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Research article

Pan-cancer analysis of the prognostic and immunological role of FKBP4

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ABSTRACT

Objectives: Our previous studies revealed the significant roles of FK506-binding protein 4 (FKBP4) in tumorigenesis, however, there has been no pan-cancer analysis of FKBP4. Using bioinformatics, the current study reported the expression and prognostic role of FKBP4, and the correlation between FKBP4 and clinicopathological parameters, methylation, molecular network, immunological traits and drug sensitivity.

Methods: RNA sequencing data, somatic mutation, and related clinical information were obtained from TCGA using UCSC Xena. The association between FKBP4 expression and clinical features was assessed using TISIDB. The relationships between FKBP4 expression and tumour stage, OS, DSS, DFS, and PFS were analysed using univariate cox regression analysis. The radar plots for TMB and MSI were obtained using "Fmsb" R package. UALCAN was used to explore the effect of FKBP4 methylation on tumour and normal samples. CBioportal was used to analyse copy number mutations in FKBP4 Gene expression and drug sensitivity data were downloaded from the CellMiner database. GO analysis was performed for the high and the low expression of FKBP4 compared with the median level of FKBP4 using clusterProfiler4.0.

Results: FKBP4 expression is significantly upregulated in various types of cancers. Cox regression analysis showed that high FKBP4 levels were correlated with poor OS, DSS, DFS, and PFS in most patients with cancer. Methylation of FKBP4 DNA was upregulated in most cancers, and FKBP4 expression is positively associated with transmethylase expression. FKBP4 and its copy were significantly associated with the expression of immune-infiltrating cells, immune checkpoint genes, immune modulators, TMB, MMR, and MSI. FKBP4 expression levels significantly correlated with 16 different drug sensitivities (all $p < 0.05$).

Conclusions: Our pan-cancer bioinformatic analysis revealed a potential mechanism underlying the effects of FKBP4 on the prognosis and progression of various cancers.

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1. Introduction

Cancer is an important cause of death globally. With a rising number of newly diagnosed cases [1], the economic burden of cancer continues to increase. Recently, targeted therapy and immunotherapies have become a prominent cancer treatment methods [2]. Therefore, it is necessary to identify innovative and sensitive biomarkers for cancer diagnosis and treatment [3]. With the continuous evolution of The Cancer Genome Atlas (TCGA), new targets for clinical treatment can be discovered by performing pan-cancer expression analyses [4].

FK506-binding protein 4 (FKBP4), a molecular chaperone, participates in numerous molecular processes by binding to different cellular receptors or targets in various cancers [5–9]. For example, FKBP4 has been predicted to interact with chaperonin-containing TCP-1 (CCT) family members to exert oncogenic functions in breast cancer [8]. Additionally, phytanoyl-CoA alpha-hydroxylase (PAHX) is a unique target of FKBP4 in the presence of immunosuppressants [10]. However, FKBP4's immunologic role remains unknown, and we are eager to find more novel details regarding its mechanism of cancer immunology in the tumour microenvironment.

In the current study, we collected data from various databases, such as TCGA, UALCAN, cBioPortal, TISIDB, TIMER, GeneMANIA, CellMiner and Kaplan-Meier Plotter to demonstrate FKBP4's connection with the prognostic role and the immunological response. We

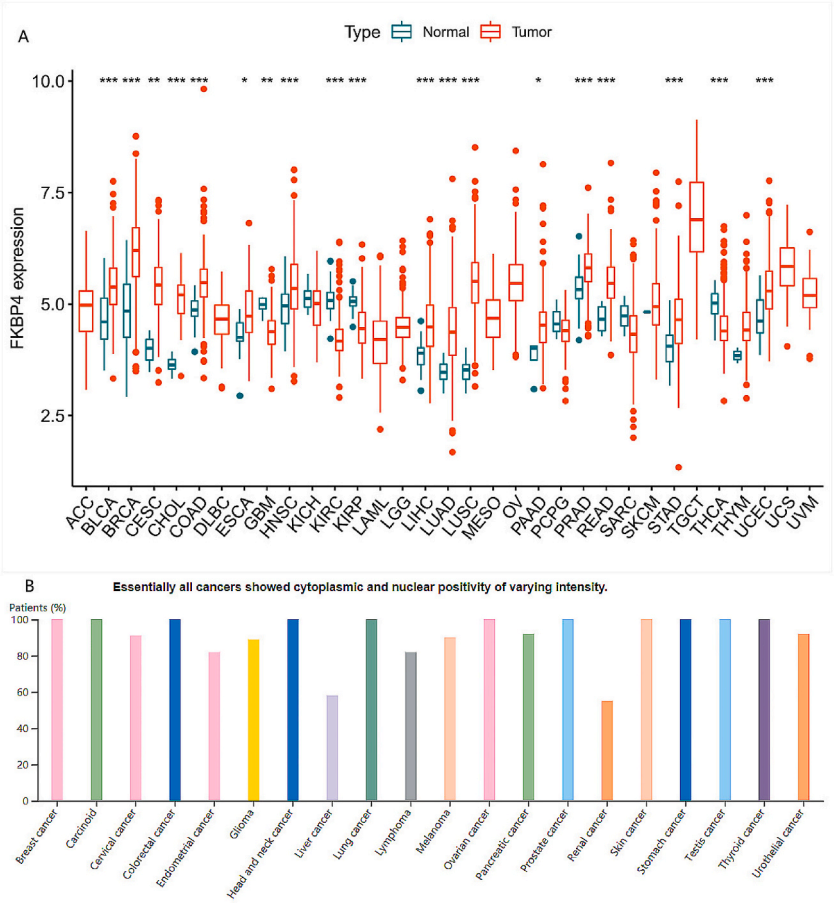

Fig. 1. FKBP4 expression at mRNA and protein levels in pan-cancer. **A** The mRNA levels of FKBP4 in diverse human cancers. The data is from TCGA. ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical and endocervical cancer; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck cancer; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumours; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma. Green represents the normal group, red represents the tumour group, the middle horizontal line in each cancer represents the median expression of FKBP4, dots represent abnormal data. **B** The protein levels of FKBP4 in different types of human cancers. The data is from HPA. The same color represents the same organ system of the human body, and the ordinate represents the proportion of patients with positive FKBP4 expression in all patients. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

then explored the relationship between FKBP4 levels and clinical stage, prognosis, DNA methylation, immune infiltration levels, immune modulators, tumour mutational burden (TMB), mismatch repair (MMR), microsatellite instability (MSI), and drug reactions. We also explored the molecular functions of FKBP4 using GO and KEGG analyses. The current pan-cancer analysis revealed the oncogenic role of FKBP4 in pan-cancer and that FKBP4 could be a promising therapeutic target for cancers in the context of immunotherapy.

2. Results

2.1. FKBP4 mRNA and protein expression level in pan-cancer

Our previous study showed that FKBP4 plays a malignant role in the luminal A subtype of breast cancer [8], suggesting that targeting FKBP4 for tumour diagnosis and treatment is novel. To assess whether FKBP4 levels correlated with other tumours, we explored FKBP4 levels in diverse cancers and adjacent tissues. Using TCGA database, we found that FKBP4 mRNA levels varied remarkably in BLCA, BRCA, CESC, CHOL, COAD, ESCA, GBM, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, PAAD, PRAD, READ, STAD, THCA and UCEC tumour tissues (Fig. 1A). Another database also revealed the upregulation of FKBP4 at the mRNA level (Figs. S1A and B). We used both the HPA and CPTAC databases to evaluate the protein expression of FKBP4. Most cancer tissues showed intense granular cytoplasmic FKBP4 expression (Fig. 1B; Fig. S1C). Collectively, FKBP4 acts as an oncogene in most cancer types.

