Article

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IKBIP is a Predictive Biomarker Related to Immunosuppressive Microenvironment in Digestive System Malignancies

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Objective: IKBKB-interacting protein (IKBIP) has rarely been reported in tumor research. This study aimed to evaluate IKBIP role in tumor progression. mRNA (messenger ribonucleic acid) expression, clinical characteristics and predictive values of IKBIP were assessed.
Methods: R package "clusterProfiler" was used to examine the potential mechanisms in which IKBIP may involve. Immune cell infiltration and its correlation with IKBIP was also analyzed. We further evaluated IKBIP influence on drug resistance.
Results: It was found that IKBIP was overexpressed and related to poorer survival in most types of tumors. IKBIP expression was strongly related to immunosuppressive cells in the TCGA (The Cancer Genome Atlas) pan-cancer samples. These immunosuppressive cells included tumor-related macrophages, tumor-related fibroblasts, and regulatory T cells. Moreover, immunosuppressive genes and immune checkpoints were positively related to IKBIP expression in several tumor types. Furthermore, patients with IKBIP overexpressed did not respond to most anti-cancer medications. It was also found that compared to control group, the number of invasive cells is four times that of IKBIP overexpression group, and the number of clone forming cells is six times that of IKBIP overexpression group. IKBIP overexpression promoted colon cancer cells invasiveness and clonogenesis by Transwell assay and colon formation assay.
Conclusions: According to current findings, IKBIP is a probable oncogene and predictive marker for most of tumor types. High IKBIP expression is associated with tumor immunosuppression.

Keywords: IKBIP; TCGA; pan-cancer; immunosuppressive microenvironment; macrophages

Introduction

IKBKB interacting protein (IKBIP), also known as IKIP, is encoded in human chromosome 12 and has not been studied in depth. Current research reports that IKBIP is a novel biomarker in glioma [1]. However, the potential function of IKBIP in tumors has been rarely reported. Thus, the role of IKBIP in malignancy and its relationship with the tumor microenvironment (TME) remain unknown.

TME has an essential participation in tumorigenesis [2]. Tumor-associated macrophages (TAM), primary TME constituents are associated to immunosuppressive TME in most tumor types [3]. TAMs are key mediators that modulate the release of metabolic factors and cytokine profiles within TME. In addition to TAMs, tumor-associated fibroblasts (TAF) and regulatory T (Treg) are immunosuppressive cells that are involved in CD8+ T cells exhaustion [4–6].

Herein, it was evaluated genetic alterations, IKBIP expression, and pan-cancer prognosis [1]. IKBIP association with TME, such as immune pathways, immune cell infiltration, and immune-related genes were explored in pan-cancer. We further evaluated IKBIP impact on the sensitivity of patients to anti-tumor medications. This work assessed IKBIP involvement in pan-cancer and highlighted a probable involvement of IKBIP in regulating the immunosuppressive TME.

Material and Methods

Data Collection

Clinical data and mRNA (messenger ribonucleic acid) expression were obtained from the UCSC Xena database (https://xenabrowser.net/datapages/). Gene alteration information was retrieved from cBioPortal database (https://www.cbioportal.org/). **Supplementary Table 1** lists the count of sampled used here.

Online Analysis

IKBIP protein levels were evaluated using UALCAN database (http://ualcan.path.uab.edu/index.html) [2] and genetic alterations were analyzed using the cBioportal database (http://www.cbioportal.org/) [3].

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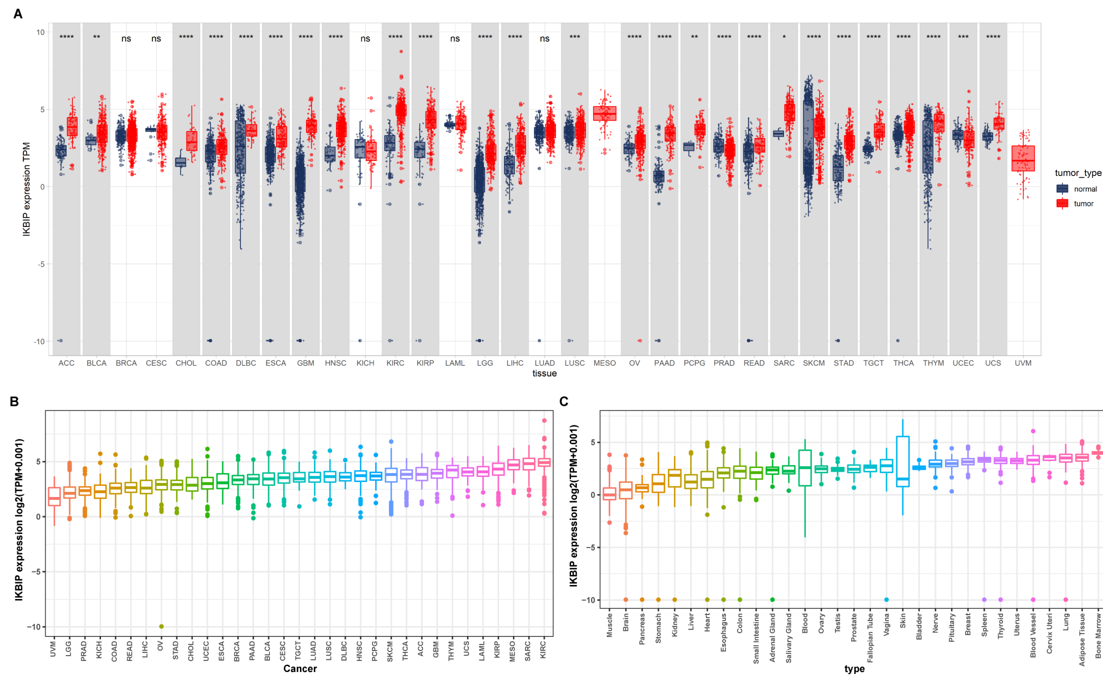

Fig. 1. IKBIP expression according to the TCGA and GTEx databases. (A) IKBIP expression in 24 tumor forms. (B) IKBIP expression according to the TCGA databases. (C) IKBIP expression according to the GTEx database. ns means $p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

Prognostic Analysis

To evaluate IKBIP relationship with overall survival (OS), disease-specific survival (DSS), diseasefree interval (DFI) and progression-free interval (PFI) in the TCGA (The Cancer Genome Atlas) cohort, univariate Cox regression (UniCox) and Kaplan–Meier analyses were performed using R tools "survival" (version 3.5-5, Mayo Foundation for Medical Education, Rochester, NY, USA) [4] and "survminer" (version 3.5-5, Mayo Foundation for Medical Education, Rochester, NY, USA) [5].

Enrichment Analysis

Gene Set Enrichment Analysis (GSEA) [6] and Gene Set Variation Analysis (GSVA) [7] were used to evaluate possible IKBIP roles in pan-cancer using the R tool "clusterProfiler." (version 3.5-5, Mayo Foundation for Medical Education, Rochester, NY, USA) [8]. The Hallmark gene sets used for GSEA enrichment analysis were collected from The Molecular Signatures Database (MsigDB) [9]. For GSVA correlation analysis, major histocompatibility complex (*MHC*) genes, chemokines and chemokine receptors were collected from MSigDB immunologic signature genes. Transforming Growth Factor-β (TGF-β), Epithelial-Mesenchymal Transition (EMT) and WNT (wingless and int-1) signaling genes were collected from MSigDB oncogenic signature gene set. TNF (tumor necrosis factor)-α, interleukin (IL)-β, IL-2 and IL-6 signaling pathways genes were collected from MSigDB Hallmark gene sets.

TME Analysis

R tool "ESTIMATE" (version 3.5-5, Mayo Foundation for Medical Education, Rochester, NY, USA) [10] was used to determine patients' stromal score, immune score, and tumor purity scores in The Cancer Genome Atlas (TCGA) [11]. TME-associated pathways were downloaded, and signature scores were determined according to previous publication [7]. IKBIP correlation with these scores was assessed.

Correlation Analysis between IKBIP and Tumor Infiltrating Cells

Infiltration information was obtained from Tumor Immune Estimation Resource 2 (TIMER2) (http://timer.cistrome.org/) [12] and the ImmuCellAI database (http://bioinfo.life.hust.edu.cn/ImmuCellAI#!/) [13].

Relationship between IKBIP Expression and Drug Response

Antitumor medications IC_{50} measures and mRNA expression in 809 tumor cell lines was obtained from the The Global South Studies Center (GSSC) database (https://www.cancerrxgene.org/). A Spearman's correlation analysis was performed between IKBIP and the IC50 of 192 antitumor medications.

