA damage binding protein 1 (DDB1) and IMiDs do not encode CRBN with normal functions [35]. Therefore, the upregulation of total CRBN mRNA may not mean the high expression of functional CRBN. Studies have found that the splicing variants of CRBN with the deletion of exon 10 exist in multiple myeloma (MM) and myelodysplastic syndromes (MDS) patients and multiple genetic alterations of CRBN are associate with the resistance of IMiDs [36,37]. The impact of the splicing variants of CRBN on immune cell infiltration and the efficacy of IMiDs, molecular glues and PROTACs deserves further investigation.

Drug design based on CRBN have developed rapidly. IMiDs have shown potent efficacy and become one of the standard approaches in hematological malignancies, the regulatory networks of this class of drugs are being explored [38]. In gastrointestinal cancer, a novel CRBN E3 ligase modulator called MDEG-541 was developed. MDEG-541 induces the degradation of G1 to S phase transition 1/2 (GSPT1/2) and the Polo-like kinase 1 (PLK1) [39]. In addition, a novel glutarimide ligand was synthesized for CRBN, which lays a solid foundation for the synthesis of advanced PROTAC protein degraders [40]. In addition to the CRBN modulator and the improved CRBN ligands mentioned above, molecular glues enable E3 ligases to recruit neo-substrates, induce the proximity of targeted protein and E3 ligase and promote the degradation of targeted protein via UPS. A set of molecular glues are CRBN binding drugs [41]. Similar to the mechanism of molecular glues, numerous PROTACs were designed to recruit CRBN E3 ligase and some PROTACs with excellent degradation efficacy have entered the phase I or phase II clinical trials [12]. Furthermore, CRBN-based novel PROTACs show excellent advantages in cancer treatment. A carbon-dot-based PROTAC can not only recruit CRBN and induce the degradation of programmed cell death ligand 1(PD-L1) but also triggers immune responses by activating the stimulator of the interferon genes (STING) pathway [42]. However, possible limitations exist in future development and application of CRBN-based drugs. A study proposed that the