2.2. Pan-cancer analysis of the connection of FKBP4 and clinical stage

To demonstrate the correlation between FKBP4 and clinicopathological features in diverse cancers, we explored FKBP4 levels in normal and patients with cancer stages I, II, III, and IV. Using the UALCAN database, FKBP4 expression was found to be strongly consistent in several advanced cancers, including ACC, BRCA, CESC, HNSC, KIRP, KICH, LUAD, LIHC, THCA, TGCT, and UCEC (Fig. 2). However, FKBP4 was not significantly expressed in other cancers with advanced clinical stages (Fig. S2).

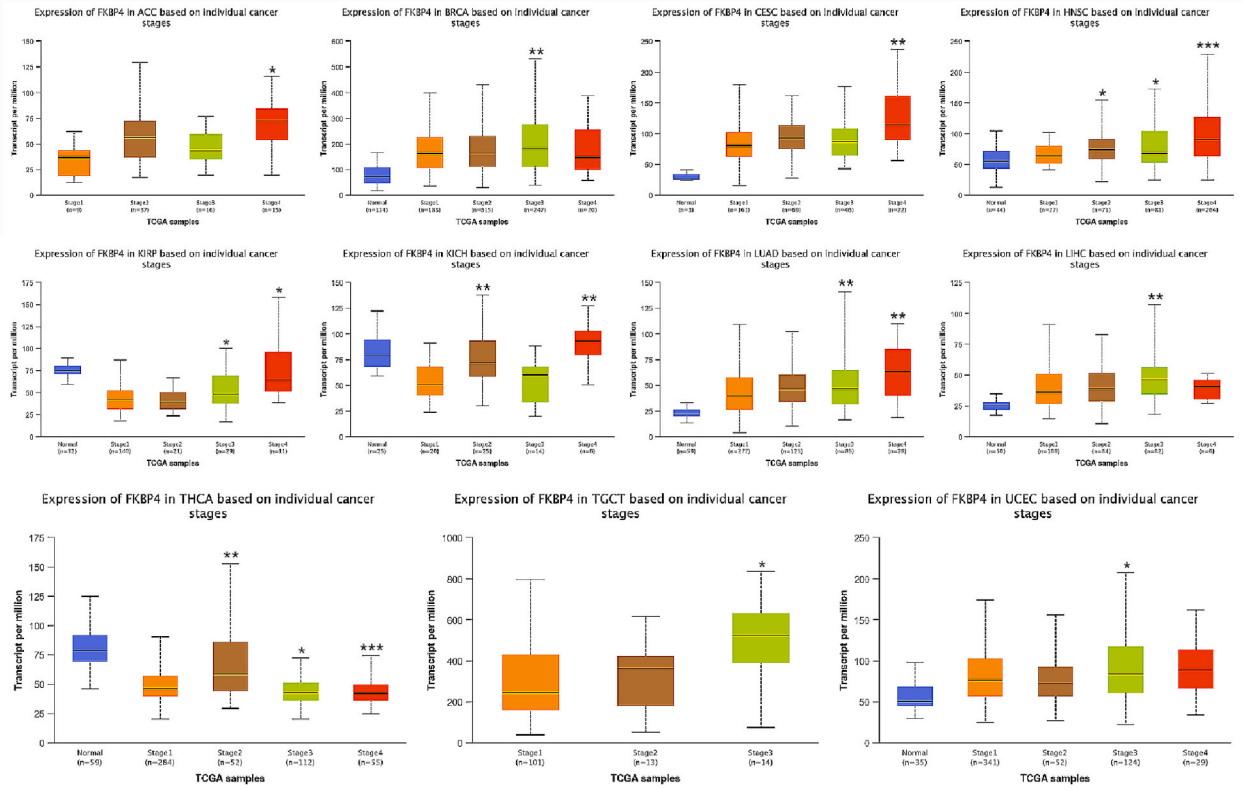

Fig. 2. Connections of FKBP4 and the main pathological stages, including stage I, stage II, stage III, and stage IV of ACC, BRCA, CESC, HNSC, KIRP, KICH, LUAD, LIHC, THCA, TGCT, and UCEC, were investigated using the UALCAN database. Different colors represent different stages, the middle horizontal line in each cancer represents the median expression of FKBP4, and the ordinate represents the expression level. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

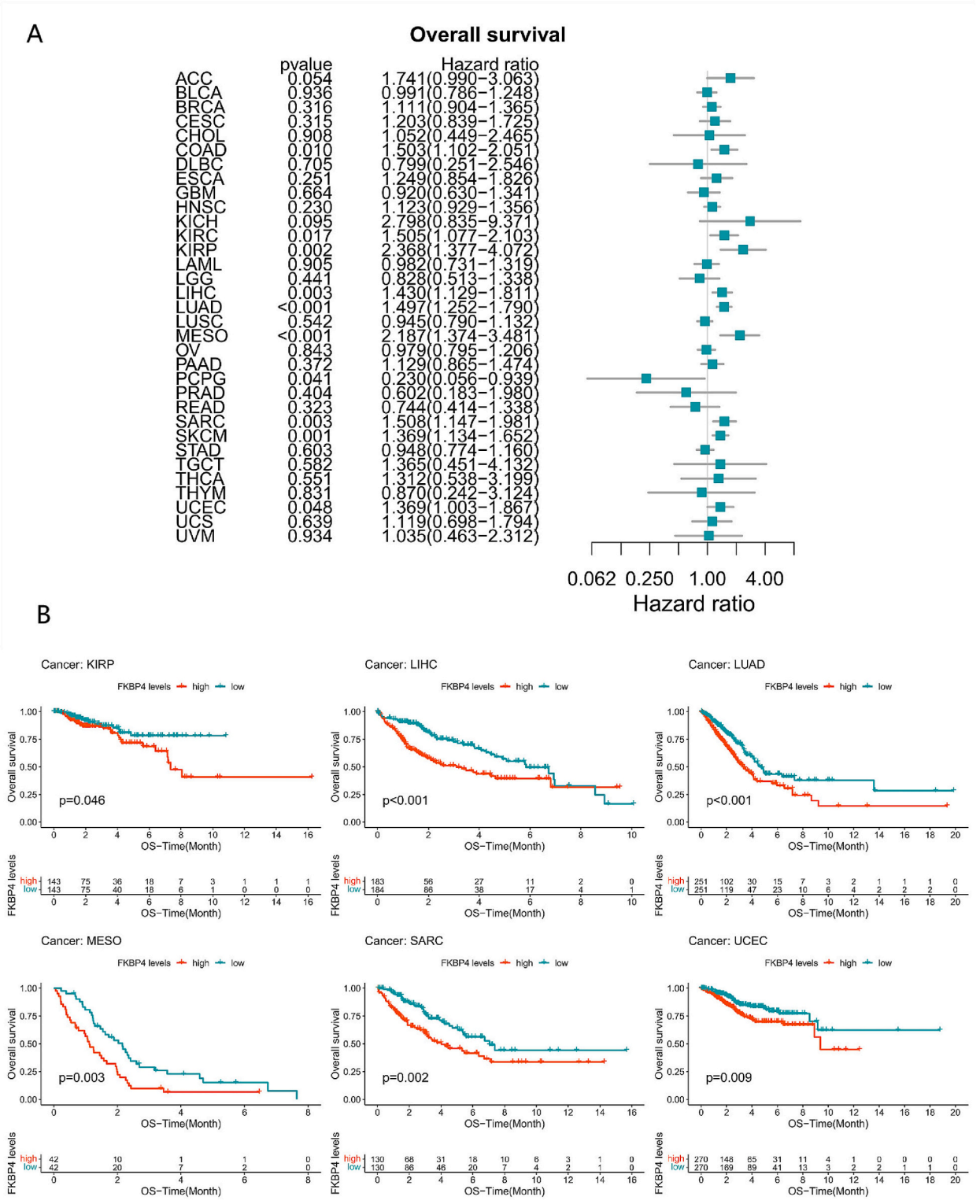

Fig. 3. Connections of FKBP4 and OS in pan-cancer. **A** The forest plot of FKBP4 in OS of pan-cancer patients, the left column shows the tumour name, p value and hazard ratio, and the right column shows the forest map, horizontal lines without intersecting with vertical lines indicate differences. **B** Kaplan-Meier survival curves of OS for patients with different FKBP4 levels in KIRC, LIHC, LUAD, MESO, SARC, and UCEC. Red represents high expression and green represents low expression of FKBP4. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