Cell Culture and Transfection

Colon cancer cells (HCT (human colon cancer cells)-116, HT-29) were purchased at American Type Culture

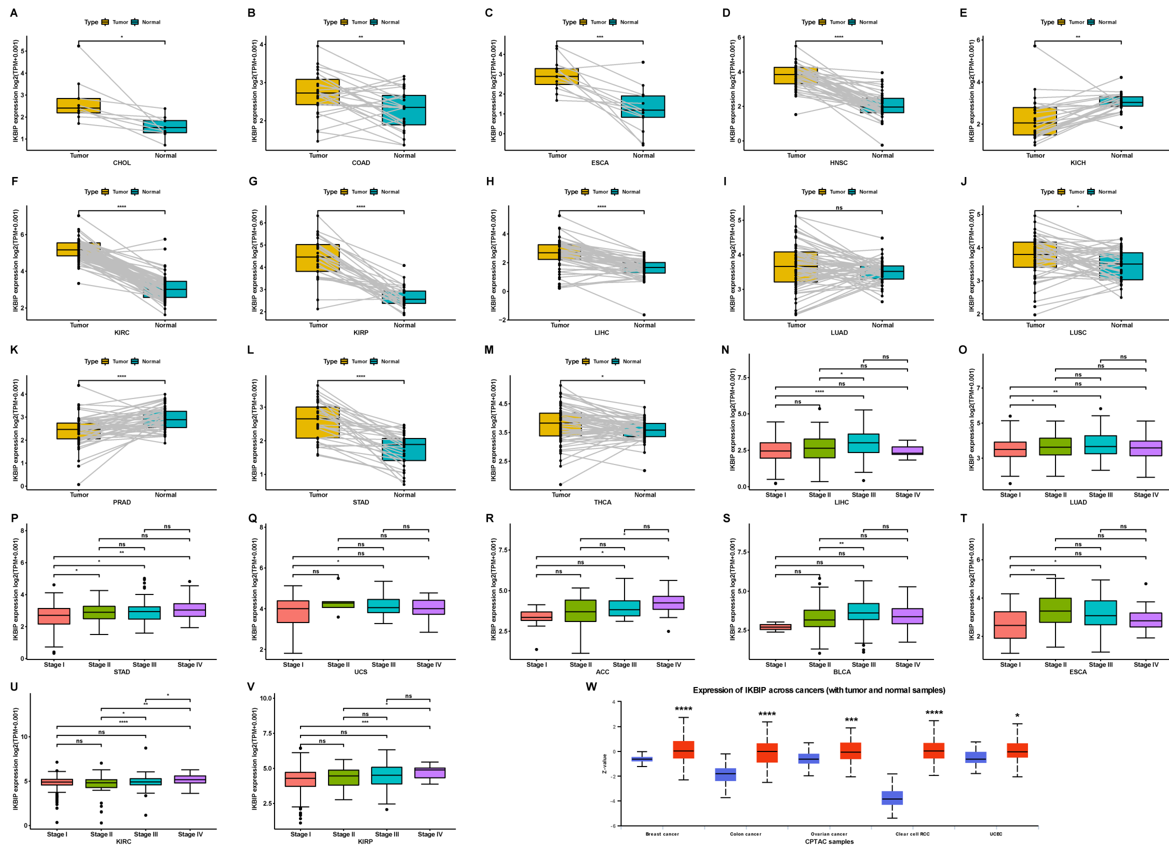

Fig. 2. IKBIP expression in different tumor tissues. (A–M) IKBIP was overexpressed in CHOL, COAD, ESCA, HNSC, KIRC, KIRP, LIHC, LUSC, STAD, and THCA, and showed a low expression in KICH and PRAD. (N–V) IKBIP expression at different tumor stages in different tumor tissues. (W) IKBIP protein value was higher in tumor tissues of BRCA, COAD, OV, KIRC, and UCEC than in healthy tissues. ns means $p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p<0.001$, $^{****}p < 0.0001$.

Collection (ATCC, Manassas, VA, USA). All cell lines tested negative for mycoplasma. Cell lines in good condition were cultivated in RPMI 1640 (Catalog Number: 21875158, Gibco, Grand Island, NY, USA), containing 10% fetal bovine serum (Catalog Number: 16000044, Life Technologies, Rockville, MD, USA) at 37 °C with 5% CO2 and 100% saturated humidity. Cells were cultivated for passage when confluence achieved 70%–80%. Moreover, cells were cultivated in a 6-well plate, and serum-free media was used to synchronise for 24 h. Cells were split into VectorNC, FGF14-IT1. Lipofectamine2000 (Life Technologies, Rockville, MD, USA) transfection kit instructions were followed.

EdU Cell Proliferation Assay

Cells were collected, the medium was discarded, washed with PBS (phosphate buffer) for once, and then digested with trypsin (Catalog Number: 25200056, Thermo Fisher, Waltham, MA, USA). Single-cell suspension was prepared and inoculated in a 96-well plate. Cells were incubated for 12 h. EdU (5-Ethynyl-2'-deoxyuridine) solution was added (Catalog Number: C0071S, Beyotime, Shanghai, China) and incubated for 2–4 h, then 100 μL paraformaldehyde fixative was added and incubated in a decolorizing shaker at room temperature for 30 min. A 2 mg/mL glycine was supplied and then incubated in decolorizing shaker for 5 min. 100 μL 1 $\times$ Apollo® dye solution was added, avoiding light. Then they were decolorized at room temperature and incubated for 30 min. A 100 μL of osmotic agent was supplied and incubated in a decolorizing shaker for 10 min. A 100 μL 1 $\times$ Hoechst33342 reaction solution (Catalog Number: C0071S, Beyotime, Shanghai, China) was added, avoiding light, and incubated in decolorizing shaker at room temperature for 30 min. Photos were taken under a fluorescence microscope (EVOS$^{\text{TM}}$ FLoid®, Thermo Fisher, Waltham, MA, USA) after elution with PBS.

Transwell Experiment

Cells were retrieved, rinsed with phosphate buffer (PBS) for 3 times and diluted to 1×10^5 cells/mL. A culture plate and 100 μL cell suspension and 200 μL serum-free media were placed into the upper chamber of the Transwell chamber (Catalog Number: PICM0RG50, Millipore, Billerica, MA, USA). 500 μL serum was placed in the lower chamber and incubated at 37 °C with 5% CO2 for 20–24 h.

Fig. 3. IKBIP genetic alterations were further explored using the cBioPortal. (A) The greatest incidence of genetic changes in IKBIP (>5%) was found in UCEC patients with "Mutation" as the primary type. (B) IKBIP expression was positively related to copy number alteration (CNA) in 23 of the 33 tumors. (C) IKBIP expression was negatively related to IKBIP DNA methylation level in 14 of the 33 tumor forms.

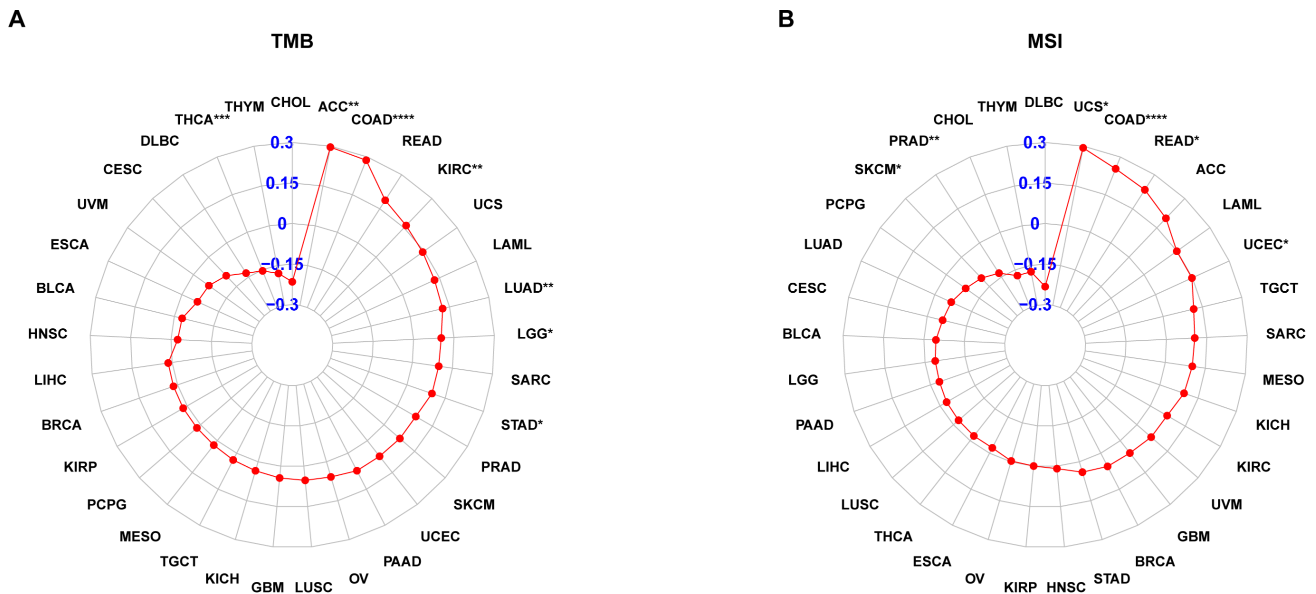

Fig. 4. The relationships between IKBIP, TMB, and MSI. (A) IKBIP was positively related to TMB in ACC, COAD, KIRC, LUAD, LGG, and STAD, and negatively related to TMB in THCA. (B) In the case of MSI, IKBIP was positively related to MSI in UCS, COAD, READ, and UCEC, but negatively related to MSI in PRAD and SKCM.

Hematoxylin (Catalog Number: B-SMS100-1, BKMAM, Changsha, China) staining was performed on the cells in the lower compartment. Five fields were randomly chosen for microscopic counting in each group and the mean value was taken.

Clone Formation Test

Cells in the experimental and control groups were digested with trypsin (Catalog Number: R001100, Thermo Fisher, Waltham, MA, USA) 48 h after transfection. Then cells were collected, reconstituted in complete culture media, quantified, and inoculated at a concentration of 1000 cells per well in 6-well plates (3 wells each for experimental group and control group). Overall, 2 mL complete culture medium was supplied to every well, and the culture plate was shaken horizontally to evenly distribute the cells. After 7 days of culture, each well culture medium was sucked, followed by PBS rinse twice, and each well was fixed with 1 mL 4% paraformaldehyde (Catalog Number: I28800, Thermo Fisher, Waltham, MA, USA) for 20 min. Suck paraformaldehyde and rinsed with PBS twice. A 1 mL crystal violet stain was supplied to every well. Then, after 20 min, the crystal violet staining solution was sucked out (Catalog Number: G1061, Solarbio, Beijing, China). The 6-well plate was rinsed under tap water, and the colony number after drying was calculated.

