Am J Transl Res 2024;16(11):6346-6364
www.ajtr.org /ISSN:1943-8141/AJTR0156008

Original Article

PIAS family gene expression: implications for prognosis, immunomodulation, and chemotherapy response

Hang Shu, Xiaoyu Chen, Jie Zhao, Ping Li, Zhen Sun

Traditional Chinese Medicine Department, People's Liberation Army Air Force Hangzhou Special Service Recuperation Center, Nanjing 210000, Jiangsu, China

Received February 20, 2024; Accepted September 23, 2024; Epub November 15, 2024; Published November 30, 2024

Abstract: Background: Cancer remains one of the leading causes of mortality worldwide, characterized by uncontrolled cell proliferation and metastasis. Protein Inhibitor of Activated STAT (PIAS) family genes, comprising PIAS1, PIAS2, PIAS3, and PIAS4, are emerging as significant players in cancer biology due to their roles in SUMOylation, transcriptional regulation, and modulation of signal transduction pathways. This study provides a comprehensive analysis of PIAS family genes from a pan-cancer viewpoint. Methodology: Detailed in silico analyses using publicly available databases and in vitro analyses involving cell culture, gene knockdown, colony formation, and wound healing assays. Results: Expression analysis revealed consistent up-regulation of PIAS1, PIAS2, PIAS3, and PIAS4 genes in tumors compared to normal tissues. Univariate Cox regression analyses indicate that high PIAS gene expression correlates with worse overall survival in specific cancers, particularly kidney renal papillary cell carcinoma (KIRP) and liver hepatocellular carcinoma (LIHC). Kaplan-Meier plots further confirm that higher PIAS gene expression is significantly associated with reduced survival probabilities in these cancers. Genetic alteration analysis showed low mutation frequencies in PIAS genes, suggesting their role in cancer progression is likely due to expression regulation rather than genetic mutations. Correlations with immune subtypes, the tumor microenvironment (TME), and immune stimulatory genes highlight the differential expression of PIAS genes across immune landscapes in KIRP and LIHC. Gene enrichment analysis emphasizes the involvement of PIAS genes in crucial cellular processes, including SUMOylation and ubiquitin-mediated proteolysis. Finally, knockdown experiments in HCC-LM3 cells demonstrate that PIAS2 and PIAS3 promote tumor growth and metastasis, reinforcing their potential as therapeutic targets. Conclusion: This study revealed the multifaceted roles of PIAS genes in KIRP and LIHC biology and their potential as prognostic biomarkers and therapeutic targets.

Keywords: PIAS family genes, cancer, biomarker, diagnosis: treatment

Introduction

Cancer remains one of the most formidable health challenges globally, with its incidence and mortality rates continuing to rise [1-4]. In 2023, cancer was the second leading cause of death worldwide, accounting for millions of new cases and deaths annually [5]. Despite advances in early detection, treatment modalities, and supportive care, the complexity and heterogeneity of cancer demand continuous research into novel biomarkers and therapeutic targets [6, 7]. Understanding the molecular mechanisms underlying cancer development and progression is crucial for improving diagnostic, prognostic, and therapeutic strategies.

Among the numerous gene families implicated in cancer, the Protein Inhibitor of Activated STAT (PIAS) family has garnered significant interest [8, 9]. The PIAS family comprises four members: PIAS1, PIAS2, PIAS3, and PIAS4, which are known to play vital roles in various cellular processes, including transcriptional regulation, DNA repair, and maintenance of genomic integrity [10]. Acting primarily as E3 SUMO ligases, PIAS proteins facilitate the sumoylation of target proteins, thereby influencing their function, localization, stability, and interactions [11]. In normal physiology, PIAS1 regulates immune responses and cell cycle by modulating Signal Transducer and Activator of Transcription 1 (STAT1) and p53 activities [12]. PIAS2 is

https://doi.org/10.62347/JRDP4018

involved in transcription regulation and cell differentiation, particularly affecting androgen receptors and MYB proto-oncogene (MYB) [13]. PIAS3 inhibits STAT3 signaling, suppressing tumorigenic processes and promoting differentiation [14]. PIAS4 plays a critical role in DNA damage response and repair by modulating proteins like BRCA1 and 53BP1, thus preventing mutation accumulation [15-17]. Collectively, the PIAS family ensures proper cellular responses to environmental cues, maintains genomic integrity and regulates immune functions, highlighting their potential as biomarkers and therapeutic targets in cancer.

Previous studies have explored the involvement of PIAS family genes in different cancers. For instance, PIAS1 has been shown to modulate the activity of tumor suppressor p53 and the oncogene Signal Transducer and Activator of Transcription 3 (STAT3), influencing cancer cell proliferation and survival [18]. PIAS3 has been reported to inhibit STAT3 signaling, thereby suppressing tumor growth in glioblastoma and other cancers [19]. Similarly, PIAS4 has been implicated in the regulation of androgen receptor signaling in prostate cancer [11]. However, these studies often focus on individual PIAS genes and specific cancer types, leaving a gap in understanding the broader implications of the entire PIAS family across diverse cancers.

Given the crucial roles of PIAS family genes in regulating key signaling pathways and maintaining genomic stability, there is a pressing need for a comprehensive pan-cancer analysis to evaluate their diagnostic, prognostic, and therapeutic potential. Such an analysis can provide valuable insights into the universal and cancer-type-specific functions of PIAS genes, aiding in the identification of novel biomarkers and therapeutic targets.

In this study, we performed an extensive pan-cancer analysis of PIAS family genes, using large-scale genomic and molecular experimental data to assess their expression patterns, prognostic significance, and potential as therapeutic targets across various cancers. Our findings aim to elucidate the multifaceted roles of PIAS genes in cancer and pave the way for improved cancer management strategies.

Methodology

PIAS family gene expression across pan-cancer

UALCAN [20] and TNMplot [21] are valuable online databases for cancer research. UALCAN provides user-friendly access to omics data from The Cancer Genome Atlas (TCGA), enabling researchers to analyze gene expression, survival data, and more factors across various cancer types. TNMplot allows comparison of gene expression between normal, tumor, and metastatic tissues, facilitating the identification of biomarkers and therapeutic targets. Both platforms offer comprehensive, high-quality data and intuitive interfaces, significantly enhancing the ability to conduct in-depth cancer genomics and transcriptomics research, ultimately advancing our understanding of cancer biology and improving patient outcomes. In this research, both UALCAN and TNMplot databases were used for the pan-cancer expression analysis of PIAS family genes.

Cox regression and survival analysis across pan-cancer

Cox univariate regression analysis was conducted to explore the impact of PIAS gene expression levels on prognosis risk. Samples were categorized into high- and low-expression groups based on the median expression levels of PIAS family genes. Subsequently, forest plots were generated using the “forestplot” package in R software (version 4.3.0). For survival analysis, the KM plotter tool [22] was utilized in this work.

Genetic alteration analysis of PIAS family genes

cBioPortal is a comprehensive, open-access database designed to explore multidimensional cancer genomics data [23]. It provides researchers and clinicians with intuitive visualization tools and advanced analytical capabilities to understand genetic alterations in cancer. By integrating data from multiple large-scale projects, such as TCGA, cBioPortal facilitates the identification of potential therapeutic targets and biomarkers, thus advancing personalized medicine and enhancing cancer research globally. In this study, cBioPortal data-

base was utilized for the genetic alteration analysis of PIAS family genes.

Correlation analysis of PIAS family genes with immune subtypes and immune stimulators

TISIDB is an integrated repository designed to facilitate the exploration of tumor-immune interactions [24]. It consolidates diverse data types, including genomic, transcriptomic, and clinical data, to provide comprehensive insights into the tumor-immune system interplay. Researchers can utilize TISIDB to identify immune-related biomarkers, analyze immune cell infiltration patterns, and evaluate the impact of various factors on tumor immunogenicity. These resources support the development of immunotherapies and enhance our understanding of cancer immunology. In this study, TISIDB database was used for the correlation analysis of PIAS family genes with immune subtypes and immune stimulators.

Correlation analysis of PIAS family genes within the tumor microenvironment (TME)

TISCH2 is a specialized database focused on the tumor microenvironment, providing a comprehensive repository of single-cell RNA sequencing (scRNA-seq) data [25]. It enables detailed exploration of cellular heterogeneity within tumors, offering insights into the complex interactions between different cell types. Researchers can access annotated scRNA-seq datasets, perform comparative analyses, and visualize cell-type-specific expression patterns. TISCH2 aids in identifying potential therapeutic targets and understanding tumor biology, thus advancing cancer research and precision oncology. In this work, the TISCH2 database was utilized for correlation analysis of PIAS family genes in the TME.