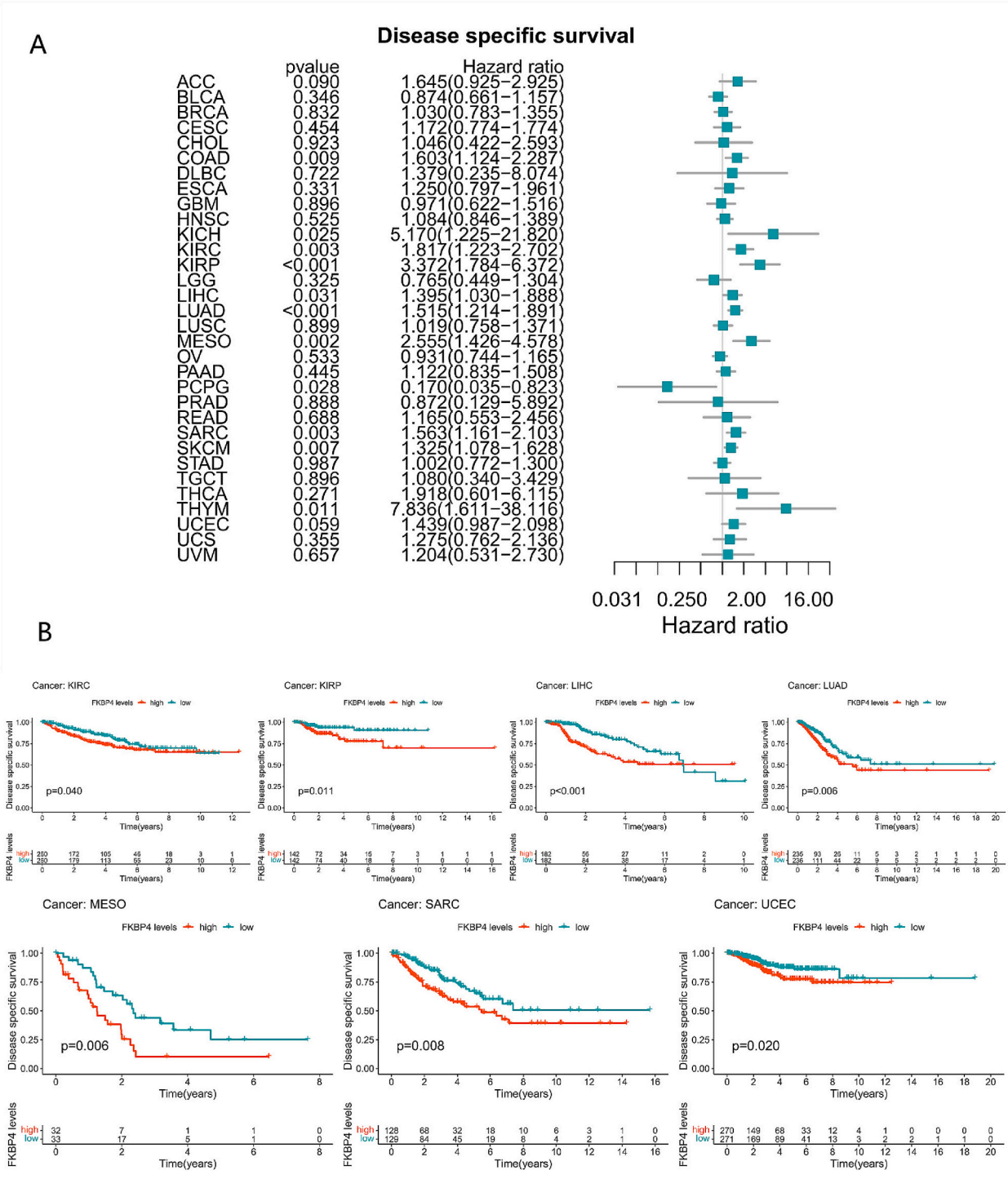

Fig. 4. Connections of FKBP4 and DSS in pan-cancer. **A** The forest plot of FKBP4 in DSS of pan-cancer patients, the left column shows the tumour name, p value and hazard ratio, and the right column shows the forest map, horizontal lines without intersecting with vertical lines indicate differences. **B** Kaplan-Meier survival curves of DSS for patients with different FKBP4 levels in KIRC, KIRP, LIHC, LUAD, MESO, SARC, and UCEC. Red represents high expression and green represents low expression of FKBP4. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2.3. Prognostic function of FKBP4 among pan-cancer

We further explored the prognostic role of FKBP4 in pan-cancer. Survival curves indicate OS, DSS, DFS, and PFS. Cox regression analysis of the results showed in 33 types of cancer FKBP4 levels were remarkably correlated with OS in 10 cancer types: COAD, KIRC, KIRP, LIHC, LUAD, MESO, PCPG, SARC, SKCM, and UCEC (Fig. 3A). Kaplan–Meier survival curves showed that an increased FKBP4 level was significantly correlated with worse OS in KIRC, LIHC, LUAD, MESO, SARC, and UCEC (Fig. 3B). We also explored the association between FKBP4 levels and DSS in patients with cancer. FKBP4 levels affected DSS in 11 cancer types: COAD, KICH, KIRC, KIRP, LIHC, LUAD, MESO, PCPG, SARC, SKCM, and THYM (Fig. 4A). Kaplan–Meier analysis showed that the upregulated FKBP4 levels were significantly associated worse DSS in patients with KIRC, KIRP, LIHC, LUAD, MESO, SARC, and UCEC (Fig. 4B). Cox regression analysis of DFS showed that FKBP4 upregulation was an oncogenic factor in MESO and UCEC (Fig. S3A). The results of the Kaplan–Meier analysis implied that increased FKBP4 levels were only associated with a worse prognosis in LIHC (Fig. S3B). We explored the association between FKBP4 and PFS and found that FKBP4 influenced PFS in patients with GBM, KIRP, LUAD, MESO, SARC, THYM, and UCEC (Fig. S4A). The results of the Kaplan–Meier analysis implied that increased FKBP4 levels correlated with a worse prognosis in LIHC, MESO, SARC, and UCEC (Fig. S4B).