Statistical Analysis

SPSS 19.0 statistical program (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Data are expressed as mean ± standard deviation. T-test method was used to assess differences between two groups using. Spearman was used to determine group-wise correlations the GSVA and GSEA analysis. $p < 0.05$ was considered statistically significant.

Results

IKBIP Expression in Pan-Cancer

IKBIP expression was analyzed according to the TCGA [11] and GTEx [14] databases. According to results, IKBIP was overexpressed in 24 tumor forms, including ACC (adrenocortical carcinoma), BLCA (bladder urothelial carcinoma), CHOL (cholangio carcinoma), COAD (colon adenocarcinoma), DLBC (diffuse large B-cell lymphoma), ESCA (esophageal carcinoma), GBM (glioblastoma multiforme), HNSC (head and neck squamous cell carcinoma), KIRC (kidney renal clear cell carcinoma), KIRP (kidney renal papillary cell carcinoma), LGG (brain lower grade glioma), LIHC (liver hepatocellular carcinoma), LUSC (lung squamous cell carcinoma), OV (ovarian serous cystadenocarcinoma), PAAD (pancreatic adenocarcinoma), PCPG (pheochromocytoma and paraganglioma), READ (rectum adenocarcinoma), SARC (sarcoma), SKCM (skin cutaneous melanoma), STAD (stomach adenocarcinoma), TGCT (testicular germ cell tumors), THCA (thyroid carcinoma), THYM (thymoma), and UCS (uterine carcinosarcoma), whereas it was only weakly expressed in PRAD (prostate adenocarcinoma) and UCEC (uterine corpus endometrial carcinoma) (Fig. 1A). IKBIP expression was the highest in KIRC and the lowest in UVM (uveal melanoma) (Fig. 1B) in the TCGA data. The highest IKBIP expression was observed in the bone marrow, and the lowest in the muscle, in the GTEx data

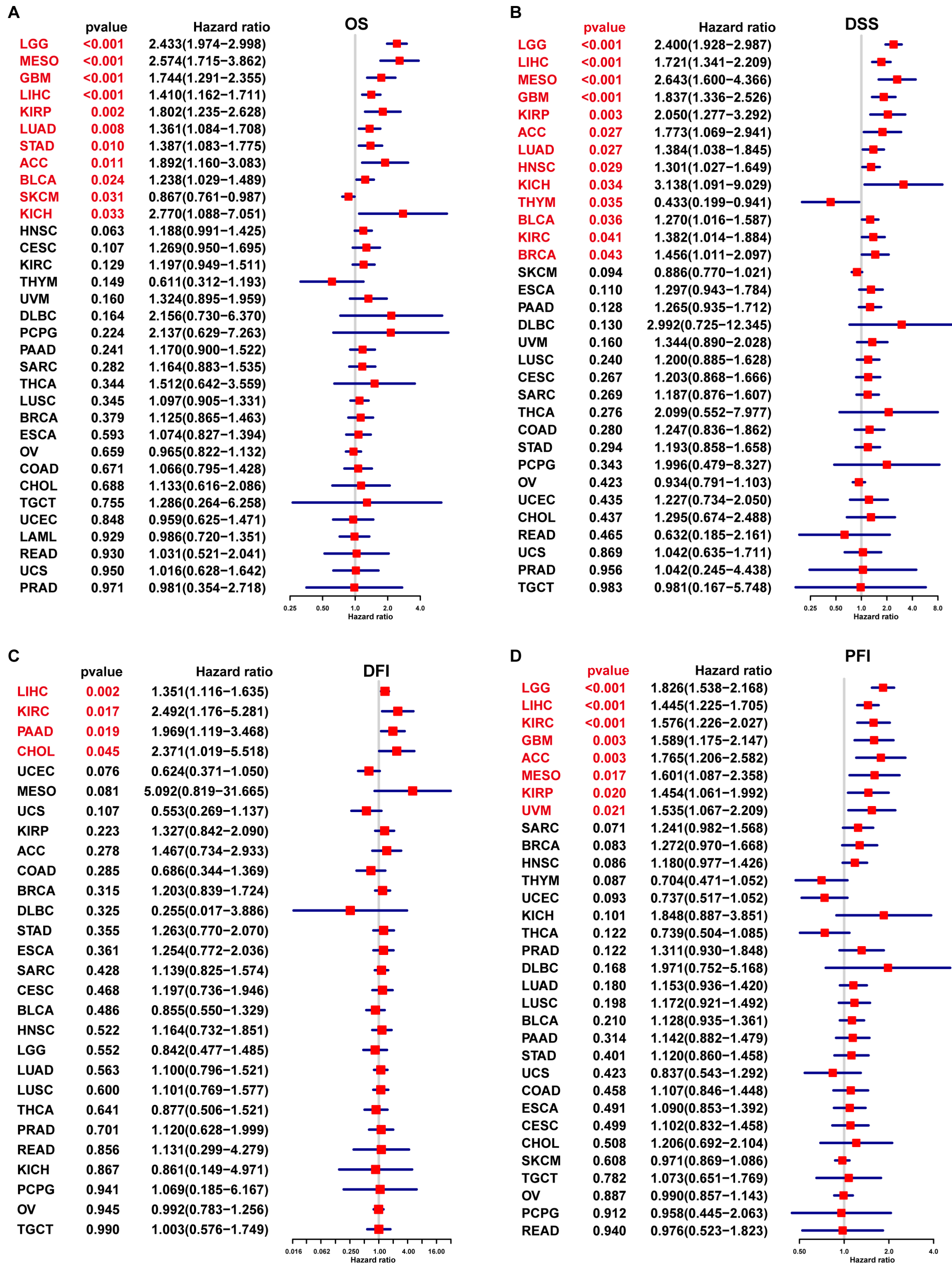

Fig. 5. IKBIP as a predictor of outcomes in patients with different tumors. (A) IKBIP increased the risk of poor OS in LGG, MESO, GBM, LIHC, KIRP, LUAD, STAD, ACC, BLCA, and KICH, and reduced the risk of poor OS in SKCM. (B) IKBIP increased the risk of poor DSS in LGG, LIHC, MESO, GBM, KIRP, ACC, LUAD, HNSC, KICH, BLCA, KIRC, and BRCA, and reduced the risk of poor DSS in THYM. (C) IKBIP increased the risk of poor DFI in LIHC, KIRC, PAAD, and CHOL. (D) IKBIP increased the risk of poor PFI in LGG, LIHC, KIRC, GBM, ACC, MESO, KIRP, and UVM.

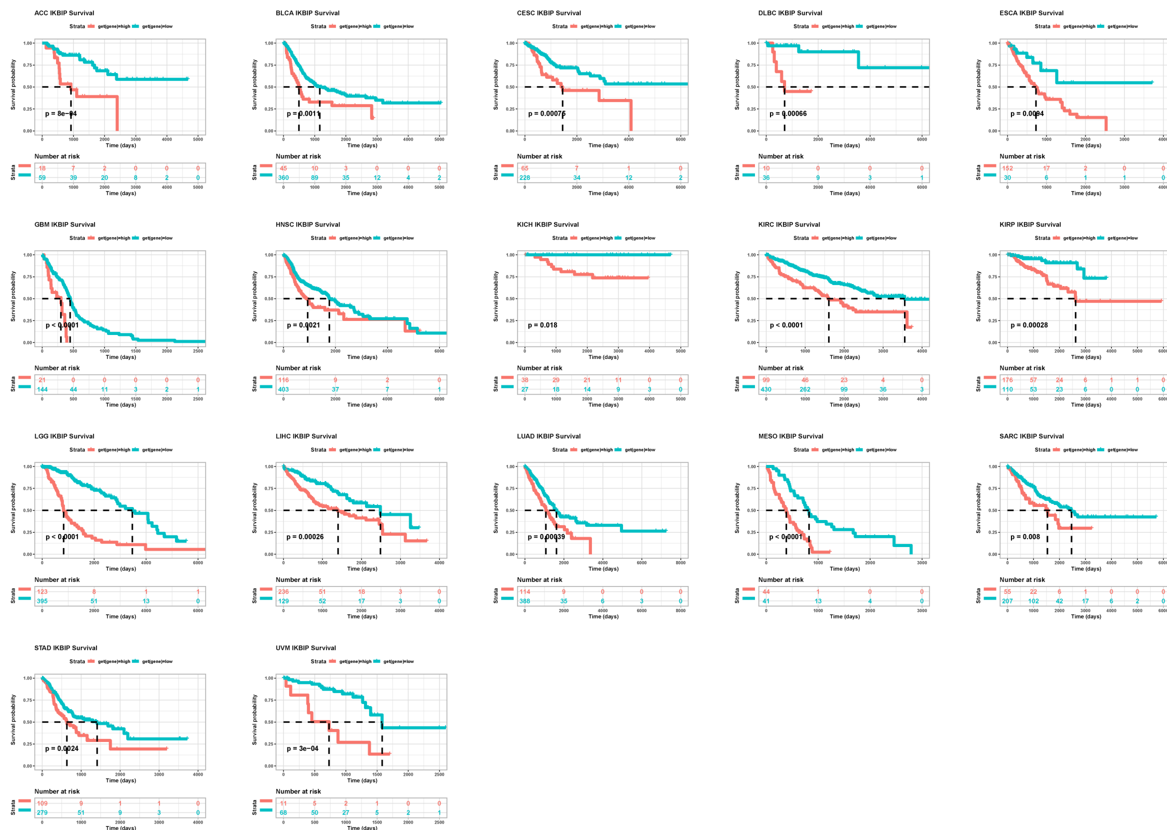

Fig. 6. Kaplan–Meier analysis with patients stratified depending in IKBIP level. IKBIP stratification was performed based on the mean expression in each tumor type.