Gene enrichment analysis

DAVID is a bioinformatics tool designed to provide functional interpretation of large lists of genes or proteins [26]. It integrates a wide array of biological data sources to offer comprehensive annotation and pathway analysis. Users can uncover biological themes, perform gene enrichment analysis, and visualize data through various graphical outputs. DAVID aids researchers in making sense of complex genomic data, facilitating discoveries in functional genomics and systems biology. In our study, we utilized DAVID for gene enrichment analysis of the PIAS family genes

Correlation analysis of PIAS family genes with immune cells and drug sensitivity

GSCA is an advanced bioinformatics tool designed to analyze and interpret gene sets within the context of cancer research [23]. It integrates multi-omics data to provide insights into the biological functions and pathways associated with specific gene sets. GSCA offers capabilities for gene set enrichment analysis, visualization of molecular interactions, and identification of potential therapeutic targets. This tool helps researchers and clinicians understand the molecular mechanisms of cancer and develop targeted treatment strategies. Herein, GSCA was used to conduct correlation analyses of PIAS family genes with immune cells and drug sensitivity.

Cell culture and cell transfection

The human hepatocellular carcinoma cell line HCC-LM3 was obtained from the American Type Culture Collection (ATCC). All cells were cultured at 37°C with 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM, Gibco, C11-995500BT) supplemented with 10% bovine serum (Gibco, 10091148) and 1% penicillin-streptomycin (Gibco, 15140122).

Two gene-specific siRNAs targeting PIAS2 and PIAS3 were designed and synthesized by GenePharma Co., Ltd. (Shanghai, China). Cells in 6-well plates (1.5 × 10^5 cells/well) were transfected with siRNAs (40 nM) using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, United States) according to the manufacturer's guidelines. Typically, gene silencing is observed at both mRNA and protein levels 72 hours after transfection; therefore, the cells were harvested and analyzed at this time point.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Quantitative analysis of PIAS2, PIAS3, and GAPDH (loading control) mRNA levels was performed by RT-qPCR method using a 2× Power SYBR Premix Ex TaqTM (TaKaRa Bio INC, Japan) in a Mastercycler ep realplex Real-Time System (Eppendorf, Germany). The ΔCt (Delta Ct) meth-

od was used for the expression analysis. The following primers were used during RT-qPCR: GAPDH-F: 5'-ACCCACTCCTCCACCTTTGAC-3', GAPDH-R: 5'-CTGTTGCTGTAGCCAAATTCG-3'; PIAS1-F: 5'-TAAGGAGGATGGCACTTGGGCA-3', PIAS1-R: 5'-TGAGACGCTACCTGATGCTCCA-3', PIAS2-F: 5'-GTTCTTGGTGTCCAATGAGACCG-3', PIAS2-R: 5'-TGCTTGCCTCACTGGCTACAGT-3', PIAS3-F: 5'-ACTCTCAGCCACTGTTCCCAAC-3', PIAS3-R: 5'-CAGTCAACTGCCTCACCAGGTA-3', PIAS4-F: 5'-CCAACCGCATTACTGTCACCTG-3', PIAS4-R: 5'-CGTCTTCAACCTCTGTAGCAGG-3'.

Western blot analysis

For Western blotting, cells were collected, and proteins were separated using 10% SDS-PAGE, then transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% bovine serum albumin (BSA, Sigma, CAS No: 9048-46-8) in Tris-buffered saline with Tween 20 (TBST). Following blocking, the membranes were incubated with specific primary antibodies at 4°C overnight. The next day, the membranes were incubated with secondary antibodies for 2 hours at room temperature (15-30°C). Finally, the protein bands were visualized using the Easysee Western Blot Kit (Transgene, Alsace, France).

Colony formation and wound healing assays

For the colony formation assay, transfected cells were seeded into 6-well plates at a density of 10^3 cells per well. After 8-10 days, the cells were gently washed with PBS, fixed with 95% ethanol, and stained with 0.1% crystal violet.

In transfected cells seeded in 12-well plates (2 × 10^5 cells/well), a wound area was carefully created by scraping the cell monolayer with a sterile 10 μL pipette tip. The cells were then washed once with Dulbecco's PBS to remove detached cells. Subsequently, the cells were incubated at 37°C with 5% CO_2. The width of the wound area was monitored at various time points using an inverted microscope.

Statistical analysis

Data were analyzed using SPSS 20.0 (IBM, Armonk, NY, USA) and GraphPad Prism 5.0 (GraphPad Prism Software Inc., San Diego, CA, USA). Correlation analysis was performed using the Pearson correlation method. Comparisons between groups were made using Student's t-test, with $P < 0.05$ considered statistically significant.

Results

Expression of PIAS family genes in pan-cancer

Figure 1 presents the expression analysis of the PIAS family genes (PIAS1, PIAS2, PIAS3, and PIAS4) across various cancers using data from the TCGA database through UALCAN and TNMplot. **Figure 1A-D** shows the differential expression of PIAS1, PIAS2, PIAS3, and PIAS4, respectively, in tumor versus normal samples across multiple cancer types via the UALCAN. PIAS1 (**Figure 1A**) demonstrates a notable increase in expression in tumor samples compared to normal samples in several cancers, including lung adenocarcinoma (LUAD), kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), lung squamous cell carcinoma (LUSC), and stomach adenocarcinoma (STAD). PIAS2 (**Figure 1B**) shows relatively higher levels in tumor samples for some cancers, but the differences are less pronounced compared to PIAS1. PIAS3 (**Figure 1C**) also shows elevated expression in tumor samples in cancers like LUAD, KIRP, LIHC, LUSC, and STAD, among others. PIAS4 (**Figure 1D**) demonstrates higher expression in tumor samples in various cancers, with some distinct differences observable in the data. **Figure 1E** provides a pan-cancer analysis from the TNMplot database, comparing the log2 gene expression levels among normal, tumor, and metastatic samples for the PIAS genes. The density plots indicate that PIAS1, PIAS2, PIAS3, and PIAS3 show a clear shift towards higher expression levels in tumor and metastatic samples compared to normal samples, suggesting a potential role in cancer progression. Overall, the data suggest that PIAS1PIAS2, PIAS3, and PIAS4 have consistent up-regulation in tumors and metastatic samples across different cancers.

Prognostic value of PIAS family genes across pan-cancers

Figure 2 presents the results of univariate Cox regression analyses evaluating the relationship between the expression of PIAS family genes (PIAS1, PIAS2, PIAS3, and PIAS4) and overall