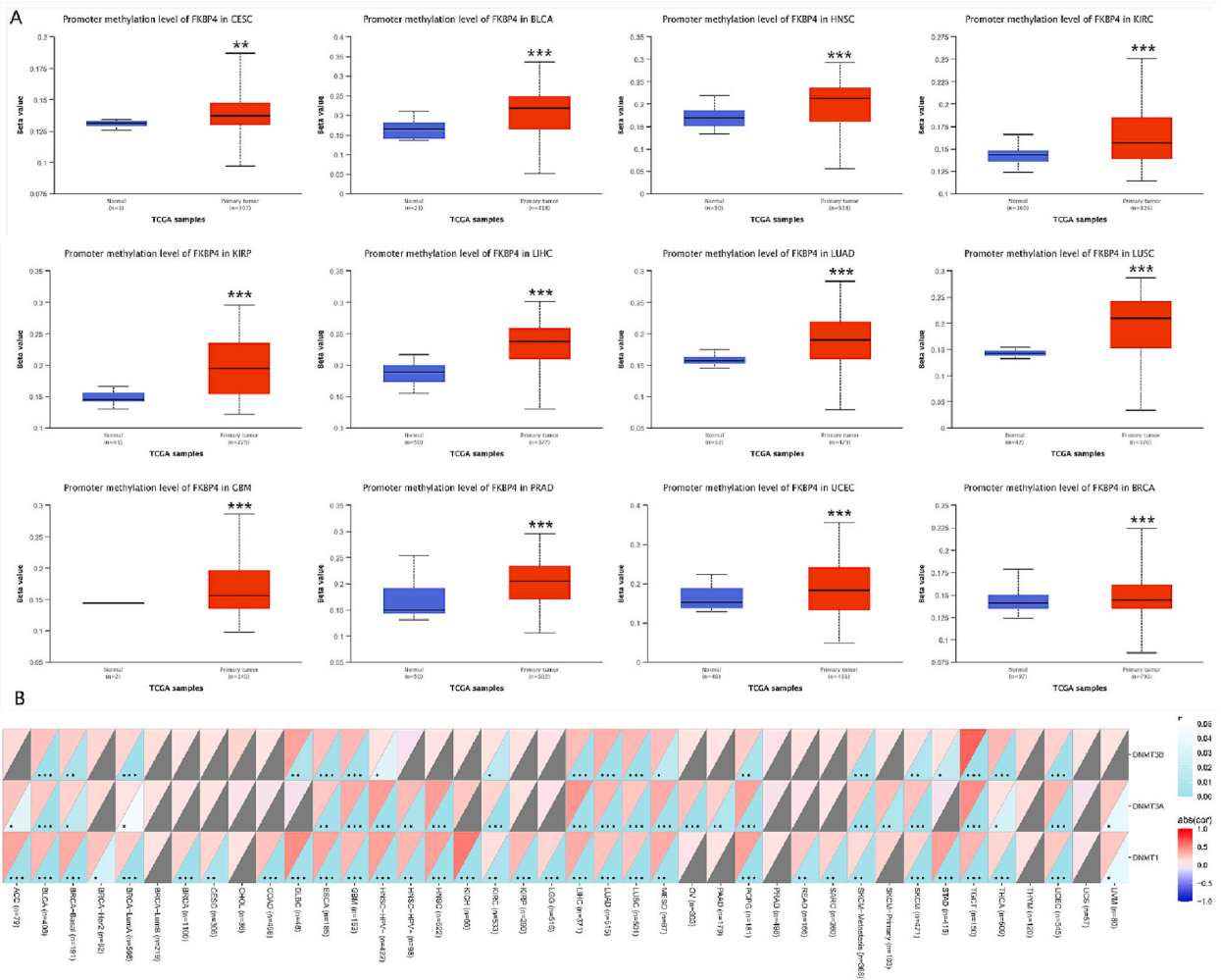

Fig. 5. Correlations between the FKBP4 expression and methylation. **A** The significant differences between normal samples and tumour samples of CESC, BLCA, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, GBM, PRAD, UCEC, and BRCA from UALCAN. The middle horizontal line in each cancer represents the median methylation level of FKBP4. Red represents high level and green represents low level of FKBP4 methylation. **B** Pearson correlation analysis of FKBP4 with DNA methyltransferases in pan-cancer. Light blue represents the significance of the *P*-value, and red or blue represents multiples of positive or negative correlations. Gray means no correlation. *p < 0.05, **p < 0.01, ***p < 0.001. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2.4. Pan-cancer analysis of the FKBP4 expression and methylation level, transmethylase

Next, using the UALCAN database, we explored DNA methylation of FKBP4. A remarkable increase in the methylation level of FKBP4 was observed in CESC, BLCA, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, GBM, PRAD, UCEC, and BRCA tissues (Fig. 5A). The methylation levels of FKBP4 in COAD, READ, and THCA tissues were significantly decreased (Fig. S5A). Nevertheless, no differences were found among the ESCA, PAAD, PCPG, SARC, STAD, THYM, and CHOL tissues (Fig. S5B).

We also found significant positive associations between FKBP4 expression and transmethylases, including DNMT1, DNMT3A, and DNMT3B in most tumours (Fig. 5B). Among the different types of cancer, TGCT was most strongly associated with upregulated FKBP4 and transmethylases.

2.5. Protein-protein interaction network and enrichment analysis of FKBP4

To assess the potential molecular mechanisms underlying FKBP4 participation in cancer promotion, using the GeneMANIA database, we constructed a protein-protein interaction (PPI) network for FKBP4 (Fig. 6). As shown in Fig. 6, FKBP4 had remarkable physical connections with GLMN (Glomulin), an E3 ubiquitin-protein ligase complex regulator.

GO and KEGG enrichment analyses showed that FKBP4 and its co-expressed factors were mainly associated with the response to heat and tau protein binding in the growth cones of prostate cancer (Figs. S6–S9).

2.6. Analysis of the FKBP4 and immune cell infiltration outcomes, immune checkpoints

Immune infiltration of the tumour microenvironment can regulate survival curves; therefore, we further explored the association between FKBP4 levels and immune infiltration outcomes. Using the TIMER database, we explored the relationship between FKBP4 and the infiltration of six immune cell types into the tumour microenvironment. We found that FKBP4 remarkably correlated with tumour purity in seven out of 32 types of cancer (Fig. S10). Additionally, the connection between FKBP4 and the infiltration of B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils and dendritic cells was significant in eight, six, four, three, seven, and six cancer types, respectively (Fig. S10).

Meanwhile, there was a significant negative correlation between FKBP4 and human leukocyte antigen (HLA)-I and -II molecular levels in most tumours (Fig. S11). FKBP4 was positively correlated with several immune checkpoint genes at the transcriptional level, including TNF8F9, ICO8LG, LAIR1, and CD70 in TGCT, THCA, and UCS (Fig. 7).

2.7. Connection of FKBP4 copy number variations and immune infiltrates in pan-cancer

Using the cBioPortal database, it was shown that the FKBP4 gene was frequently changed (Fig. S12). The relationship between

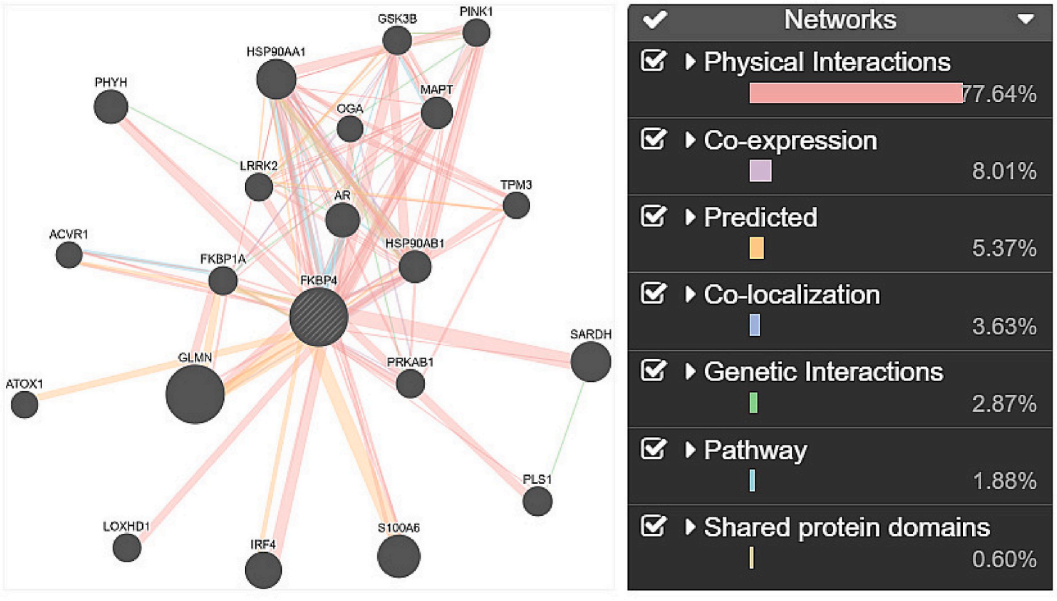

Fig. 6. Protein-protein interaction network construction. Protein-protein interaction network was formed by all 20 DEGs using GeneMANIA database. Dots represent protein names and lines represent interactions. Different colors of the edge meaned: physical interaction, co-expression, predicted, co-localization, genetic interaction, pathway, and shared protein domains. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