(Fig. 1C). IKBIP was overexpressed in CHOL, COAD, ESCA, HNSC, KIRC, KIRP, LIHC, LUSC, STAD, and THCA, and showed a low expression in KICH (kidney chromophobe) and PRAD for paired tumors and adjacent healthy tissues in the TCGA (Fig. 2A–M). Regarding IKBIP expression in a variety of tumor stages, it was found that IKBIP expression was higher in more advanced tumor stages of LIHC, LUAD (lung adenocarcinoma), STAD, UCS, ACC, BLCA, ESCA, KIRC, and KIRP (Fig. 2N–V). Moreover, it was observed that IKBIP protein concentration was elevated in tumor tissue of BRCA (breast invasive carcinoma), COAD, OV, KIRC, and UCEC compared to healthy tissues (Fig. 2W).

IKBIP Gene Alterations in Pan-Cancer

Genetic alterations in IKBIP were further evaluated using the cBioPortal. The greatest rate of genetic mutations in IKBIP (>5%) was found in patients with UCEC with "Mutation" as the main form (Fig. 3A). In addition, IKBIP expression was positively correlated with the copy number alteration (CNA) in 23 of the 33 tumors (Fig. 3B) and negatively related to IKBIP DNA methylation level in 14 of the 33 tumor forms (Fig. 3C).

It was also assessed the relationships between IKBIP, TMB (tumor mutation burden), and MSI (microsatellite instability). The results indicated that IKBIP was positively correlated with TMB in ACC, COAD, KIRC, LUAD, LGG, and STAD, and negatively correlated to TMB in THCA (Fig. 4A). In the case of MSI, IKBIP was positively correlated to MSI in UCS, COAD, READ, and UCEC, but negatively correlated to MSI in PRAD and SKCM (Fig. 4B).

IKBIP Prognostic Analysis

To identify the association of IKBIP with tumorous patients outcomes, a survival analysis was conducted. IKBIP increased the risk of poor OS in LGG, MESO (mesothelioma), GBM, LIHC, KIRP, LUAD, STAD, ACC, BLCA, and KICH, and reduced the risk of OS in SKCM (Fig. 5A). For DSS, IKBIP increased the risk of poor DSS in LGG, LIHC, MESO, GBM, KIRP, ACC, LUAD, HNSC, KICH, BLCA, KIRC, and BRCA, and reduced the risk of poor DSS in THYM (Fig. 5B). In patients with DFI, IKBIP acts as a risk variable in LIHC, KIRC, PAAD, as well as CHOL (Fig. 5C). For PFI, IKBIP was a risk factor for LGG, LIHC, KIRC, GBM, ACC, MESO, KIRP, and UVM (Fig. 5D). Among patients with ACC, BLCA, CESC (cervical squamous cell carcinoma and endocervical adenocarcinoma), DLBC, ESCA, GBM, HNSC, KICH, KIRC, KIRP, LGG, LIHC, LUAD, MESO, SARC, STAD, and UVM, those with high IKBIP levels had worse OS (Fig. 6).

Fig. 7. GSEA was used to evaluate IKBIP potential pathway in different tumor forms. (A–F) IKBIP was associated with immune-related pathways in ESCA, LIHC, PAAD, COAD, READ, and STAD.

IKBIP Gene Function Analysis

To analyze potential IKBIP pathways, we conducted GSEA and GSVA in pan-cancer. GSEA results, taking digestive system malignancies as example, IKBIP was associated with immunerelated pathways in ESCA, LIHC, PAAD, COAD, READ, and STAD (Fig. 7A–F). IKBIP expression was associated to GSVA scores (Fig. 8). IKBIP was related to tumorassociated pathways in most tumors, involving some cell cycle related pathways, including cell cycle and cell cycle, metotic. Tumor-related pathways included epithelial mesenchymal transition, angiogenesis, and TGF beta signaling. Additionally, immune-related pathways were significantly associated between IKBIP expression including innate and adaptive immune system, cytokine signaling in immune system, demonstrating that individuals with an increased in IKBIP expression had high immune cell infiltrate.

TME Analysis of IKBIP

IKBIP association with stromal and immune scores was evaluated in pan-cancers. Findings suggest that IKBIP was positively related to immune, stromal, and ESTIMATE scores in most tumor forms, especially digestive system malignancies (Fig. 9A). In addition, TME-associated signatures were downloaded and measured in accordance to a previous publication [15]. According to results, IKBIP was closely associated with all of these pathways in the pan-cancer analysis (Fig. 9B).

Immune Infiltrating Analysis

According to the results of the above analysis, it was hypothesized that IKBIP plays an important role in the immune microenvironment. Accordingly, the relation between IKBIP with TME was assessed. Based on the immune cell infiltrating records from the TIMER2 database, it was found that IKBIP was significantly related to the majority of immunosuppressive cells, involving TAMs and TAFs, at the pan-cancer level (Fig. 10A). Immune cell infiltration association analysis in the basis of the ImmuCellAI database showed identical findings: Immunosuppressive cells, including TAMs and Tregs, were positively associated to IKBIP (Fig. 10B).

Additionally, it was found that IKBIP was highly associated to immunosuppressive genes (Fig. 11A), immune checkpoints (Fig. 11B–G), *MHC* genes (Fig. 12A), chemokines (Fig. 12B), and chemokine receptors (Fig. 12C) in pan-cancer, especially in digestive tumors. Moreover, TNF-α, IL-2, and IL-6 signaling were associated to the immunosuppressive TME (**Supplementary Figs. 1,2,3**). Additionally, it was found an association between IKBIP with the genes involved in these pathways

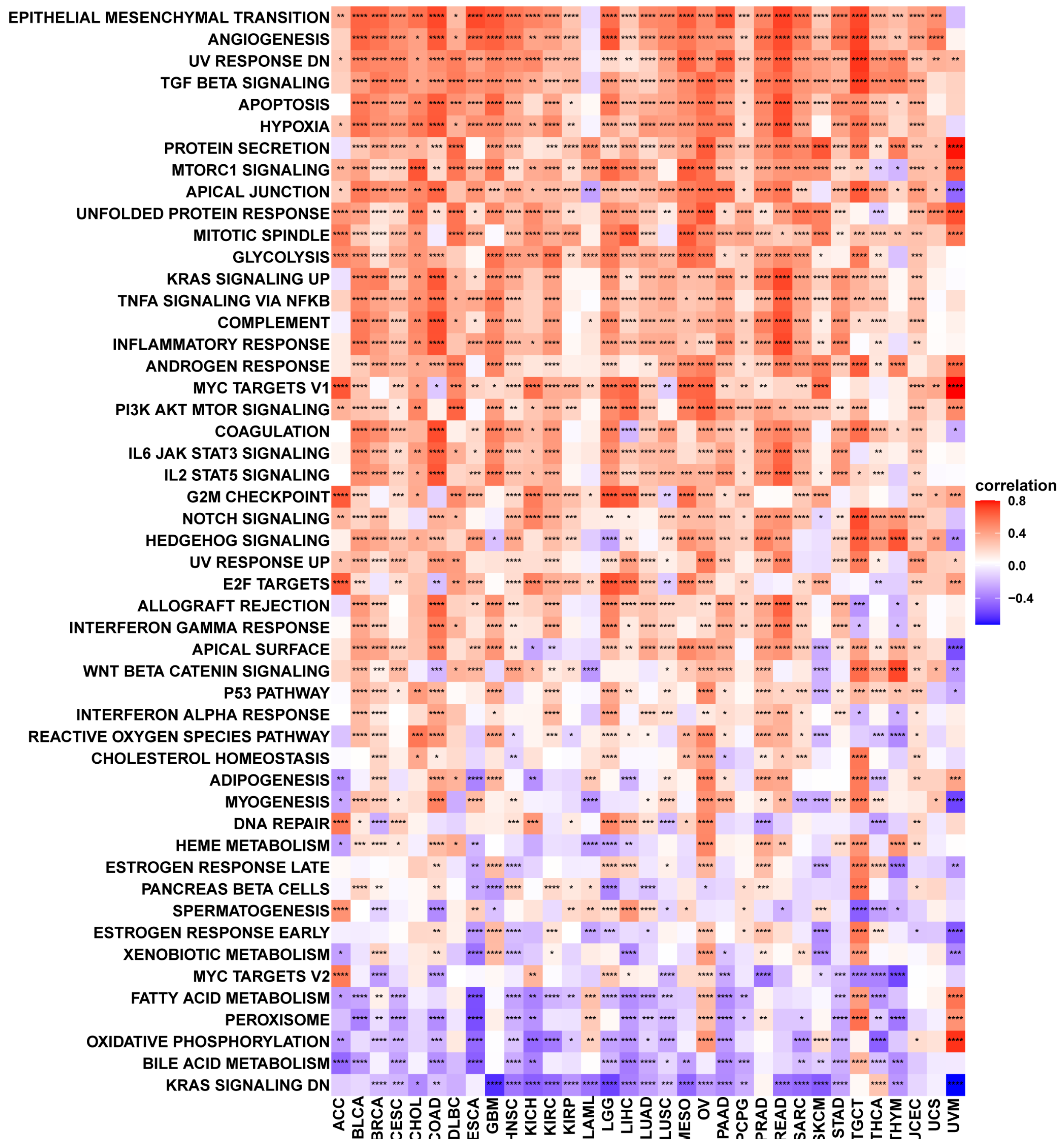

Fig. 8. IKBIP expression correlation with GSVA scores. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

(Fig. 13A–C). These findings indicate that patients having increased IKBIP expression are in an immunosuppressive TME.