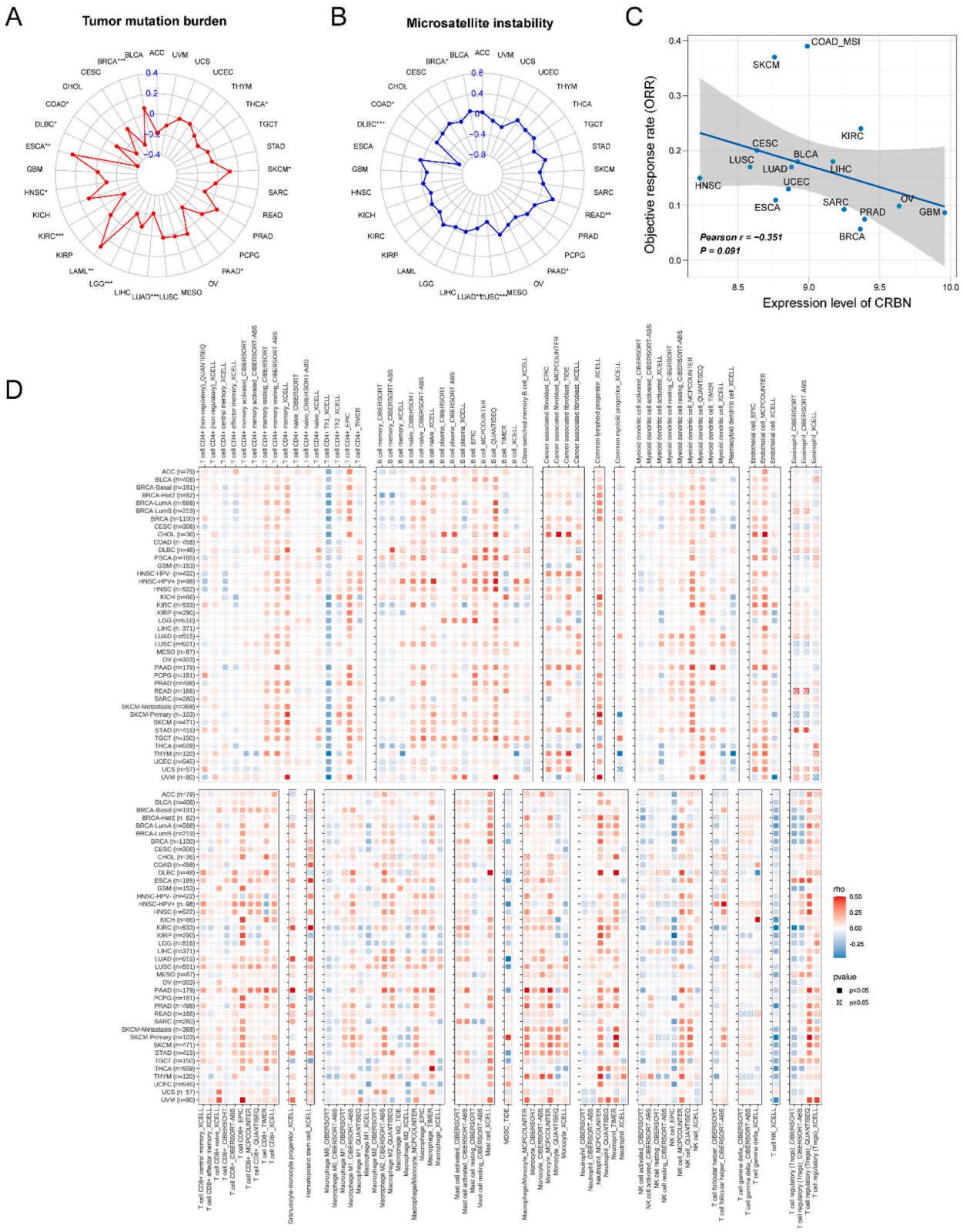

Fig. 9. Prognostic value of CRBN in immunotherapy. (A, B) Association between CRBN expression and TMB and MSI in pan-cancer. (C) Correlations between CRBN expression and objective response rate in different cancers. (D) Relationship between CRBN and immune cell infiltration in the TME based on 7 different algorithms.

reduction of cereblon protein after IMiDs treatment attributed to the resistant to lenalidomide and pomalidomide for MM cell lines [34]. In addition to the downregulation of CRBN gene expression, the mutation or copy loss of CRBN gene and splice variants of CRBN mRNA may significantly affect the degradation efficacy of CRBN recruiting PROTACs even if in the cell lines with high CRBN expression level.

Although the study is the first one to multidimensionally analyze CRBN in pan-cancer, some limitations still exist. Firstly, all the analyses were based on the data from online databases, which lack of verification by molecular and animal experiments, which should be the main limitation of the study. To make our analysis more reliable, we explore and collect representative IHC images from HPA database to verify our conclusions. Secondly, the definite physiological mechanisms of CRBN in cancer are not provided in current articles, so a deeper and more targeted exploration fails to carry out. Thirdly, the sample size in the TCGA database was limited, which may lead to some bias in the analysis results.

5. Conclusion

Pan-cancer analysis reveals the positive role of CRBN expression in tumorigenesis inhibition and its versatile immunologic roles in different cancer types. It is also promising for CRBN to be a prognostic biomarker. However, low expression of CRBN in most tumor groups penalizes the application of PROTAC. The upregulation of CRBN presumably benefits the therapeutic strategies of cancer more than expected.

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Author contributions

SW, QX and JH designed the manuscript. SZ and NZ wrote and completed the manuscript. XZ, SM, HH made contribution to the acquisition and analysis of data. YA and MX edited the figures and a table of this article. JS, CL and JX reviewed and revised the manuscript. All authors have made significant scientific contributions to the research and agreed to submit the final manuscript.

Data availability statement

All data analyzed in this study are included in the article, and all data/ codes have been uploaded and are available from the following Jianguo Cloud links: https://www.jianguoyun.com/p/DS6uNFsQ95SsCxjT4vQEIAA.

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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