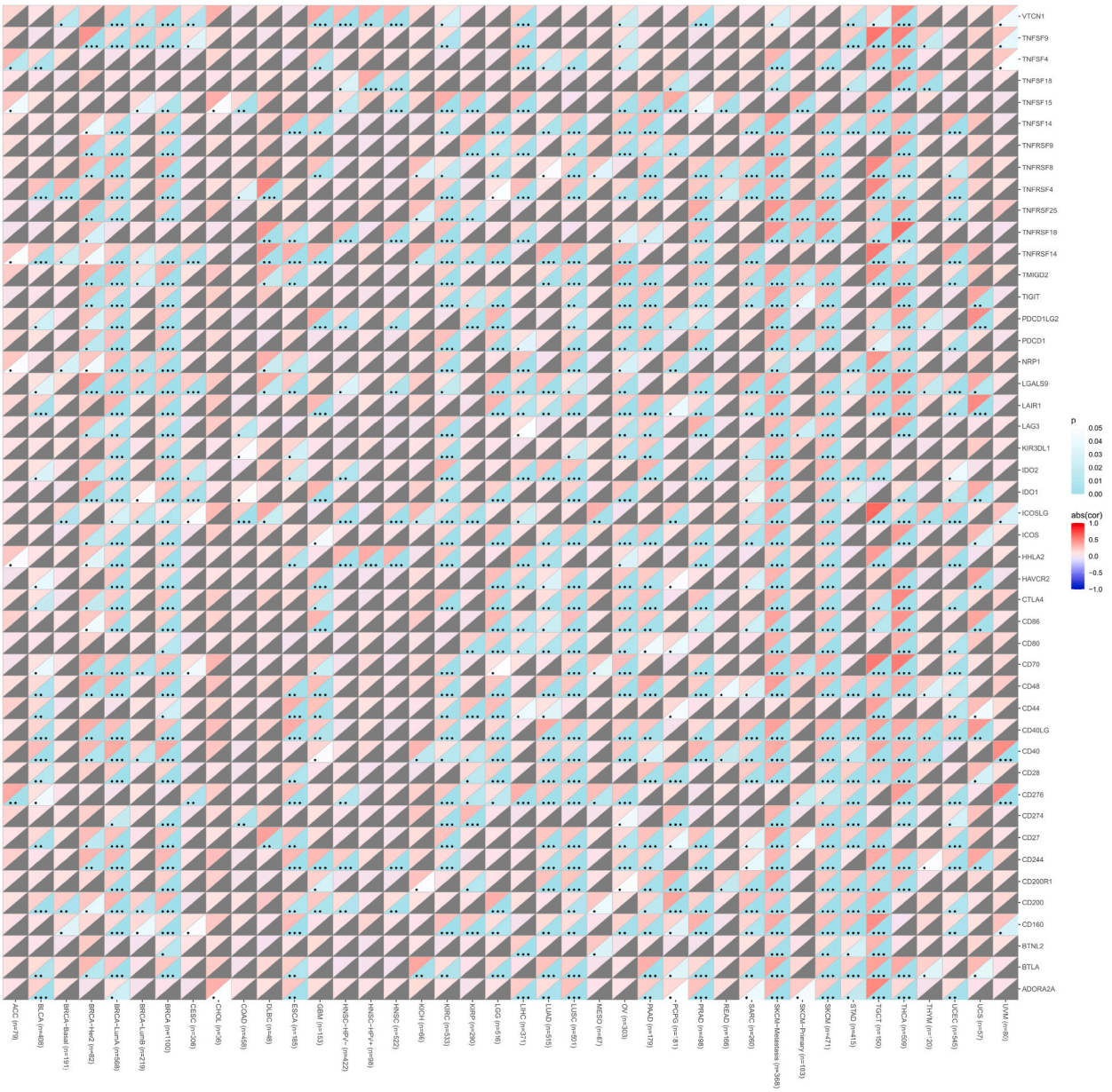

Fig. 7. Pearson correlation analyses of FKBP4 with immune checkpoint genes from TCGA in pan-cancer. Light blue represents the significance of the *P*-value, and red or blue represents multiples of positive or negative correlations. Gray means no correlation. *p < 0.05, **p < 0.01, ***p < 0.001. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

FKBP4 copy number variations and immune cell infiltration in diverse cancers was explored. Fig. S13 shows the remarkable relationship between changes in FKBP4 copy number variations and immune infiltrates among the 26 cancer types. In particular, the deletion of FKBP4 was significantly associated with lower immune infiltration levels in BLCA, BRCA, DLBC, ESCA, PAAD, READ, STAD, THYM, and UCEC. However, the amplification of FKBP4 was significantly associated with lower immune infiltration levels in GBM, HNSC, LUAD, LUSC, PRAD, SKCM, THCA, UCEC, and UCS. These findings suggest the potential molecular function of FKBP4 in predicting the immune therapy response.

2.8. Analysis of connection of the FKBP4 and immune modulators, TMB, MMR, and MSI

To assess the connection between FKBP4 and the TME in pan-cancer, we further determined the relationship between FKBP4 and two immune modulators. Remarkably, we found that FKBP4 was negatively correlated with most immunostimulators and immunoinhibitors in all cancer types (Fig. 8).