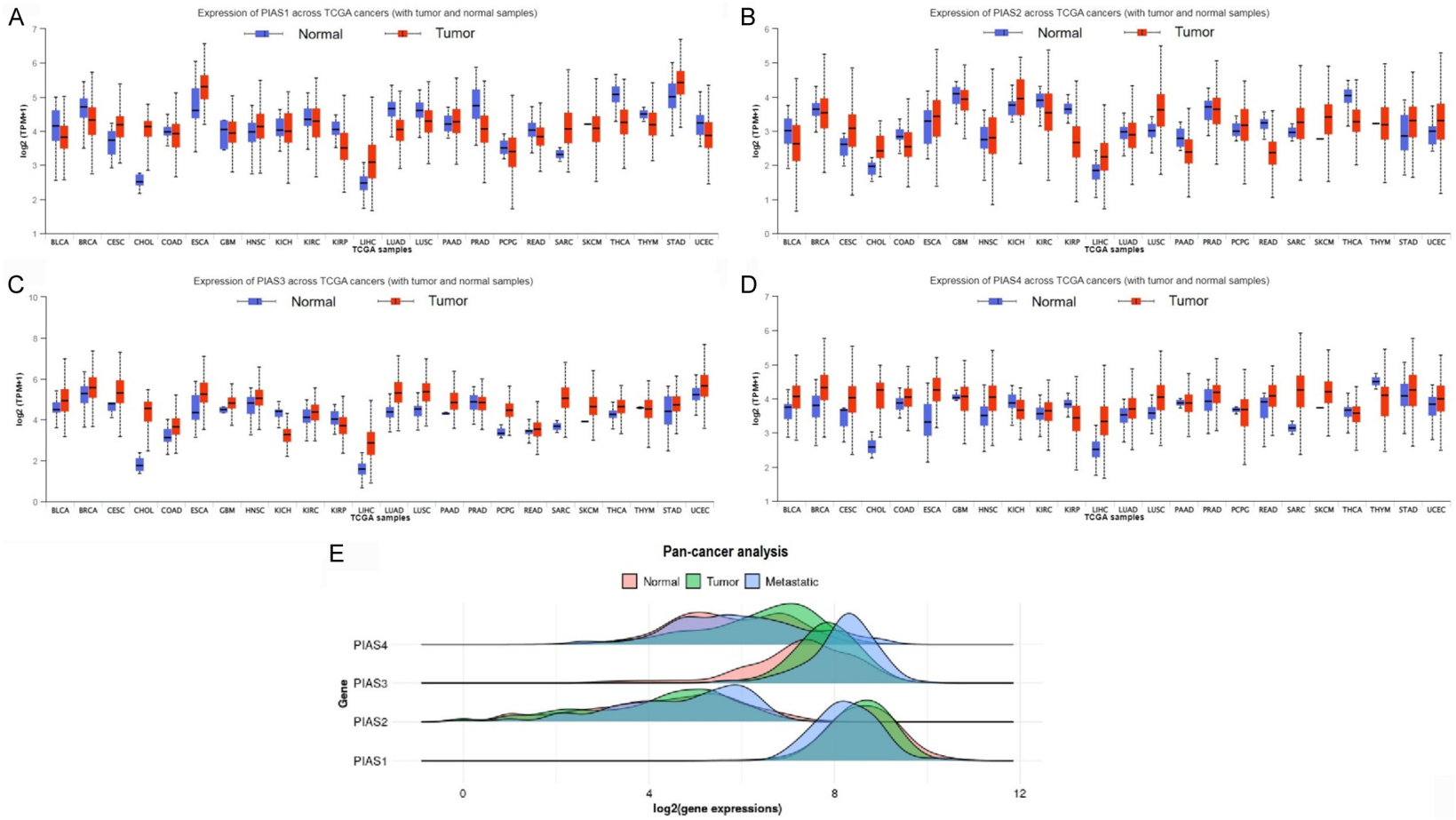


Figure 1. Expression analysis of PIAS gene family across various cancers using the cancer genome atlas (TCGA) data. A. Expression of PIAS1 across TCGA cancers (with tumor and normal samples) using UALCAN. B. Expression of PIAS2 across TCGA cancers (with tumor and normal samples) using UALCAN. C. Expression of PIAS3 across TCGA cancers (with tumor and normal samples) using UALCAN. D. Expression of PIAS4 across TCGA cancers (with tumor and normal samples) using UALCAN. E. Pan-cancer analysis of PIAS1, PIAS2, PIAS3, and PIAS4 expression across normal, tumor, and metastatic samples using TNMplot. Density plots show log2 (gene expressions) distributions for each gene across different sample types: normal (red), tumor (blue), and metastatic (green). *P*-value < 0.05. UVM = Uveal Melanoma, UCS = Uterine Carcinosarcom, UCEC = Uterine Corpus Endometrial Carcinoma, THYM = Thymoma, THCA = Thyroid Carcinoma, TGCT = Testicular Germ Cell Tumors, STAD = Stomach Adenocarcinoma, SKCM = Skin Cutaneous Melanoma, SARC = Sarcoma, READ = Rectum Adenocarcinoma, PRAD = Prostate Adenocarcinoma, PCPG = Pheochromocytoma and Paraganglioma, PAAD = Pancreatic Adenocarcinoma, OV = Ovarian Serous Cystadenocarcinoma, MESO = Mesothelioma, LUSC = Lung Squamous Cell Carcinoma, LUAD = Lung Adenocarcinoma, LIHC = Liver Hepatocellular Carcinoma, LGG = Brain Lower Grade Glioma, LAML = Acute Myeloid Leukemia, KIRP = Kidney Renal Papillary Cell Carcinoma, KIRC = Kidney Renal Clear Cell Carcinoma, KICH = Kidney Chromophobe, HNSC = Head and Neck Squamous Cell Carcinoma, GBM = Glioblastoma Multiforme, ESCA = Esophageal Carcinoma, DLBC = Lymphoid Neoplasm Diffuse Large B-cell Lymphoma, COAD = Colon Adenocarcinoma, CHOL = Cholangiocarcinoma, CESC = Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma, BRCA = Breast Invasive Carcinoma, BLCA = Bladder Urothelial Carcinoma, and ACC = Adrenocortical Carcinoma.

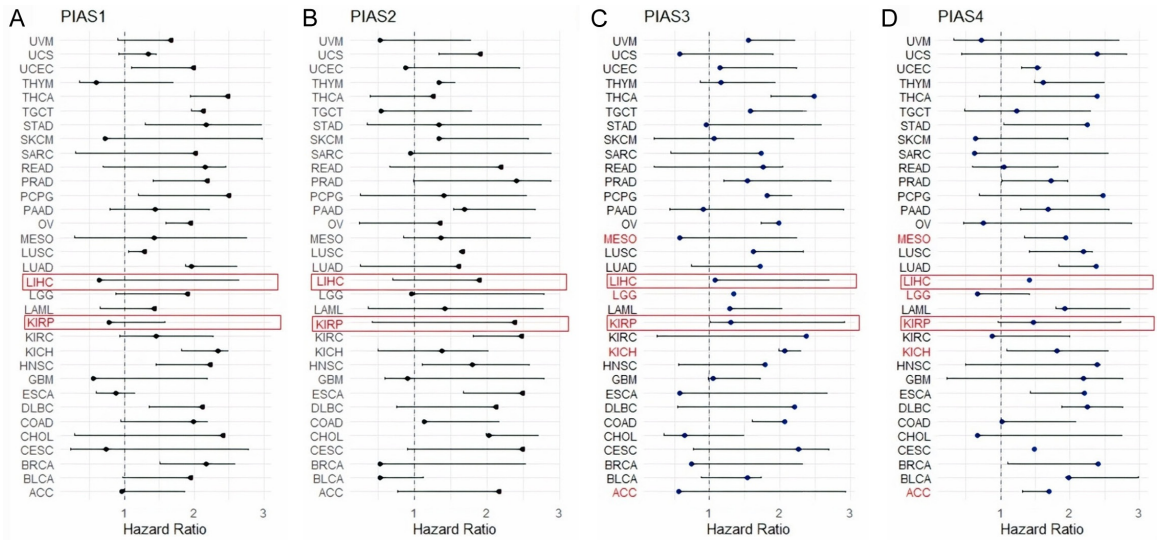


Figure 2. Univariate cox regression analyses of PIAS family genes across various cancers. A. Cox regression analyses of PAIS1. B. Cox regression analyses of PAIS2. C. Cox regression analyses of PAIS3. D. Cox regression analyses of PAIS4. *P*-value < 0.05. UVM = Uveal Melanoma, UCS = Uterine Carcinosarcom, UCEC = Uterine Corpus Endometrial Carcinoma, THYM = Thymoma, THCA = Thyroid Carcinoma, TGCT = Testicular Germ Cell Tumors, STAD = Stomach Adenocarcinoma, SKCM = Skin Cutaneous Melanoma, SARC = Sarcoma, READ = Rectum Adenocarcinoma, PRAD = Prostate Adenocarcinoma, PCPG = Pheochromocytoma and Paraganglioma, PAAD = Pancreatic Adenocarcinoma, OV = Ovarian Serous Cystadenocarcinoma, MESO = Mesothelioma, LUSC = Lung Squamous Cell Carcinoma, LUAD = Lung Adenocarcinoma, LIHC = Liver Hepatocellular Carcinoma, LGG = Brain Lower Grade Glioma, LAML = Acute Myeloid Leukemia, KIRP = Kidney Renal Papillary Cell Carcinoma, KIRC = Kidney Renal Clear Cell Carcinoma, KICH = Kidney Chromophobe, HNSC = Head and Neck Squamous Cell Carcinoma, GBM = Glioblastoma Multiforme, ESCA = Esophageal Carcinoma, DLBC = Lymphoid Neoplasm Diffuse Large B-cell Lymphoma, COAD = Colon Adenocarcinoma, CHOL = Cholangiocarcinoma, CESC = Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma, BRCA = Breast Invasive Carcinoma, BLCA = Bladder Urothelial Carcinoma, and ACC = Adrenocortical Carcinoma.

survival (OS) across thirty-three cancer types from the TCGA dataset. For each PIAS gene, the hazard ratios (HR) and confidence intervals (CI) are displayed for different cancers. A hazard ratio greater than 1 suggests that higher expression of the PIAS gene is associated with worse OS, while a hazard ratio less than 1 indicates better OS. Most cancer types show HRs close to 1, indicating no significant association between PIAS (PIAS1, PIAS2, PIAS3, and PIAS4) expression and OS (**Figure 2A-D**). However, specific cancer types, notably, KIRP and LIHC, consistently exhibit trends or significant associations where higher expression of PIAS genes correlates with worse OS (**Figure 2A-D**). This pattern suggests that these genes may play a more critical role in the prognosis of KIRP and LIHC.

Next, we also used the KM plotter tool to further evaluate the prognostic significance of PIAS family genes in KIRP and LIHC. The KM survival curves in **Figure 3** evaluate the prognostic roles of PIAS1, PIAS2, PIAS3, and PIAS4 in KIRP and LIHC. The results consistently show that higher expression levels of these PIAS genes are associated with worse overall survival in both cancer types. Specifically, for KIRP, high expression of PIAS1 (HR = 1.51, P = 0.017), PIAS2 (HR = 1.76, P = 0.0012), PIAS3 (HR = 2.49, P = 0.0038), and PIAS4 (HR = 2.66, P = 0.0093) significantly correlate with reduced survival probabilities (**Figure 3A**). Similarly, for LIHC, high expression of PIAS1 (HR = 1.53, P = 0.042), PIAS2 (HR = 1.76, P = 0.0012), PIAS3 (HR = 1.76, P = 0.003), and PIAS4 (HR = 1.67, P = 0.0047) is also linked to poorer overall survival (**Figure 3B**). These findings suggest that PIAS gene expression could serve as a valuable prognostic biomarker in KIRP and LIHC patients.