Anti-Tumor Medications Efficacy Analysis

The relationship between IKBIP with the IC_{50} of 192 antitumor medications was evaluated. IKBIP was positively related to the IC_{50} of 64 out of 192 medications (**Supplementary Table 2**), including PF-4708671, TAF1_5496, acetalax, afatinib, OSI-027, dihydrorotenone, ABT737, osimertinib, and LGK974 (Fig. 14), indicating that patients having elevated IKBIP expression could be resistant to these drugs.

IKBIP Overexpression Promotes the Malignancy of Colon Cancer Cells

Colon cancer cell lines were selected to confirm the impact on colon cancer. IKBIP was overexpressed in HCT-116 and HT-29 colon cancer cells to further define its role in malignant phenotypes development in these tumors (Fig. 15A). IKBIP overexpression effect on colon cancer cells was detected by EdU assay, Transwell assay, and clone formation assay. Findings indicated that, HCT-116 and HT-

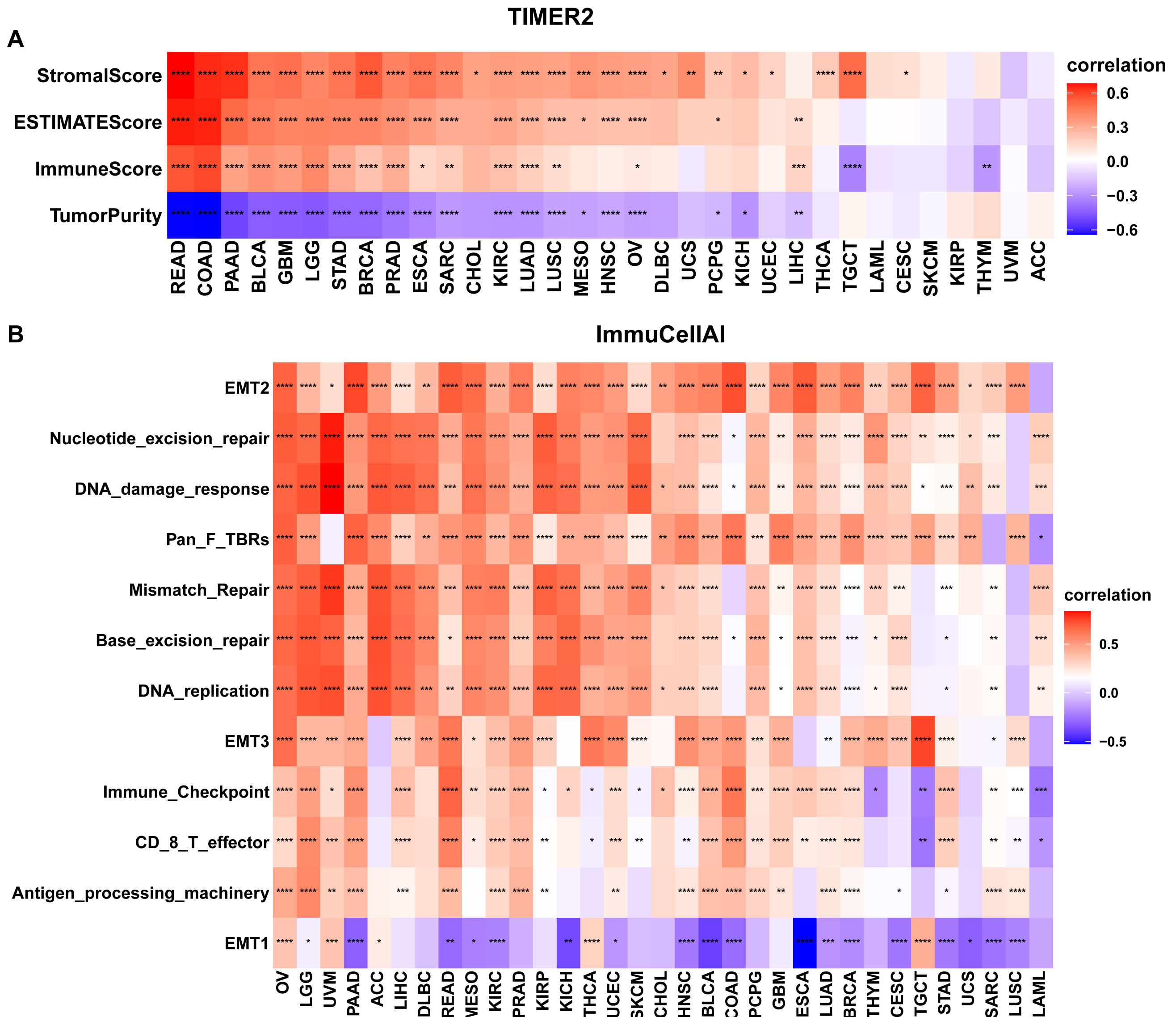

Fig. 9. IKBIP association with stromal and immune scores was evaluated in pan-cancers. (A,B) Correlation between IKBIP, pancancer matrix and immune score from the TIMER2 and ImmuCellAI databases. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

29 cells proliferative activity showed a significant increase after IKBIP was overexpressed compared to the vector-NC group (Fig. 15B). Further tests revealed that IKBIP overexpression promoted the invasiveness and clonogenesis of colon cancer cells. The cells of the experimental group and control group were inoculated in a Transwell chamber. The culture medium containing 10% FBS was used to induce cell movement. After 24 h, the cells crossing the basement membrane were stained with crystal violet. Numbers were counted under microscope and analyzed statistically. It was observed that invasion and clone formation of HCT-116 and HT-29 cells were activated after IKBIP overexpression (Fig. 15C,D).

Discussion

IKBIP function in disease progression has rarely been studied. Previously Yang Y *et al.* [1] reported IKBIP as a novel EMT-related biomarker in glioma using bioinformatic analysis with transcriptomic data, collected from patients of the TCGA and CGGA databases. In their work, IKBIP interreacted synergistically with key EMT molecules including N-cadherin, snail, slug, vimentin and TWIST1. Liang J *et al.* [8] also reported that IKBIP knockdown strongly suppressed cell proliferation and tumor growth in a hGBM (human glioblastoma) mouse xenograft model, possibly by inhibiting the ubiquitination and degradation of CDK4 in *in-vitro* tests. These findings were in line with our hypothesis, whereas IKBIP is linked to tumor-associated pathways like EMT, angiogenesis and TGF beta signaling

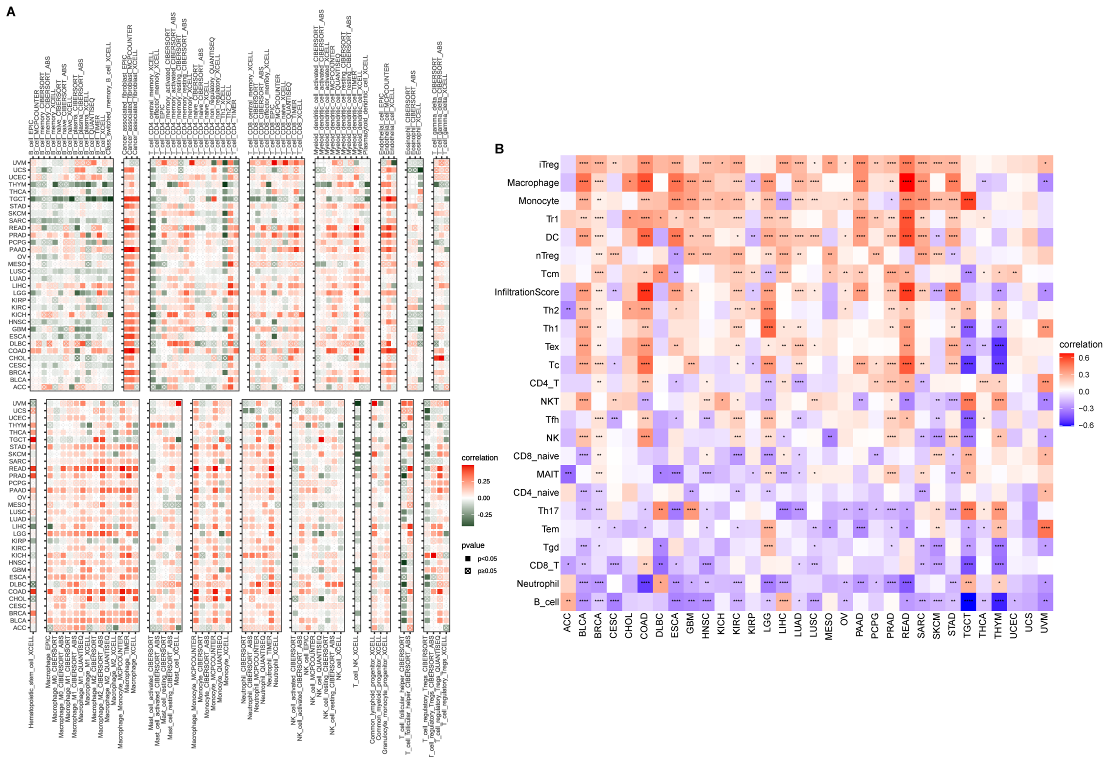

Fig. 10. Correlation between IKBIP with TME. (A) At the pan-cancer level, IKBIP was highly related to most immunosuppressive cells, including TAMs and TAFs at pan-cancer level. (B) immunosuppressive cells, including TAMs and Tregs, were positively associated to IKBIP. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