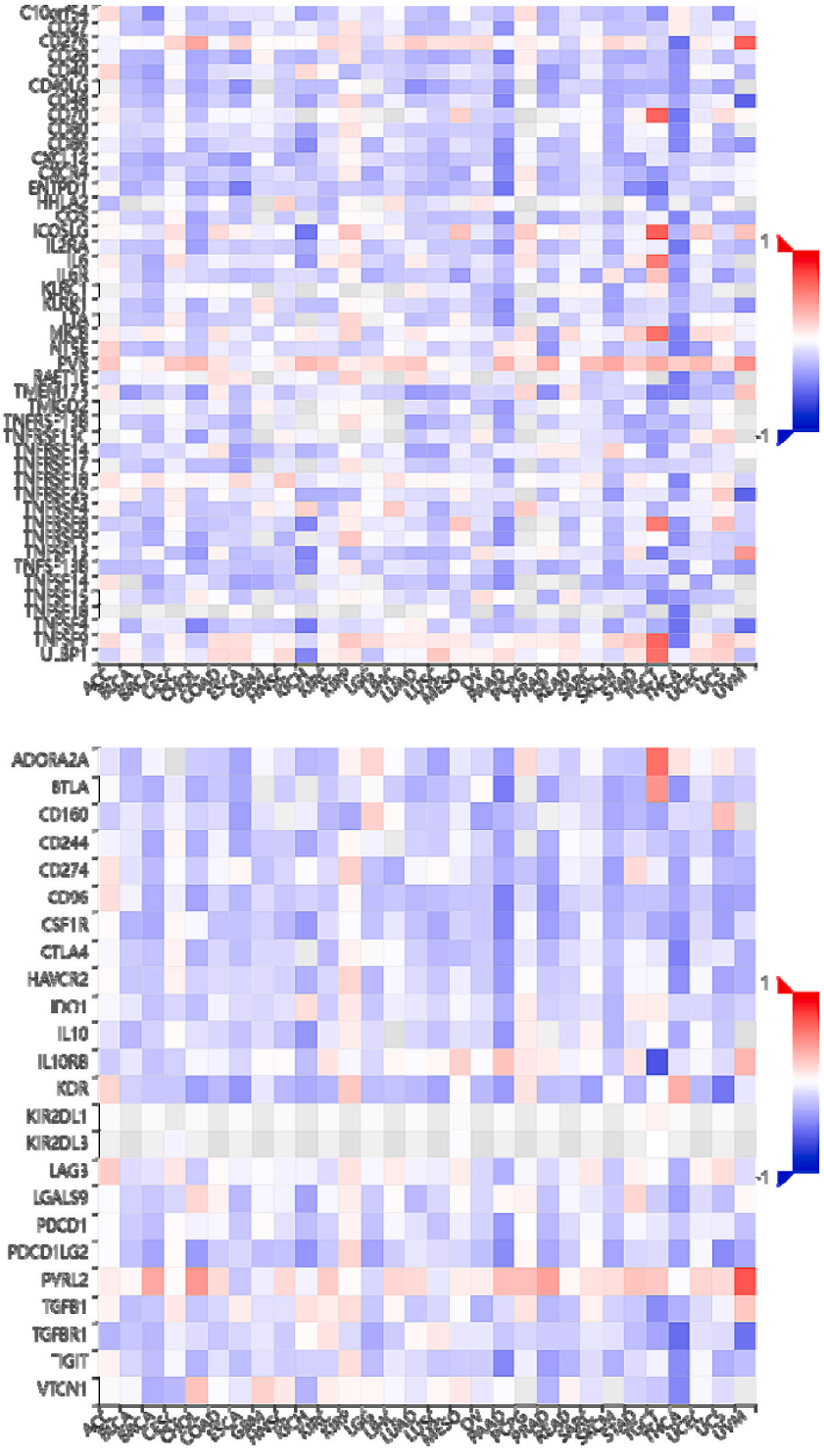

Fig. 8. Heatmap of correlation analyses of the FKBP4 expression with immunostimulators and immunoinhibitors from TISIDB database in pan-cancer. The horizontal coordinates represent different tumour types, and the vertical coordinates represent different immune genes. Red or blue represents multiples of positive or negative correlations of FKBP4 and immune related genes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Tumour mutational burden (TMB), mismatch repair (MMR), and microsatellite instability (MSI) are the three main factors associated with the immunotherapy response. First, the association between FKBP4 expression and TMB was determined. FKBP4 was strongly associated with TMB in several tumours, including BLCA, COAD, HNSC, KICH, LAML, LIHC, LUAD, LUSC, PAAD, PRAD, STAD, and UCEC (Fig. 9A). We also found significant positive associations between FKBP4 expression and MMR, including PMS2, MSH6, MSH2, MLH1, and EPCAM levels in most tumours (Fig. 9B). The association of FKBP4 with MSI was explored: ACC, CESC, COAD, GBM, HNSC, KICH, LIHC, LUSC, STAD, and UCEC showed positive correlations, whereas READ and PCPG showed negative correlations in 33 cancer types (Fig. 9C).

2.9. Connection of FKBP4 and drug sensitivity

FKBP4 was significantly associated with sensitivity to 16 drugs in the CellMiner database (Fig. 10). As the FKBP4 expression level increased, the drug sensitivity of cells, including ICG-001 (R = −0.323, p = 0.012), Vinorelbine (R = −0.299, p = 0.020), Pluripotin (R = −0.285, p = 0.027), and Depsipeptide (R = −0.281, p = 0.030), was downregulated.

FKBP4 had remarkably positive connections with 5−Fluoro deoxy l (R = 0.351, p = 0.006), Cpd−401 (R = 0.343, p = 0.007), Cladribine (R = 0.337, p = 0.009), Bisacodyl, active (R = 0.320, p = 0.013), Amuvatinib (R = 0.313, p = 0.015), Seliciclib (R = 0.306, p = 0.017), (+)−JQ1 (R = 0.286, p = 0.027), SNS−314 (R = 0.283, p = 0.029), XL−147 (R = 0.276, p = 0.033), Floxuridine (R = 0.265, p = 0.041), IWR−1 (R = 0.263, p = 0.042), and 8−Chloro−adeno (R = 0.258, p = 0.047).