Genetic alteration analysis of PIAS family genes

The genetic analysis of the PIAS family genes using cBioPortal reveals distinct mutation patterns in KIRP and LIHC. In KIRP, mutations were found in 5 out of 281 samples (1.78%), with

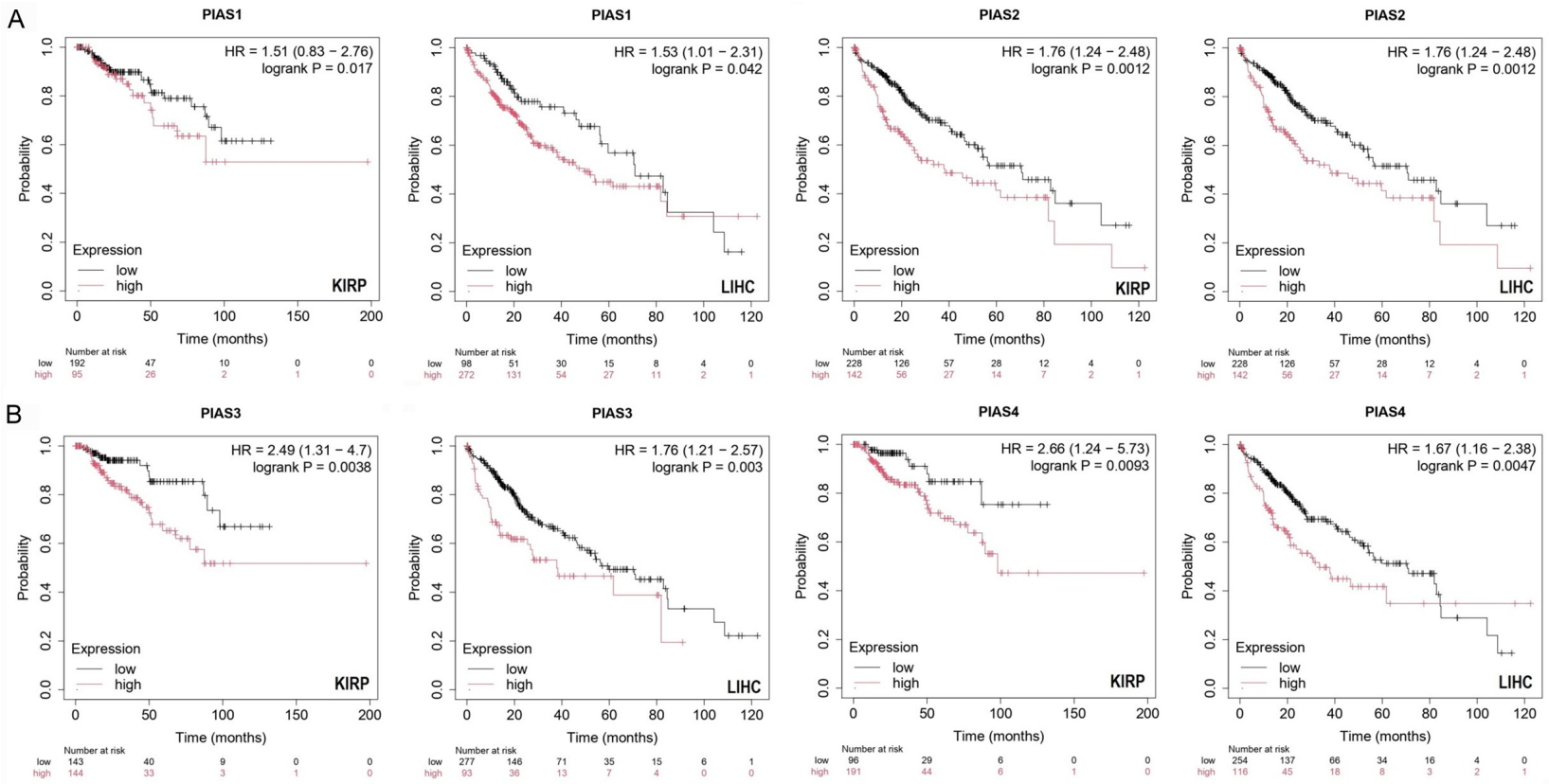


Figure 3. Survival analysis of PIAS gene family using KM plotter. A. Kaplan-Meier overall survival (OS) curves of PIAS family genes for kidney renal papillary cell carcinoma (KIRP) patients. B. Kaplan-Meier OS curves of PIAS family genes for kidney renal papillary cell carcinoma (KIRP) patients and liver hepatocellular carcinoma (LIHC) patients. *P*-value < 0.05. HR = Hazard Ratio.

PIAS2 being the most frequently altered gene (40%), followed by PIAS1, PIAS3, and PIAS4, each at 20% (**Figure 4A**). These mutations are exclusively missense mutations and SNPs, with a notable presence of T>C and C>T transitions (**Figure 4B**). Conversely, in LIHC, only 1 out of 364 samples (0.27%) exhibited mutations, specifically in the PIAS3 gene (**Figure 4C**). Like KIRP, these mutations are missense and single nucleotide polymorphisms (SNPs), predominantly T>G and T>C transitions (**Figure 4D**). Overall, the data indicate that genetic alterations in the PIAS genes are infrequent in both KIRP and LIHC, with missense mutations being the most common type of alteration. The low frequency of mutations suggests that while these genes may play a role in cancer progression and prognosis, genetic alterations are not the primary mechanism of their involvement.

Correlation of PIAS family genes with immune subtypes

Figure 5 illustrates the correlation of PIAS family gene expression with immune subtypes in KIRP and LIHC using the TISIDB database. In KIRP, the expression of PIAS1 and PIAS3 shows significant variability across immune subtypes, with PIAS1 being highest in C3 and C6, and PIAS3 peaking in C3. PIAS2 and PIAS4 exhibit less pronounced variation, with PIAS2 being slightly higher in C1 and C5, and PIAS4 in C2 and C3 (**Figure 5A**). Conversely, in LIHC, PIAS gene expression is more uniform across immune subtypes (**Figure 5B**). PIAS2 and PIAS3 show the most variability, with PIAS2 peaking in C6 and PIAS3 in C3, while PIAS1 and PIAS4 display minimal variation, with PIAS1 being slightly higher in C6 and PIAS4 in C2 and C6 (**Figure 5B**). This analysis suggests differential regulation of PIAS family genes across immune subtypes in KIRP and LIHC, indicating their distinct roles in the tumor microenvironment of these cancers.

Correlation of PIAS family genes with TME

Figure 6 evaluates the correlation of PIAS family gene expression with the TME in KIRP and LIHC using the TISCH2 database. In KIRP (**Figure 6A**), the expression of PIAS1, PIAS2, and PIAS3 is primarily observed in the Monocytes/Macrophages cluster, indicating a significant role in immune cell regulation within the TME, while PIAS4 shows minimal expression (**Figure 6A**). In contrast, LIHC (**Figure 6B**) demonstrates that PIAS1, PIAS2, and PIAS3 are predominantly expressed in the Malignant and Monocytes/Macrophages clusters, with PIAS2 showing the highest intensity (**Figure 6B**). PIAS4 has low expression levels mainly in the Malignant cluster (**Figure 6B**). These patterns suggest that PIAS genes play crucial roles in both immune and tumor cells within the TME of these cancers, potentially affecting tumor progression and immune response.

Correlation of PIAS family genes with immune stimulators

The correlation analysis of PIAS family genes with immune modulator genes was conducted using the TISIDB database. The heatmaps in **Figure 7** reveal that PIAS family genes (PIAS1, PIAS2, PIAS3, and PIAS4) demonstrate notable negative correlations with key immune stimulatory genes in KIRP and LIHC. Specifically, in both KIRP and LIHC, the PIAS genes show negative correlations with ICOSLG (Inducible T-cell Costimulator Ligand), IL12A (Interleukin 12A), IL6R (Interleukin 6 Receptor), TNFRSF14 (TNF Receptor Superfamily Member 14), and TNFRSF18 (TNF Receptor Superfamily Member 18) (**Figure 7A-D**). These correlations suggest that higher expression of PIAS genes might be associated with reduced expression or activity of these crucial immune stimulators, potentially impacting the immune landscape and tumor microenvironment in these cancers (**Figure 7**). This negative correlation emphasizes the possible role of PIAS genes in modulating immune responses in KIRP and LIHC, which could have significant implications for understanding tumor immune evasion and developing targeted therapies.