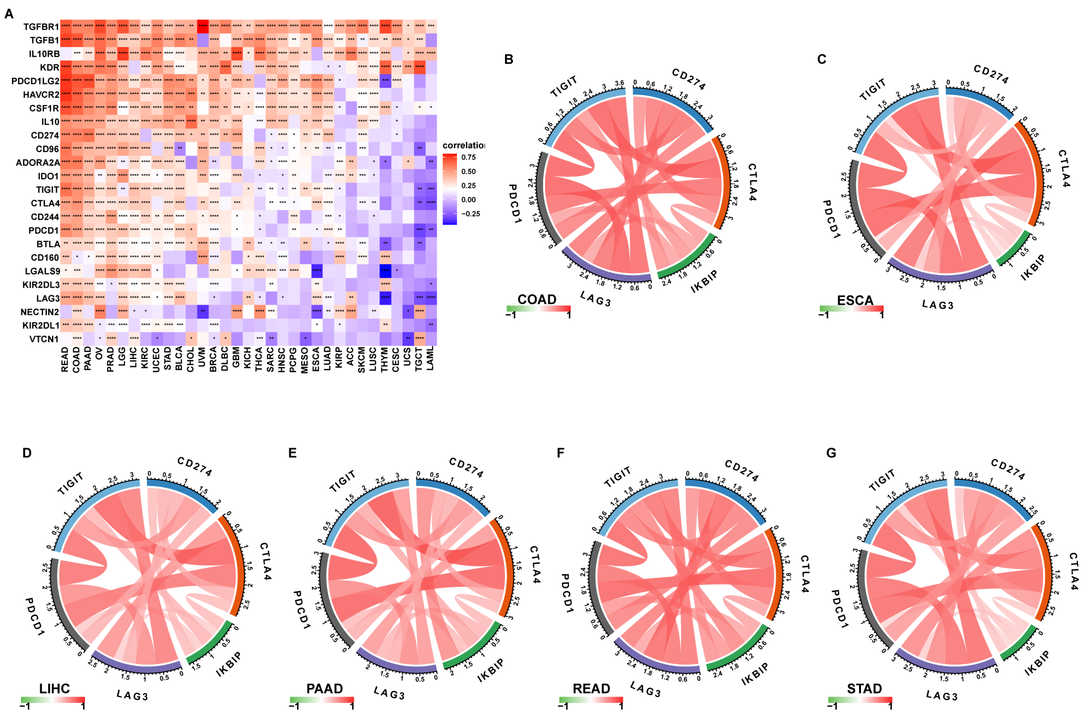

Fig. 11. The relationship between IKBIP and immunosuppressive genes and immune checkpoints in gastrointestinal tumors. (A–G) IKBIP was highly associated with immunosuppressive genes and immune checkpoints. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

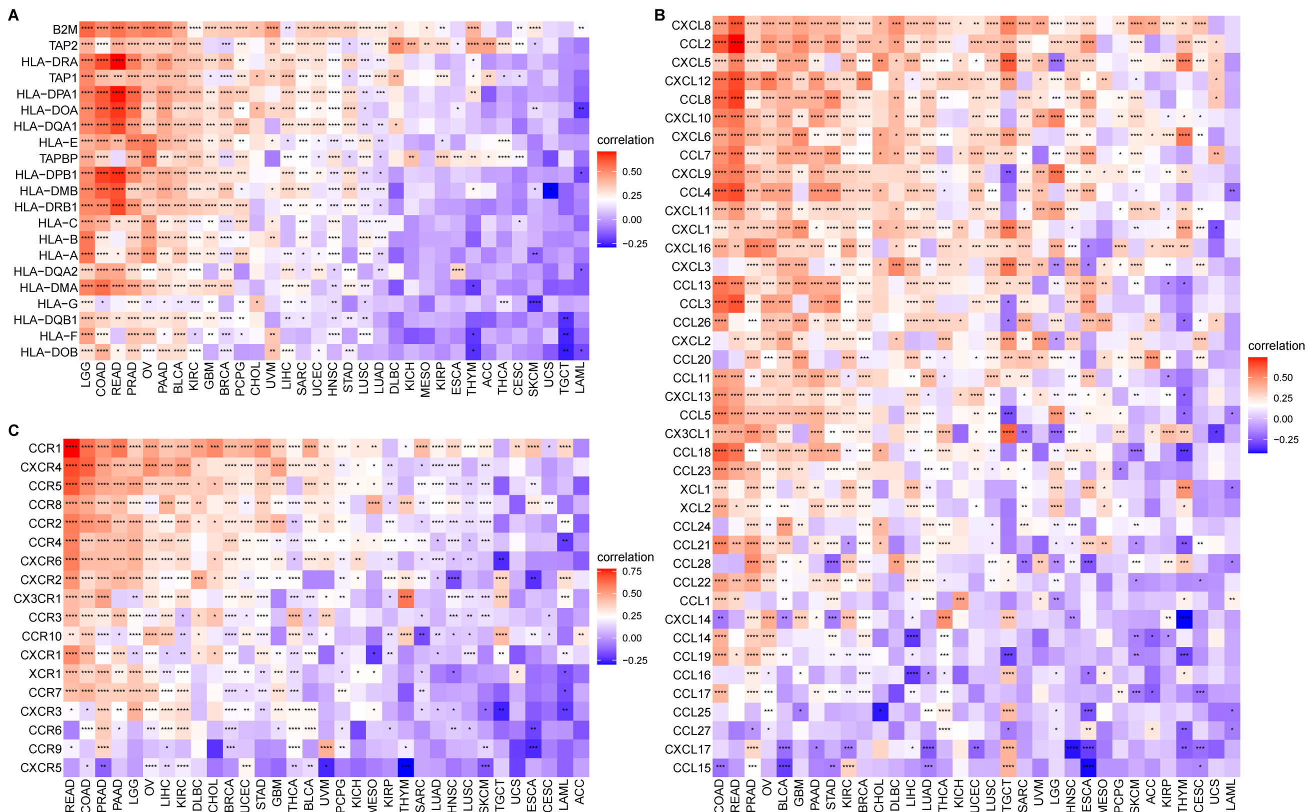

Fig. 12. The relationship between IKBIP and *MHC* genes, chemokines, and chemokine receptors in gastrointestinal tumors. (A–C) IKBIP was associated with *MHC* genes, chemokines, and chemokine receptors in pan-cancer. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

pathways. Further, Chen TY *et al.* [9] reported high IKBIP expression as a biomarker for poor prognosis that was correlated with immune infiltrates in hepatocellular carcinoma (HCC). Nevertheless, the significantly upregulated IKBIP expression in HCC was related to the ACT-CD4+ T cells. However, its role in most tumors is uncertain.

Herein, it was identified that IKBIP showed a significant upregulation in 24 out of 33 tumors. Elevated IKBIP estimated poorer OS, DSS, DFI, and PFI in tumor patients, especially in GBM, LGG, and LIHC. In addition, it was observed that IKBIP DNA methylation was negatively related to IKBIP, and IKBIP CNA level was positively related to IKBIP expression, indicating that IKBIP epigenetics may regulate IKBIP mRNA expression. GSEA and GSVA was conducted to identify IKBIP function in tumorigenesis and cancer establishment. Moreover, it was observed that IKBIP was significantly related to several canonical tumor-related malignant pathways, such as epithelial mesenchymal transition, angiogenesis, and TGF beta signaling pathways, suggesting that patients with elevated IKBIP expression may have increased tumor burdens.

Next, immune score and stromal score of tumor tissues was calculated and it was observed that IKBIP was strongly correlated with immune score and stromal score in pan-cancer, indicating that IKBIP is highly correlated with TME immune cell infiltration. Tregs and Th17 cells are derived from CD4+ T cells differentiation [10]. Previous studies have shown that the balance of differentiation between these two cell types is affected by the TME. Among them, Treg cells are classic immune cells that participate in the tumorigenesis and tumor establishment through tumor immunity and in addition induce immune escape [11–13]. Tumor cells often modify TAMs and TAFs in the TME to enhance tumorigenesis [14,15]. Further analysis of tumor immune infiltration revealed that IKBIP expression was significantly correlated with most immunosuppressive cells (such as TAMs, TAFs, and Tregs) in the pan-cancer TME. Additionally, we observed that IKBIP was highly related to immunosuppressive genes, immune checkpoints, *MHC* genes, chemokines, and chemokine receptors in pan-cancer, especially in digestive tumors. Further, TNF-A, IL2 and IL6 signaling pathways were closely associated with the immunosuppressive TME, and IKBIP was positively associated with genes involved in these pathways. The present findings indicate that patients with increased IKBIP have TME immunosuppressed, which could contribute to worse survival status of cancer patients.