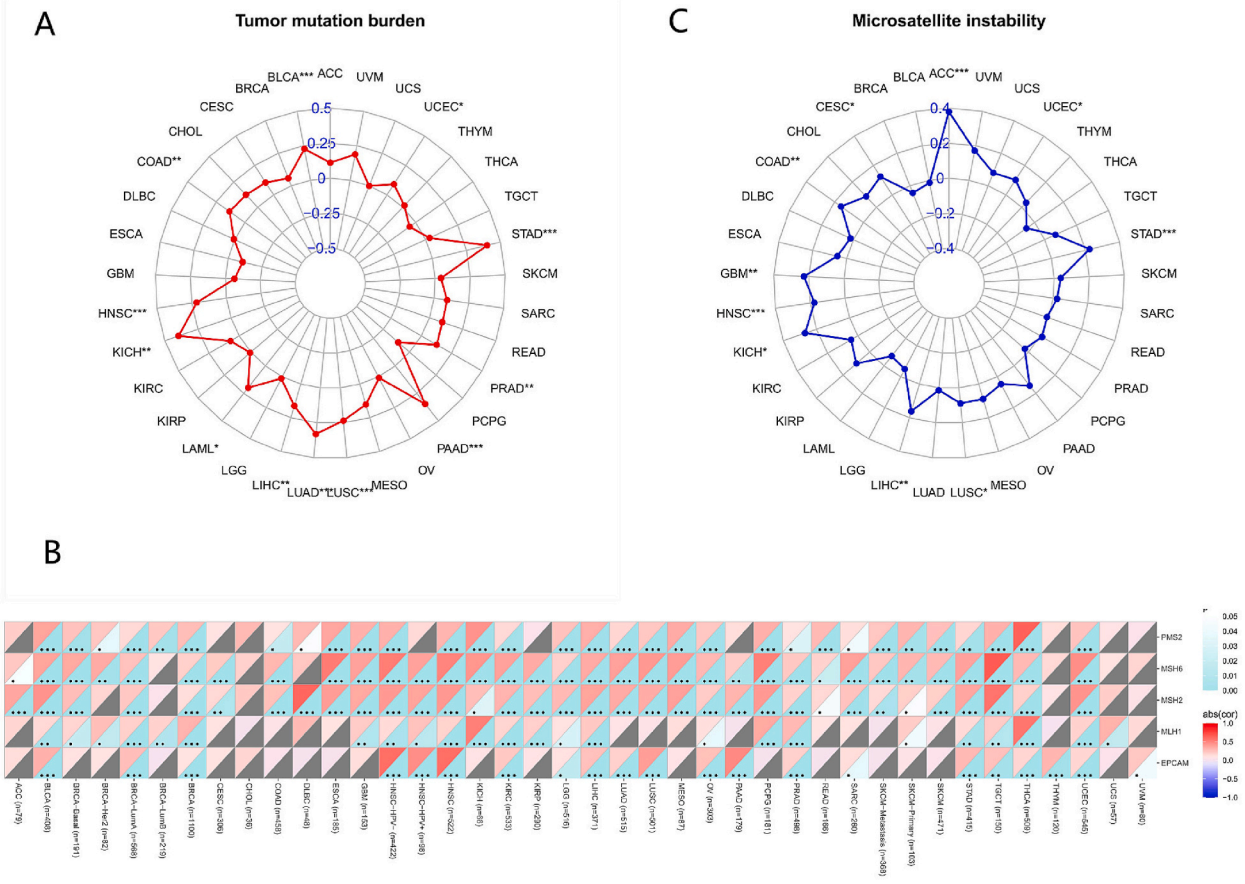

Fig. 9. Connections of FKBP4 and immunity, including TMB, MMR and MSI in pan-cancer. **A** Radar map of connection of FKBP4 and TMB. The expression value of each point represents the TMB value of the corresponding tumour, and the area of the line is the general trend of TMB for all tumours. The number represents the TMB distribution frequency. **B** Pearson correlation analyses of FKBP4 and MMR. Light blue represents the significance of the *P*-value, and red or blue represents multiples of positive or negative correlations. Gray means no correlation. **C** Radar map of connection of FKBP4 and MSI. The expression value of each point represents the MSI value of the corresponding tumour, and the area of the line is the general trend of MSI for all tumours. The number represents the MSI distribution frequency. *p < 0.05, **p < 0.01, ***p < 0.001. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

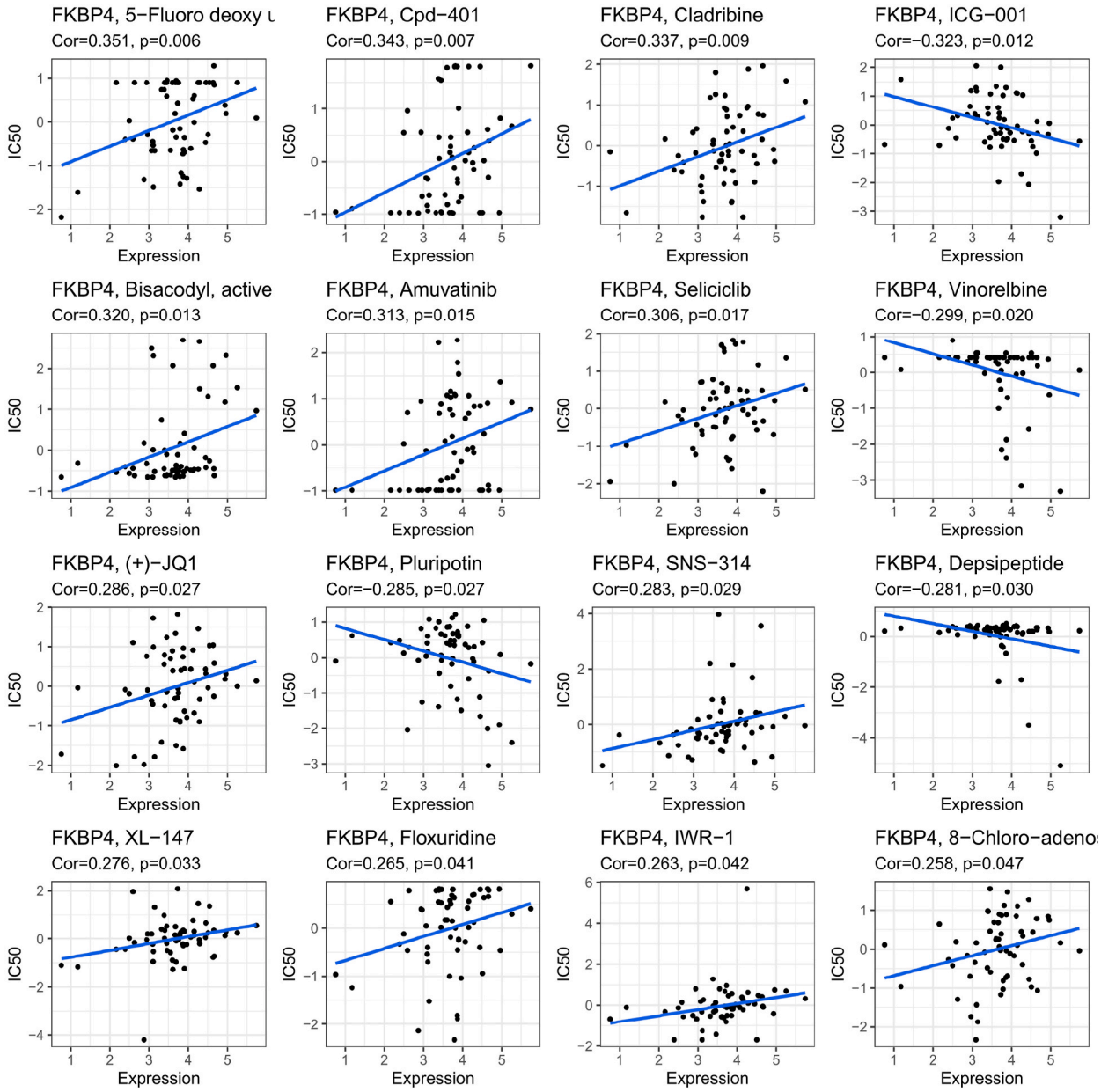

Fig. 10. Connection analysis of FKBP4 and drug sensitivity. The horizontal axis meaned gene expression, the vertical axis meaned drug sensitivity. Dots represent the number of samples. Blue lines represent the trend of correlations. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3. Discussion

Targeted therapy exerts stronger antitumour activity by targeting the characteristic changes in tumour cells [11]. Although recent advances in immunotherapy and molecular targeted therapy have shown great benefits for clinical patients, resistance to targeted drugs may occur [12]. More novel molecular markers should be explored to enhance the efficacy of therapeutic targets in patients with cancer. Pan-cancer analysis can reveal the differences and similarities among diverse cancers, which are important for the identification of sensitive biomarkers [13]. To our knowledge, this is the first study to comprehensively demonstrate FKBP4 levels in a pan-cancerous background. First, we found from TCGA that FKBP4 was remarkably upregulated in BLCA, BRCA, CESC, CHOL, COAD, ESCA, GBM, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, PAAD, PRAD, READ, STAD, THCA, and UCEC tissues compared to normal tissues. Increased FKBP4 levels were associated with worse OS, DSS, DFS, and PFS in most cancers, consistent with a previous study on LUAD by Wang et al. [14]. FKBP4 expression was significantly associated with clinical stage, methylation level, immune cell infiltration,

immune checkpoints, immune modulators, TMB, MMR, and MSI in various types of cancers. Consistent with previous studies, our GO and KEGG analyses implied that FKBP4 was significantly associated with various molecular pathways. Collectively, our study provides deep insights into FKBP4 as a potential prognostic factor in pan-cancer and immune-oncology [15].

FKBP4 acts as a malignancy biomarker in many aspects of tumour biology [16]. Researchers have shown that FKBP4 acts as an oncogene with AR, Hsp90, mTORC2, and PI3K [17,18]. DNA methylation is an important form of epigenetic modification that inhibits gene expression by regulating DNA stability and conformation [19]. Recently, links between DNA methylation and various cancers have been identified [20]. Although Brait et al. and our team found that cancer-associated genes methylation in testicular cancer was strongly associated with FKBP4 [21], the concrete mechanism of FKBP and transmethylase (DNMT1, DNMT3A, and DNMT3B) needs to be further clarified.