Gene enrichment analysis

The gene enrichment analysis for PIAS family genes, illustrated in the attached figure via DAVID, reveals significant associations across various biological processes, cellular components, molecular functions, and pathways. **Figure 8A** highlights the enriched cellular components, with PIAS genes being prominently associated with the “PML body, nuclear speck, and nuclear body, indicating their localization and functional relevance within these nuclear substructures”. **Figure 8B** focuses on molecular functions, showing strong enrichment for

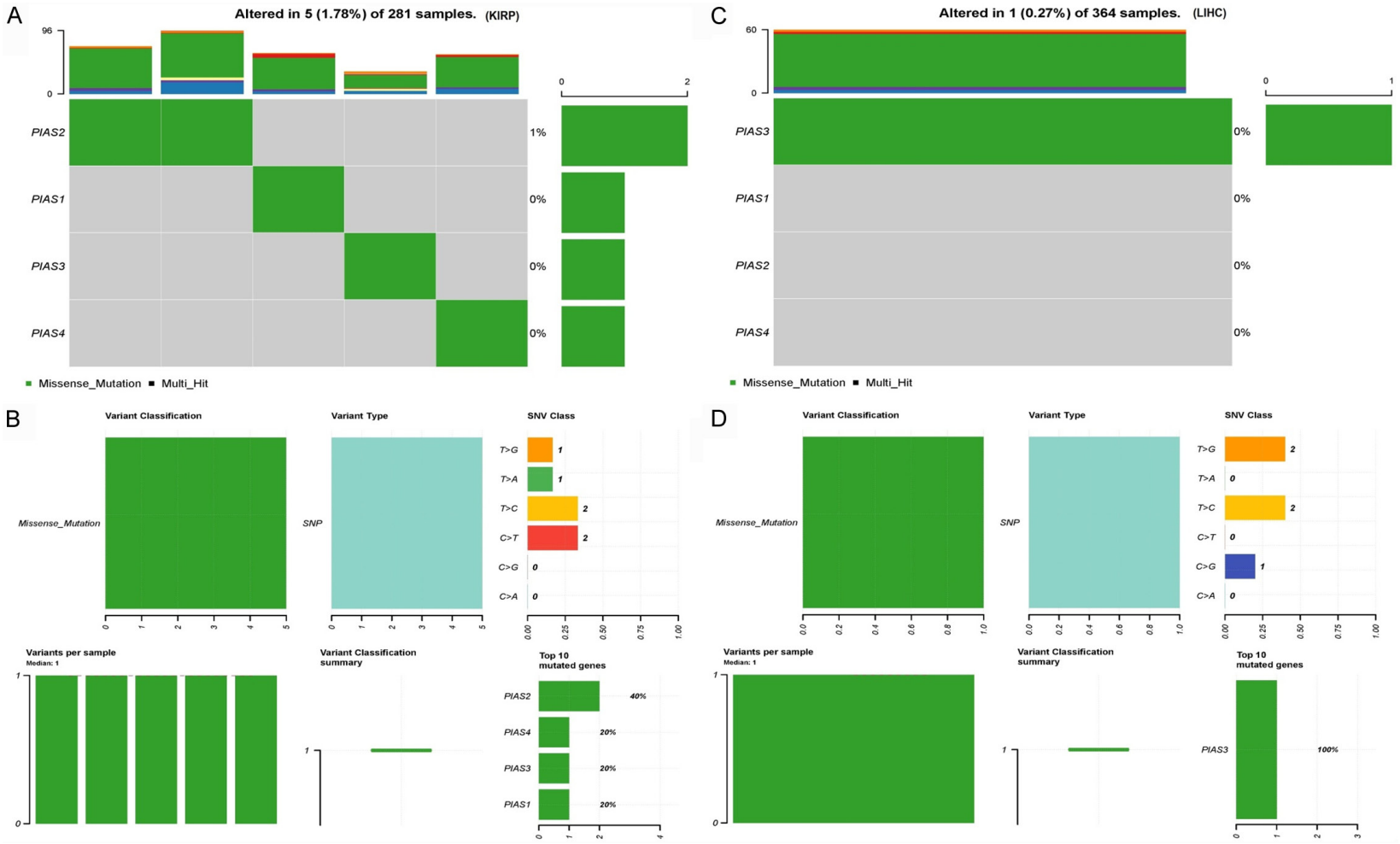


Figure 4. Genetic alteration analysis of PIAS gene family in kidney renal papillary cell carcinoma (KIRP) and liver hepatocellular carcinoma (LIHC) using cBioPortal. A. Genetic alterations in PIAS1, PIAS2, PIAS3, and PIAS4 in KIRP samples (n = 281). B. Variant classification of observed mutations in KIRP. C. Genetic alterations in PIAS1, PIAS2, PIAS3, and PIAS4 in LIHC samples (n = 364). D. Variant classification of observed mutations in LIHC. SNP = Single nucleotide polymorphism.

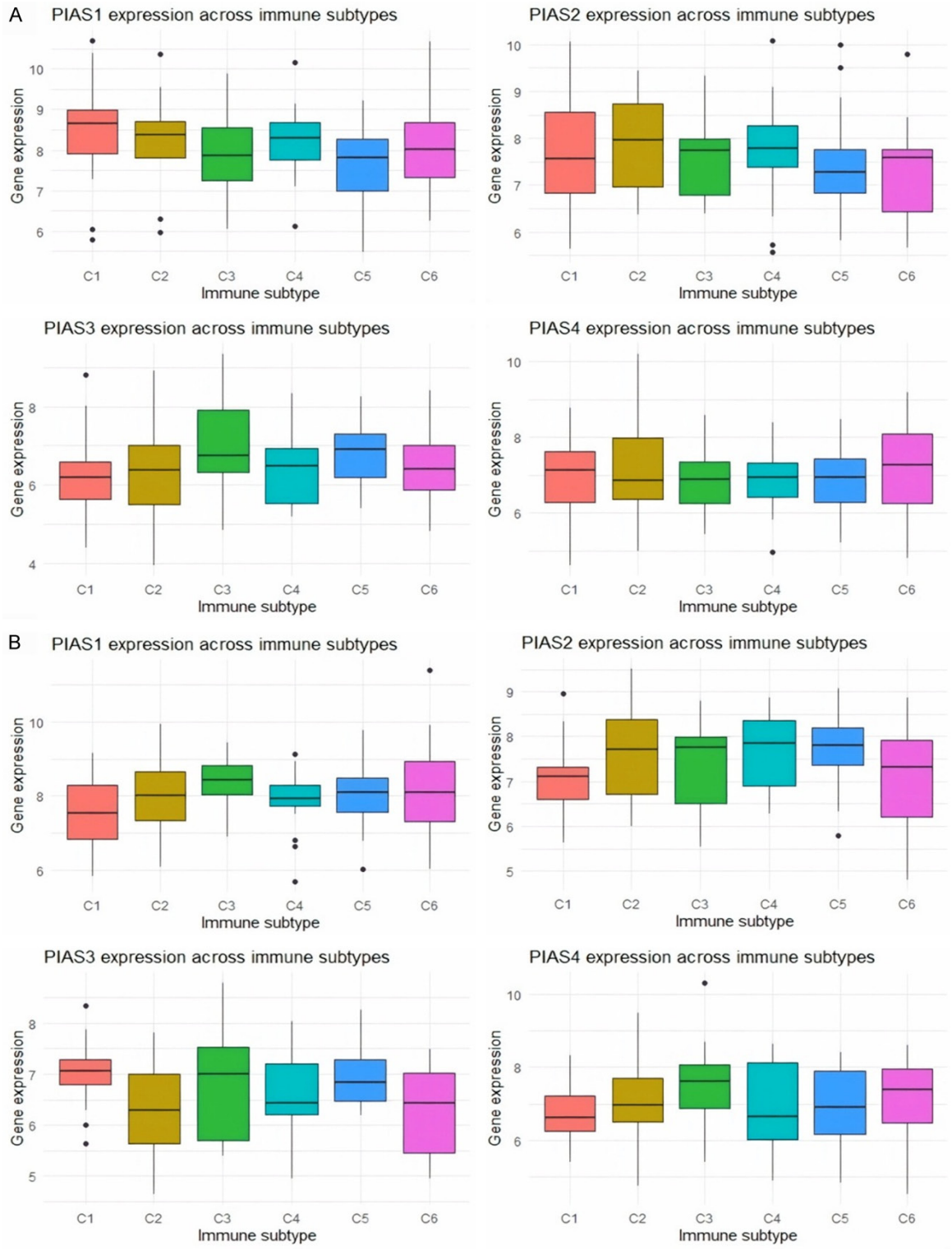


Figure 5. Correlation analysis of PIAS family genes with immune subtypes in kidney renal papillary cell carcinoma (KIRP) and liver hepatocellular carcinoma (LIHC) using TISIDB database. A. This panel presents box plots showing the expression levels of PIAS1, PIAS2, PIAS3, and PIAS4 across six immune subtypes (C1 to C6) in KIRP. B. This panel B displays similar box plots for LIHC. Each plot delineates the distribution of gene expression within each immune subtype, indicating the median, interquartile range, and outliers. *P*-value < 0.05.