Moreover, we examined IKBIP relation with antitumor medications and observed that patients having increased IKBIP levels could be unresponsive to most medi-

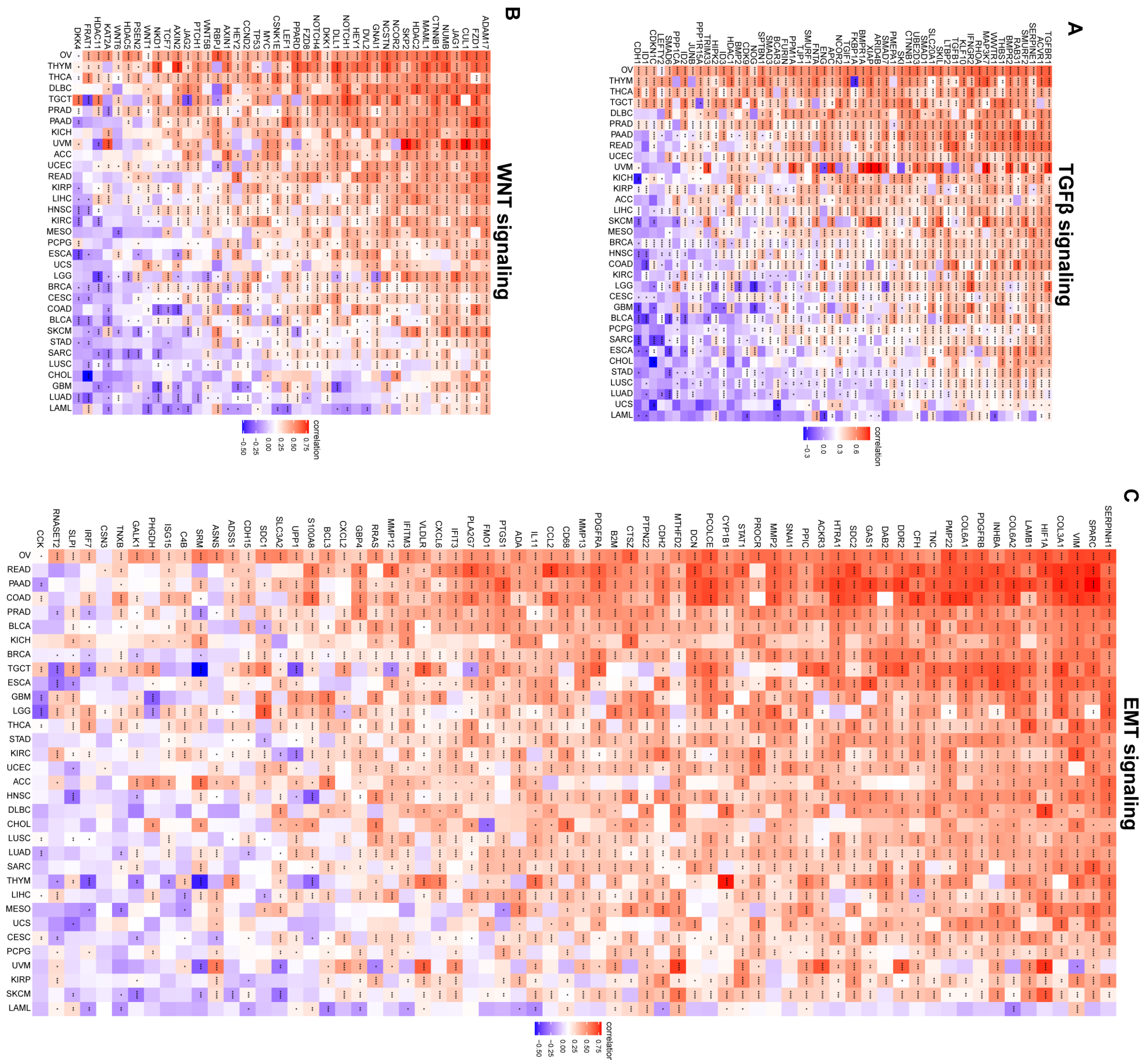

Fig. 13. IKBIP is positively correlated with genes involved in tumor-associated pathway. (A) The relationship between IKBIP and TGF-β signaling. (B) The relationship between IKBIP and WNT signaling. (C) The relationship between IKBIP and EMT signaling. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p<0.001$, $^{****}p < 0.0001$.

cations, including PF-4708671, TAF1_5496, acetalax, afatinib, OSI-027, dihydrorotenone, ABT737, osimertinib, and LGK974, indicating that tumor patients having elevated IKBIP expression could be resistant to these drugs. However, the mechanism that increased IKBIP expression that might induce drug-resistance is not clear.

Finally, *in-vitro* experiments with EdU assay, Transwell assay, and clone formation assay suggested that IKBIP upregulation inhibited colon cancer cells invasiveness and clonogenesis.

One of the limitations of this study is of that only the correlation between IKBIP and cancer cell proliferation and migration in colon cancer cells was evaluated. However, since IKBIP is closely associated with TME immune cell infiltration in several types of cancers, further research is required to investigate IKBIP expression effect on immunosuppressive cells, such as TAMs, TAFs, and Tregs in the pan-cancer TME. In special how IKBIP impact on immune-related signaling pathways, including TNF-A, IL-2 and IL-6, besides of the underlying mechanisms and its biological implications.

In summary, it was found that IKBIP might participate in various cancer types occurrence and development by both tumor-associated and immune-related signaling pathways. IKBIP overexpression may lead to unresponsive anticancer therapy, suggesting that IKBIP might be a novel target as well as a biomarker for colon cancer treatment.

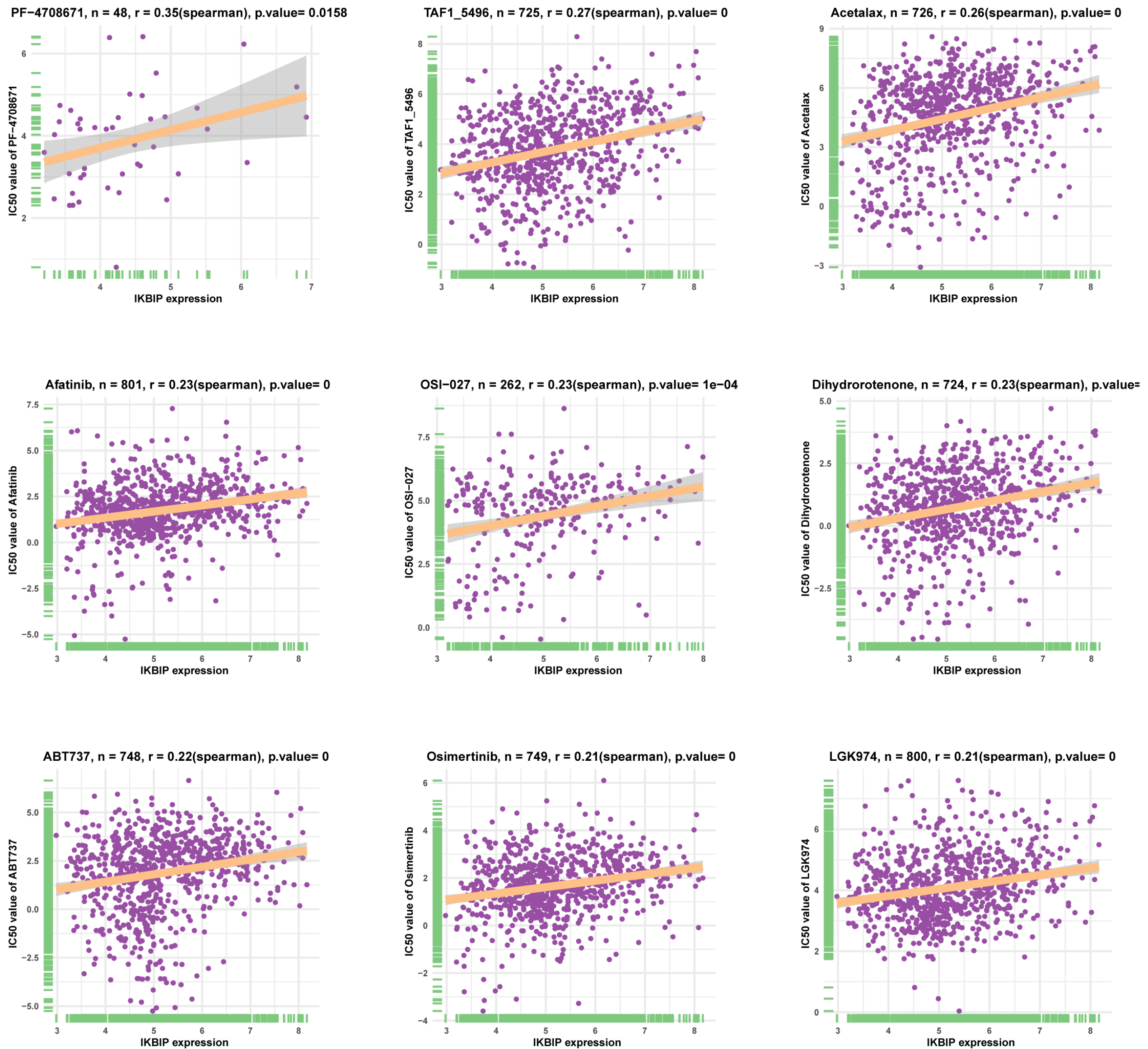

Fig. 14. IKBIP correlation with the IC_{50} of 192 antitumor medications was evaluated.

Conclusions

High IKBIP expression is associated with immunosuppressive TME in pan-cancer. Targeting IKBIP may activate the immune microenvironment and enhance the survival of patients having tumors.

Contribution to the Field

IKBKB interacting protein, is located on human chromosome 12, and has yet to be studied thoroughly. However, IKBIP may have potential as a diagnostic biomarker. In our study, we assessed IKBIP expression, genetic alterations, and prognosis in various diverse cancer-causing tumor types (also known as pan-cancer). Additionally, it was investigated the correlation of IKBIP correlation with TME. TME plays a crucial part in tumor progression and metastasis. Lastly, IKBIP effect on patients resistance to anti-tumor drugs was evaluated. Current data demonstrated that IKBIP is a potential oncogene and predictive marker in most tumor types. High IKBIP expression is associated with tumor immunosuppression. Further, patients with high IKBIP expression may be resistant to therapy with certain tumor-suppressant drugs. This research offers novel insights about IKBIP function in malignancy and its relationship with the tumor microenvironment. The results of this study can help to develop improved tumor diagnostic methods and treatment strategies.