Numerous studies on invertebrate and vertebrate genetic systems have demonstrated the critical influence of immunity on tumour progression and poor cancer treatment efficacy [22]. Young et al. reviewed the major functional and clinical studies on PD-1/PD-L1, including the effectiveness and biomarker function of PD-1 and PD-L1 blockade [23], which prompted us to elucidate the immunological role of FKBP4 in pan-cancer to determine whether immunity affects the prognostic function of FKBP4. Our previous work found that the FKBP4/NR3C1/NRF2 axis promotes dendritic cell differentiation and maturation in breast cancer cells, consistent with the above-mentioned findings: our current analysis revealed that FKBP4 expression had a significant connection with the infiltration outcomes of B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils and dendritic cells in eight, six, four, three, seven, and six cancer types. These results imply that FKBP4 plays a complex role in regulating adaptive and innate immune systems in the tumour microenvironment.

TMB, MMR and MSI are latent biomarkers that predict response to immune checkpoint blockade [24,25]. Li et al. reported that SLC41A3, a member of the 41st family of solute carriers, exhibited significant connection with TMB, MMR, and MSI in patients with cancer [26]. Therefore, our study sheds light on understanding the potential role of FKBP4 in TMB, MMR and MSI. For example, FKBP4 was significantly associated with TMB in BLCA, COAD, HNSC, KICH, LAML, LIHC, LUAD, LUSC, PAAD, PRAD, STAD, and UCEC. FKBP4 is also associated with sensitivity to diverse chemotherapeutic drugs, implying that FKBP4 could be used to analyse whether certain chemotherapy drugs are clinically suitable for patients.

Although we performed a substantial exploration of FKBP4 in the prognostic and immunological aspects, several limitations remain. First, our analyses were based on pan-cancer data of patients from TCGA, and the specific details of the patient's medication or surgical treatment might be lacking, thus affecting patient prognosis. Second, multiple analyses based on diverse databases yielded conflicting results. Future prospective studies focusing on FKBP4 are needed to verify our bioinformatic analysis. Third, we found that FKBP4 was strongly associated with TMB and MSI. However, only COAD, HNSC, KICH, LIHC, LUSC, and STAD were affected by possible influencing factors that remain to be identified and verified.

4. Methods

4.1. Data collection, preprocessing, and differentially expressed gene (DEG) analysis

RNA sequencing data, somatic mutations, and related clinical information were obtained from TCGA using UCSC Xena (https://xena.ucsc.edu/) with a sample size of 11007. FKBP4 expression data were converted to base-2 logarithm with two sets of t-tests conducted on these tumour types; $p < 0.05$ implied significant differential expression between the tumour and normal samples. To understand the difference in FKBP4 levels between tumour and normal samples more accurately, we used a *t*-test analysis of paired samples. We used R software (Version 4.0.2; https://www.R-project.org) and the R package "ggpubr" to draw figures. The mRNA levels of FKBP4 were further verified using TIMER2 (http://timer.cistrome.org/) and GEPIA2 (http://gepia2.cancer-pku.cn/#index), and TCGA and GTEx data were combined. The protein levels of FKBP4 were analysed using data from the Clinical Proteomic Tumor Analysis Consortium (CPTAC) with UALCAN (http://ualcan.path.uab.edu/analysis-prot.html). The association between FKBP4 expression and clinicopathological features, such as immune and molecular subtypes, was determined using TISIDB (http://cis.hku.hk/TISIDB/).

4.2. Analysis of connections of FKBP4 and clinical stage, prognosis

Relationships between FKBP4 expression and tumour stage, OS, DSS, DFS, and PFS [27] were analysed using univariate cox regression analysis. P value, HR, and 95 % CI of each variable were obtained using the "forestplot" R package. Survival curves for diverse cancers were obtained using the Kaplan–Meier method.

4.3. Analysis of connections of FKBP4 and TMB, MSI, MMR, immune microenvironment and immune checkpoint genes

TMB contains somatic mutations in its exon [28]. MSI and MMR deficiency predict the efficacy of immunotherapy in pan-cancer [29]. The radar plots for TMB and MSI were obtained using the "Fmsb" R package. Estimation of stromal and immune cells in malignant tumour tissues using expression data (ESTIMATE) has been employed to show stromal and immune infiltration [30]. The connections between FKBP4 and scores are shown as scatter plots. The connections between FKBP4 and immune checkpoint (ICP) genes in diverse cancers were examined using ICP genes retrieved from a previous study [31].

4.4. Methylation analysis of FKBP4

DNA methyltransferase can alter chromatin structure and gene expression [32]. Comprehensive methylation of FKBP4 was analysed by comparing FKBP4 expression with that of three methyltransferases (DNMT1, DNMT3A, and DNMT3B) using Pearson's correlation analysis. UALCAN was used to explore the effect of FKBP4 methylation on tumour and normal samples.

4.5. Copy number variation analysis of FKBP4

CBioportal (https://www.cbioportal.org/) was used to analyse copy number mutations of FKBP4 expression.

4.6. Drug sensitivity analysis

The CellMiner database (https://discover.nci.nih.gov/cellminer/home.do) was used to download the gene expression and drug sensitivity data. Next, we applied FKBP4 expression to drug sensitivity data to conduct a Pearson's correlation assay. Finally, the relationship between FKBP4 expression and drug sensitivity was established.

4.7. Gene enrichment and protein–protein interaction network construction analysis

GO analysis was performed in the high- and low-expression FKBP4 compared with the median level of FKBP4 using clusterProfiler4.0 [33]. The top terms in the GO and KEGG analyses are listed (Figs. S6–S9). GeneMANIA (http://www.genemania.org), which generates hypotheses on gene function, can be used to construct a PPI network. This network integration algorithm demonstrated physical interaction, co-expression, co-localization, gene enrichment analysis, genetic interaction, and website prediction.

5. Statistics

Data are shown as mean ± SD from three independent experiments. A two-tailed Student's t-test and ANOVA were used in the current study [34–36]. Statistical significance is indicated as $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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CRediT authorship contribution statement

Hanchu Xiong: Writing – review & editing, Writing – original draft, Supervision, Resources, Conceptualization. **Zihan Chen:** Formal analysis, Data curation. **Yucheng Li:** Formal analysis. **Zhuazhua Wu:** Formal analysis. **Da Qian:** Methodology. **Long Chen:** Methodology. **Qiang Li:** Software. **Huaxin Liu:** Software. **Weijun Chen:** Validation. **Baihua Lin:** Validation. **Yongshi Jia:** Methodology. **Cheng Wang:** Writing – review & editing.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29098.

Abbreviations

ACC	adrenocortical carcinoma
BLCA	bladder urothelial carcinoma
BRCA	breast invasive carcinoma
CESC	cervical and endocervical cancer
CHOL	cholangiocarcinoma
COAD	colon adenocarcinoma
DLBC	diffuse large B-cell lymphoma
ESCA	esophageal carcinoma
GBM	glioblastoma multiforme
HNSC	head and neck cancer
KICH	kidney chromophobe
KIRC	kidney renal clear cell carcinoma
KIRP	kidney renal papillary cell carcinoma
LAML	acute myeloid leukemia
LGG	lower grade glioma
LIHC	liver hepatocellular carcinoma
LUAD	lung adenocarcinoma
LUSC	lung squamous cell carcinoma
MESO	mesothelioma
OV	ovarian serous cystadenocarcinoma
PAAD	pancreatic adenocarcinoma
PCPG	pheochromocytoma and paraganglioma
PRAD	prostate adenocarcinoma
READ	rectum adenocarcinoma
SARC	sarcoma
SKCM	skin cutaneous melanoma
STAD	stomach adenocarcinoma
TGCT	testicular germ cell tumours
THCA	thyroid carcinoma
THYM	thymoma
UCEC	uterine corpus endometrial carcinoma
UCS	uterine carcinosarcoma
UVM	uveal melanoma

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