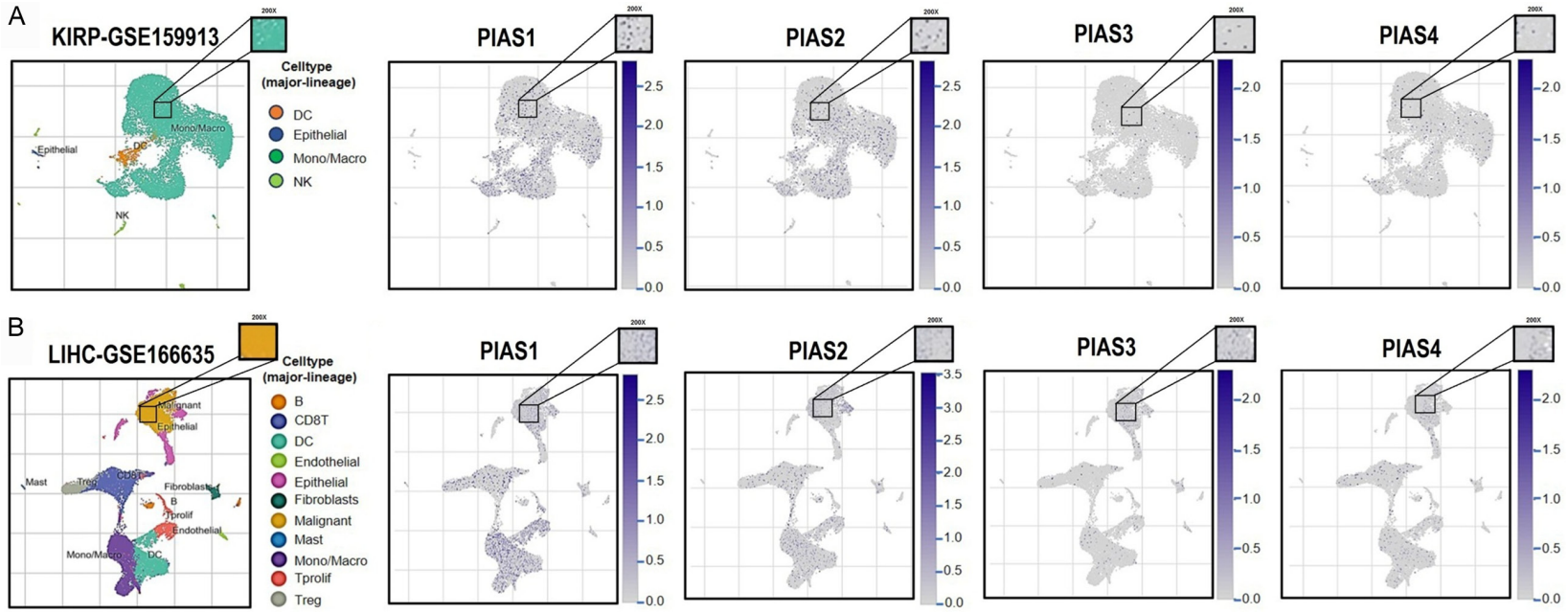


Figure 6. Correlation of PIAS family genes with tumor microenvironment in KIRP and LIHC using TISCH2 database. A. UMAP plots depict the major cell types in the TME of KIRP (GSE159913) and the expression levels of PIAS1, PIAS2, PIAS3, and PIAS4. The major cell types include dendritic cells (DC), epithelial cells, monocytes/macrophages (Mono/Macro), and natural killer cells (NK). Each plot shows the spatial distribution and expression intensity of the respective PIAS gene across these cell types. B. UMAP plots illustrating the major cell types in the TME of LIHC (GSE166635) and the expression levels of PIAS1, PIAS2, PIAS3, and PIAS4. The major cell types include B cells, CD8+ T cells, dendritic cells (DC), endothelial cells, epithelial cells, fibroblasts, malignant cells, mast cells, monocytes/macrophages (Mono/Macro), proliferating T cells (Tprolif), and regulatory T cells (Treg). Each plot represents the spatial distribution and expression intensity of the respective PIAS gene across these cell types. *P*-value < 0.05.

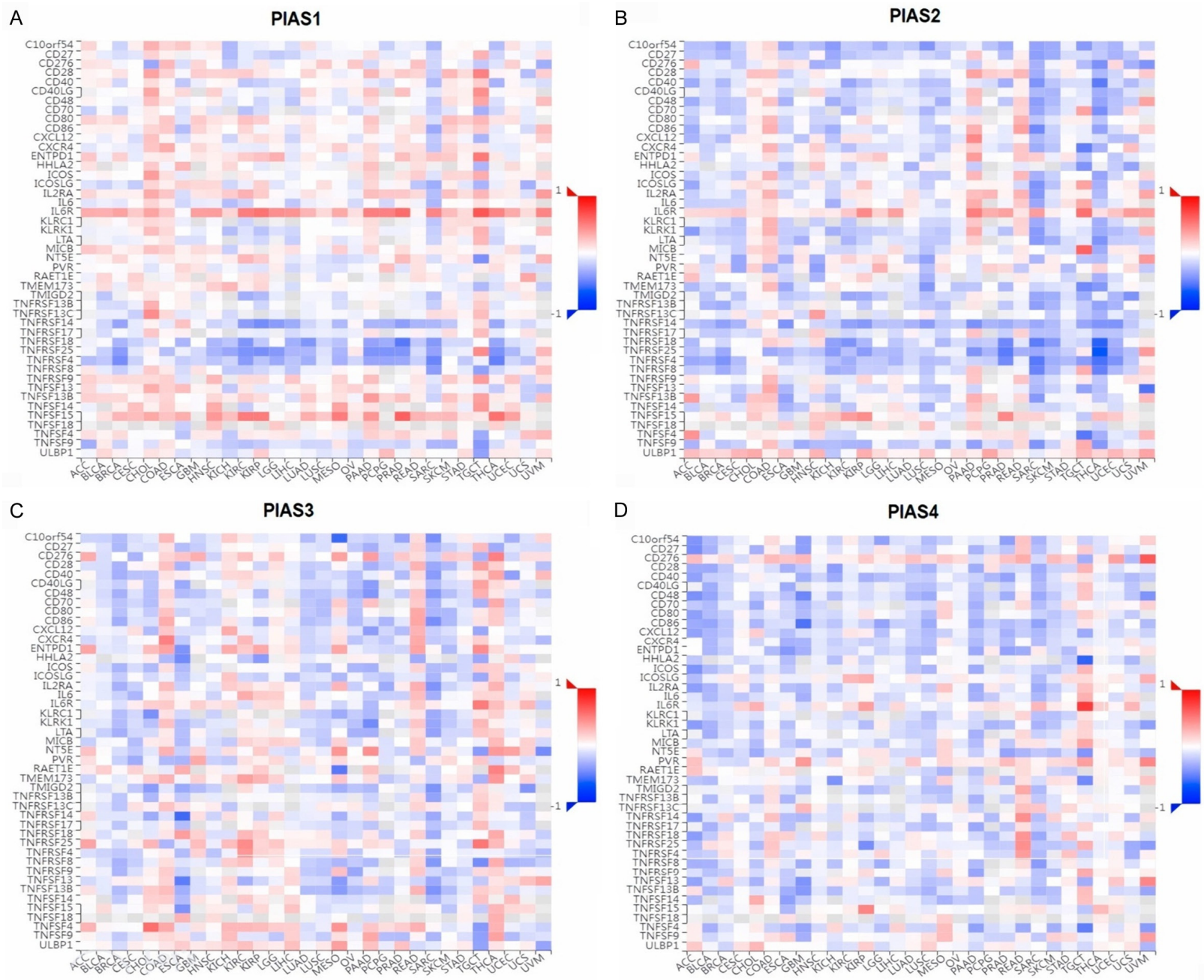

A
PIAS1
B
PIAS2
C
PIAS3
D
PIAS4
C10orf54
CD27
CD276
CD28
CD40
CD40LG
CD48
CD70
CD80
CD86
CXCL12
CXCR4
ENTPD1
HHLA2
ICOS
ICOSLG
IL2RA
IL6
IL6R
KLRC1
KLRK1
LTA
MICB
NT5E
PVR
RAET1E
TMEM173
TMIGD2
TNFRSF13B
TNFRSF13C
TNFRSF14
TNFRSF17
TNFRSF18
TNFRSF25
TNFRSF4
TNFRSF8
TNFRSF9
TNFSF13
TNFSF13B
TNFSF14
TNFSF15
TNFSF18
TNFSF4
TNFSF9
ULBP1
1
-1
ACC BLCA BRCA CESC CHOL COAD ESCA GBM HNSC KICH KIRC KIRP LGG LIHC LUAD LUSC MESO OV PAAD PCPG PRAD READ SARC SKCM STAD TGCT THCA UCEC UCS UVM

Figure 7. Correlation analysis of PIAS family genes with immune stimulator genes across various cancers using TISIDB database. A. Correlation analysis of PIAS1 with immune stimulator genes. B. Correlation analysis of PIAS2 with immune stimulator genes. C. Correlation analysis of PIAS3 with immune stimulator genes. D. Correlation analysis of PIAS4 with immune stimulator genes. The color scale ranges from blue (negative correlation) to red (positive correlation), with the intensity of the color indicating the strength of the correlation. *P*-value < 0.05.