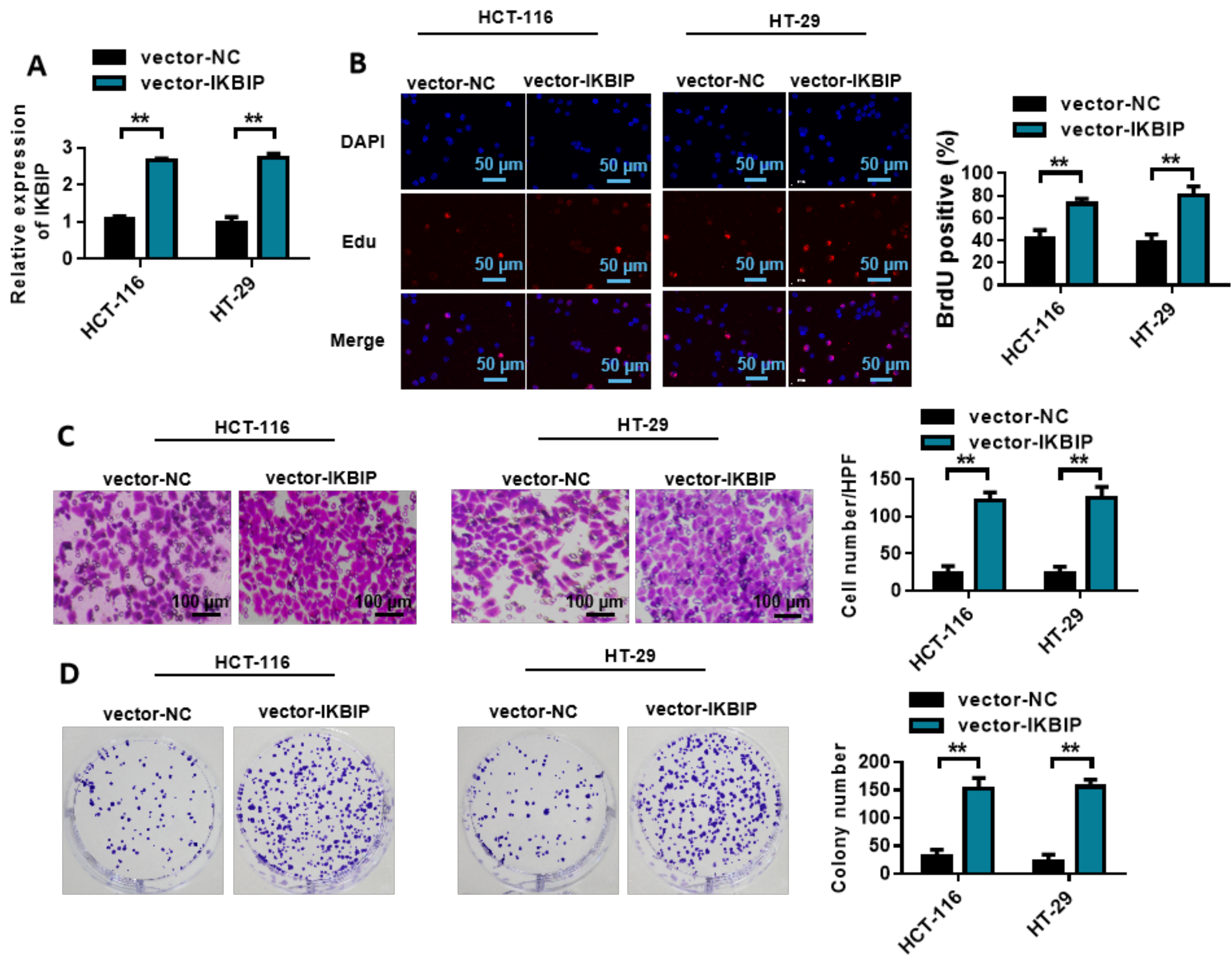

Fig. 15. IKBIP overexpression promotes colon cancer cells malignant behavior. (A) IKBIP expression levels in HCT-116 and HT-29 cells. (B) IKBIP level on HCT-116 and HT-29 proliferation by EdU. (C) IKBIP effect on HCT-116 and HT-29 invasion ability examined by Transwell assay. (D) IKBIP effect on the capacity of HCT-116 and HT-29 to make clones by cloning detection. $^{**}p < 0.05$.

Abbreviations

IKBIP, IKBKB interacting protein; TME, tumor microenvironment; TAM, tumor-associated macrophages; TAF, tumor-associated fibroblasts; Treg, regulatory T; OS, overall survival; DSS, disease-specific survival; DFI, disease-free interval; PFI, progression-free interval; Uni-Cox, univariate Cox regression; GSEA, Gene Set Enrichment Analysis; GSVA, Gene Set Variation Analysis; MsigDB, The Molecular Signatures Database; *MHC*, major histocompatibility complex; TGF-β, Transforming Growth Factor-β; EMT, Epithelial-Mesenchymal Transition; IL, interleukin; TCGA, The Cancer Genome Atlas; TIMER2, Tumor Immune Estimation Resource; GSSC, The Global South Studies Center; ATCC, American Type Culture Collection; PBS, phosphate buffer.

Availability of Data and Materials

Not applicable.

Author Contributions

QJL and ZGZ—designed the study, and drafted and revised the manuscript; FFP and CZK—conducted the experiments; FD and QJL—confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202335174.7.

References

[1] Yang Y, Wang J, Xu S, Lv W, Shi F, Shan A. *IKBIP* is a novel EMT-related biomarker and predicts poor survival in glioma. *Transl Neurosci*. 2021;12(1):9–19. doi: 10.1515/tnsci-2021-0002

[2] Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. *Nat Immunol*. 2013;14(10):1014–1022. doi: 10.1038/ni.2703

[3] Cai H, Zhu XD, Ao JY, *et al.* Colony-stimulating factor-1-induced AIF1 expression in tumor-associated macrophages enhances the progression of hepatocellular carcinoma. *Oncoimmunology*. 2017;6(9):e1333213. doi: 10.1080/2162402X.2017.1333213

[4] Chen X, Song E. Turning foes to friends: Targeting cancer-associated fibroblasts. *Nat Rev Drug Discov*. 2019;18(2):99–115. doi: 10.1038/s41573-018-0004-1

[5] Farhood B, Najafi M, Mortezaee K. $CD8^{+}$ cytotoxic T lymphocytes in cancer immunotherapy: A review. *J Cell Physiol*. 2019;234(6):8509–8521. doi: 10.1002/jcp.27782

[6] Wang H, Franco F, Ho PC. Metabolic Regulation of Tregs in Cancer: Opportunities for Immunotherapy. *Trends Cancer*. 2017;3(8):583–592. doi: 10.1016/j.trecan.2017.06.005

[7] Zeng D, Li M, Zhou R, *et al.* Tumor Microenvironment Characterization in Gastric Cancer Identifies Prognostic and Immunotherapeutically Relevant Gene Signatures. *Cancer Immunol Res*. 2019;7(5):737–750. doi: 10.1158/2326-6066.CIR-18-0436

[8] Liang J, Li X, Xu J, Cai GM, Cao JX, Zhang B. hsa_circ_0072389, hsa_circ_0072386, hsa_circ_0008621, hsa_circ_0072387, and hsa_circ_0072391 aggravate glioma via miR-338-5p/IKBIP. *Aging (Albany NY)*. 2021;13(23):25213–25240. doi: 10.18632/aging.203740

[9] Chen TY, Liu Y, Chen L, Luo J, Zhang C, Shen XF. Identification of the potential biomarkers in patients with glioma: A weighted gene co-expression network analysis. *Carcinogenesis*. 2020;41(6):743–750. doi: 10.1093/carcin/bgz194

[10] Yoshie O. CCR4 as a Therapeutic Target for Cancer Immunotherapy. *Cancers (Basel)*. 2021;13(21):5542. doi: 10.3390/cancers13215542

[11] Qianmei Y, Zehong S, Guang W, Hui L, Lian G. Recent advances in the role of Th17/Treg cells in tumor immunity and tumor therapy. *Immunol Res*. 2021;69(5):398–414. doi: 10.1007/s12026-021-09211-6

[12] Yu WQ, Ji NF, Gu CJ, Wang YL, Huang M, Zhang MS. Coexpression of Helios in $Foxp3^{+}$ Regulatory T Cells and Its Role in Human Disease. *Dis Markers*. 2021;2021:5574472. doi: 10.1155/2021/5574472

[13] Lee J, Lozano-Ruiz B, Yang FM, Fan DD, Shen L, González-Navajas JM. The Multifaceted Role of Th1, Th9, and Th17 Cells in Immune Checkpoint Inhibition Therapy. *Front Immunol*. 2021;12:625667. doi: 10.3389/fimmu.2021.625667

[14] Zhu Y, Herndon JM, Sojka DK, *et al.* Tissue-Resident Macrophages in Pancreatic Ductal Adenocarcinoma Originate from Embryonic Hematopoiesis and Promote Tumor Progression. *Immunity*. 2017;47(2):323–338.e6. doi: 10.1016/j.immuni.2017.07.014

[15] Qiu D, Hu J, Hu J, *et al. PMEPA1* Is a Prognostic Biomarker That Correlates With Cell Malignancy and the Tumor Microenvironment in Bladder Cancer. *Front Immunol*. 2021;12:705086. doi: 10.3389/fimmu.2021.705086