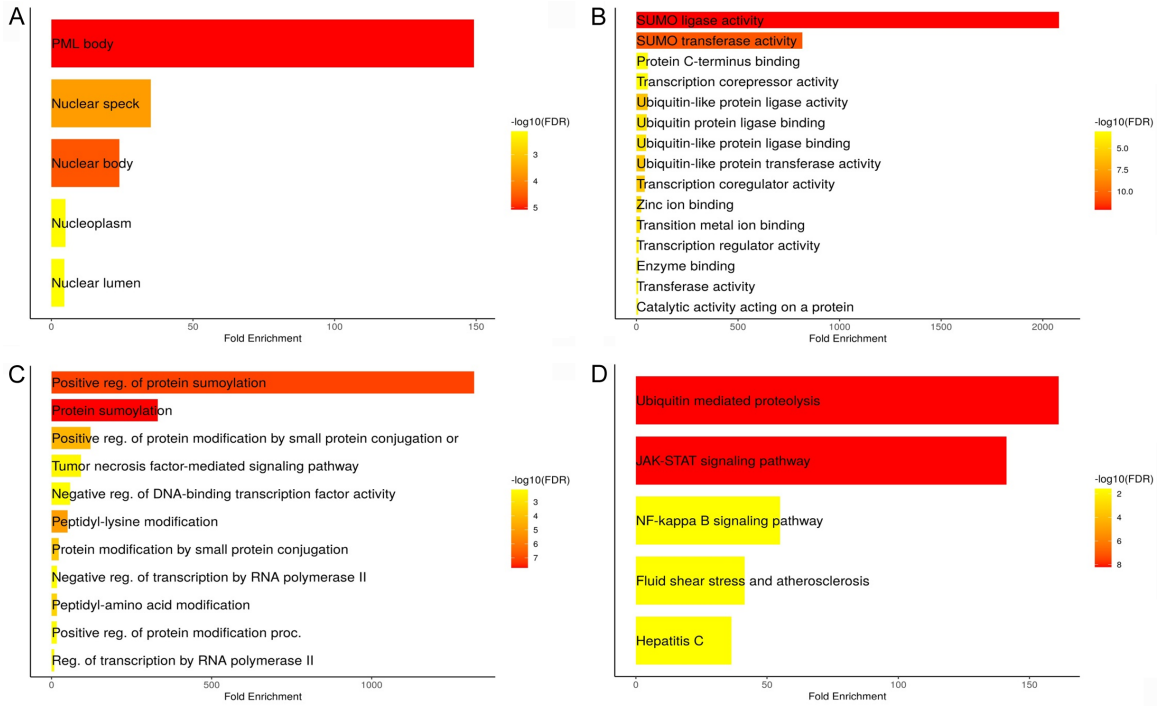


Figure 8. Gene ontology (GO) and KEGG pathway enrichment analysis of PIAS family genes using DAVID tool. A. Cellular components. B. Molecular functions. C. Biological process. D. Pathways. *P*-value < 0.05.

"SUMO ligase activity and SUMO transferase activity, emphasizing the role of PIAS genes in the SUMOylation process". This panel also identifies associations with "transcription corepressor activity and ubiquitin-like protein ligase activities, suggesting a broader involvement in protein modification and gene regulation". **Figure 8C** delineates biological processes, with significant enrichment in positive regulation of "protein SUMOylation, and protein modification by small protein conjugation, underscoring the critical regulatory functions of PIAS genes in post-translational modifications". Lastly, **Figure 8D** highlights pathway enrichment, with notable associations in "ubiquitin-mediated proteolysis and JAK-STAT signaling pathways, implicating PIAS genes in crucial signaling and protein degradation pathways". These enrichment results collectively emphasize the multifaceted roles of PIAS genes in cellular processes, protein modification, and signaling pathways, potentially contributing to their involvement in cancer biology.

Correlation of PIAS family genes with immune cells and drug sensitivity

The correlation analysis of PIAS genes with immune cells and drug sensitivity in KIRP and LIHC was carried out using the GSCA database. In **Figure 9A**, the expression of PIAS genes in KIRP shows significant correlations with various immune cell types. PIAS1 and PIAS4 display a strong negative correlation with CD4+ T cells, CD8+ T cells, and macrophages, suggesting a potential role in immune suppression (**Figure 9A**). Conversely, PIAS2 shows a positive correlation with these immune cells, indicating a possible immunostimulatory effect (**Figure 9A**). PIAS3 has a less pronounced correlation with immune cells compared to other PIAS genes (**Figure 9A**). In **Figure 9B**, examining LIHC, PIAS1, and PIAS4 again demonstrate a strong negative correlation with multiple immune cell types, including Tregs, CD4+ T cells, and macrophages, while PIAS2 exhibits a positive correlation with these cells, similar to its

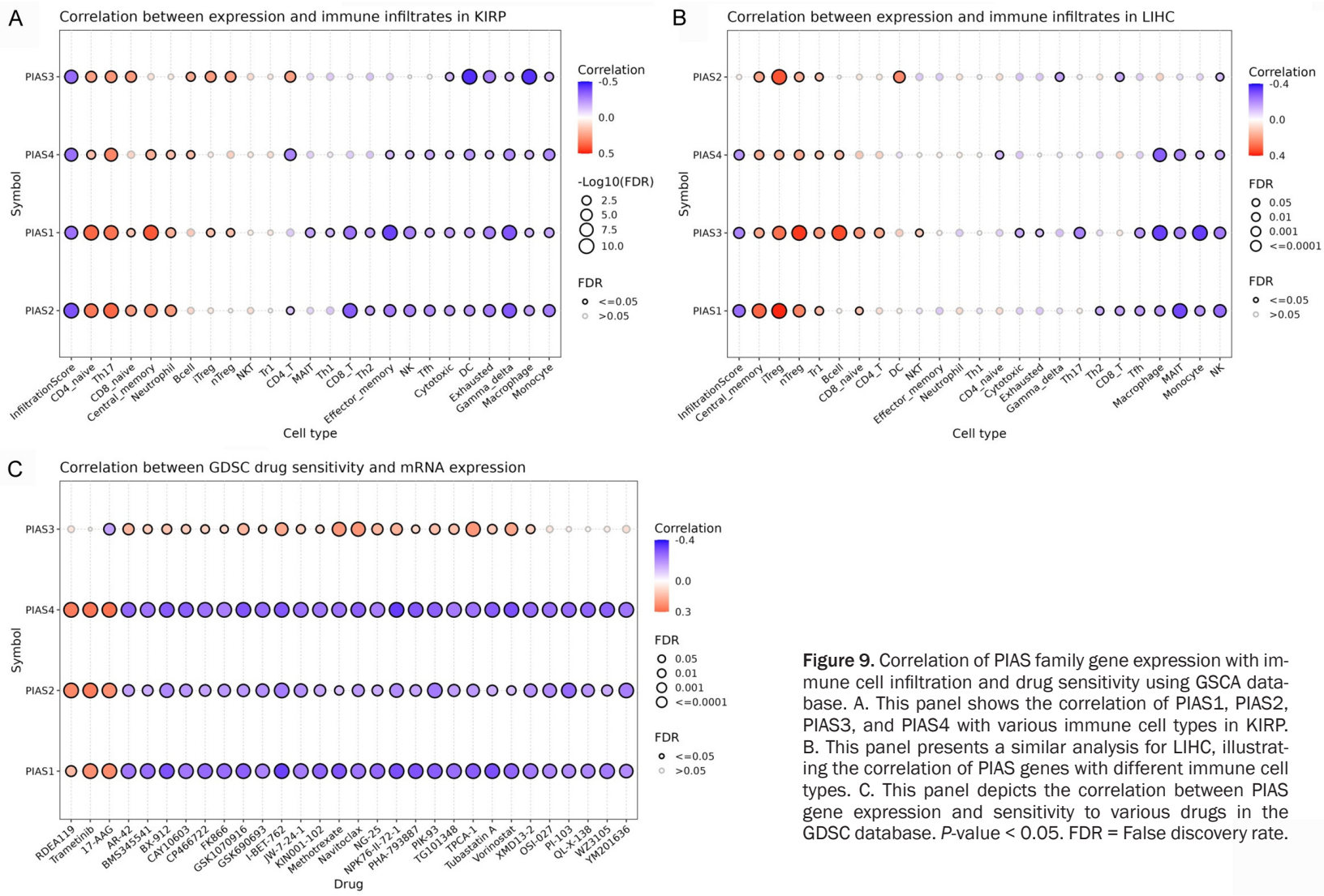


Figure 9. Correlation of PIAS family gene expression with immune cell infiltration and drug sensitivity using GSCA database. A. This panel shows the correlation of PIAS1, PIAS2, PIAS3, and PIAS4 with various immune cell types in KIRP. B. This panel presents a similar analysis for LIHC, illustrating the correlation of PIAS genes with different immune cell types. C. This panel depicts the correlation between PIAS gene expression and sensitivity to various drugs in the GDSC database. *P*-value < 0.05. FDR = False discovery rate.

pattern in KIRP (**Figure 9B**). This suggests a consistent immunoregulatory role for PIAS genes across different cancer types. PIAS3 in LIHC shows a positive correlation with Tregs and a negative correlation with CD8+ T cells, indicating a complex interaction with the immune microenvironment (**Figure 9B**). **Figure 9C** highlights the correlation between PIAS gene expression and drug sensitivity. All PIAS genes show a significant negative correlation with numerous drugs, implying that higher expression of PIAS genes is associated with increased drug resistance. This negative correlation is particularly notable with drugs like Trametinib, TAK-715, and Afatinib, suggesting that PIAS genes may contribute to chemotherapy resistance in cancer treatment. Overall, these results indicate that PIAS genes play critical roles in modulating immune responses and drug sensitivity in KIRP and LIHC, which could have important implications for developing targeted therapies and overcoming drug resistance in these cancers.

Correlation of PIAS family genes expression with tumor metastasis

Figure 10 presents the results of experiments evaluating the role of PIAS2 and PIAS3 genes in tumor metastasis using gene knockdown strategies in HCC-LM3 cells. Gene expression analysis (**Figure 10A**) and Western blotting (**Figure 10B** and Supplementary Figure 1) confirm successful knockdown of PIAS2 and PIAS3, with reduced expression levels and diminished protein bands, respectively. **Figure 10C**, **10D** demonstrates that the Ctrl-HCC-LM3 cells have the highest colony formation, while the knockdown of PIAS2 and PIAS3 results in fewer colonies, suggesting these genes promote tumor growth. **Figure 10E**, **10F** displays cell migration through wound healing assays, with PIAS2-HCC-LM3 and PIAS3-HCC-LM3 conditions showing significantly enhanced wound healing compared to the Ctrl-HCC-LM3 condition, indicating increased cell migration due to gene knockdown. Overall, the results suggest that PIAS2 and PIAS3 genes are crucial in tumor metastasis by promoting colony formation and increasing cell migration, making them potential targets for cancer therapy.

Discussion

Cancer remains a leading cause of morbidity and mortality worldwide, characterized by uncontrolled cell proliferation and the potential for metastasis [27, 28]. The Protein Inhibitor of Activated STAT (PIAS) family, comprising PIAS1, PIAS2, PIAS3, and PIAS4, plays crucial roles in regulating various cellular processes, including transcriptional regulation and post-translational modifications through SUMOylation [11, 29]. Our study provides a comprehensive analysis of the expression, prognostic value, genetic alterations, and functional implications of PIAS family genes across multiple cancer types.

Our results show a consistent up-regulation of PIAS1, PIAS2, PIAS3, and PIAS4 in tumor and metastatic samples across various cancer types compared to normal tissues. This observation aligns with previous studies that have reported the overexpression of PIAS genes in different cancers. For instance, PIAS1 has been shown to be up-regulated in lung adenocarcinoma and gastric cancer, suggesting its role in promoting tumorigenesis [30]. Similarly, PIAS3 overexpression has been linked to poor prognosis in lung and breast cancers [31, 32]. Our data extends these findings by demonstrating the elevated expression of PIAS genes in a broader range of cancers, underscoring their potential as universal biomarkers for cancer progression.

The univariate Cox regression analysis highlights the prognostic significance of PIAS gene expression in KIRP and LIHC. Notably, high expression levels of PIAS1, PIAS2, PIAS3, and PIAS4 correlate with worse overall survival in these cancer types. This is consistent with previous reports where high PIAS1 expression was associated with poor prognosis in prostate cancer [33], and elevated PIAS3 levels were linked to reduced survival in glioblastoma [34]. Our findings reinforce the prognostic value of PIAS genes, particularly in KIRP and LIHC, suggesting that they could serve as valuable biomarkers for patient stratification and targeted therapy.

Our genetic alteration analysis revealed infrequent but notable mutations in PIAS genes, predominantly missense mutations and SNPs. This low frequency of genetic alterations suggests that while PIAS genes are crucial in cancer progression, their involvement might not primarily depend on genetic mutations. Instead, their role could be attributed to aberrant expression or post-translational modifications. This aligns with previous studies that have high-

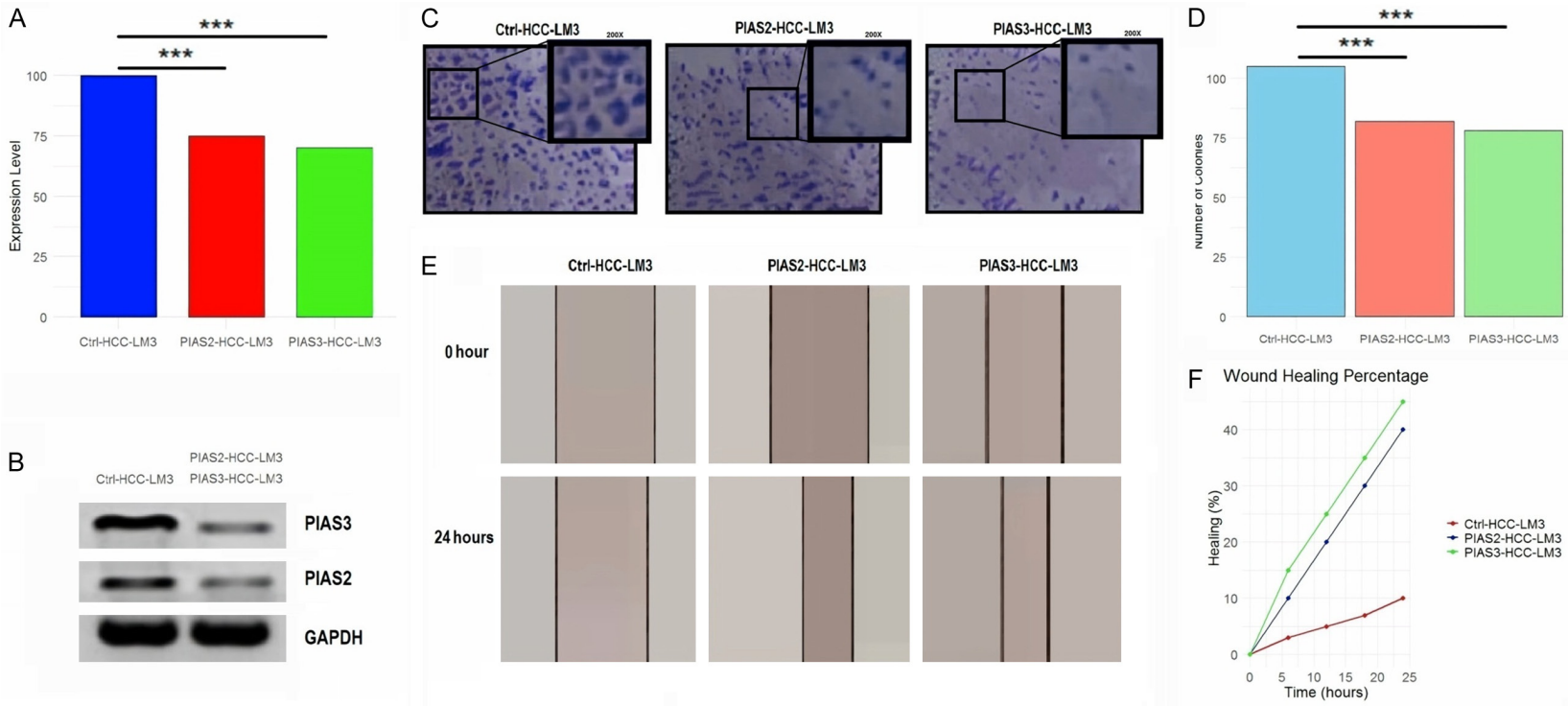


Figure 10. Functional characterization of PIAS2 and PIAS3 in HCC-LM3 cells: gene expression, invasion, colony formation, and wound healing assays. A. This panel shows that the expression levels of PIAS2 and PIAS3 are reduced in transfected cells as compared to the control (Ctrl-HCC-LM3). B. This panel confirms the protein expression of PIAS2 and PIAS3 via Western blot, with GAPDH as a loading control in transfected and control cells. C. This panel illustrates that colony formation is highest in Ctrl-HCC-LM3 cells, with fewer colonies in PIAS2-HCC-LM3 and PIAS3-HCC-LM3 cells. D. This panel depicts colony numbers in graphical form. E. This panel shows wound healing assay images highlighting that PIAS2-HCC-LM3 and PIAS3-HCC-LM3 cells achieve greater wound closure at 24 hours compared to control cells. F. This panel quantifies this effect, with line graphs indicating significantly higher wound healing percentages in PIAS2-HCC-LM3 and PIAS3-HCC-LM3 cells over time. ***P-value < 0.001.

lighted the functional importance of PIAS proteins in cancer through mechanisms such as SUMOylation rather than genetic mutations [35, 36].

Our analysis indicates differential expression of PIAS genes across immune subtypes in KIRP and LIHC, with significant variability, particularly for PIAS1 and PIAS3. These genes showed prominent expression in monocytes/macrophages and malignant cell clusters, suggesting their involvement in modulating the TME. Previous research has demonstrated that PIAS proteins can influence immune responses, such as PIAS3's role in suppressing STAT3 activity, which is crucial for immune evasion in tumors [11, 37]. Our findings suggest that PIAS genes may contribute to shaping the TME by regulating immune cell infiltration and activity, thereby influencing tumor progression and immune escape mechanisms.

Our correlation analysis revealed notable negative associations between PIAS gene expression and key immune stimulatory genes, such as ICOSLG and IL12A, in KIRP and LIHC. This negative correlation suggests that high PIAS expression may dampen immune stimulatory pathways, potentially aiding tumor immune evasion. Additionally, our analysis showed a significant correlation between PIAS gene expression and increased drug resistance, particularly with targeted therapies like Trametinib and Afatinib. These results are consistent with previous findings where PIAS1 was implicated in resistance to chemotherapy in breast cancer by modulating DNA damage repair pathways [38, 39].

The knockdown experiments in HCC-LM3 cells demonstrated that reducing PIAS2 and PIAS3 expression significantly impaired colony formation and cell migration, highlighting their roles in tumor growth and metastasis. This functional evidence aligns with earlier studies where PIAS2 knockdown inhibited cell proliferation and invasion in endometrial cancer cells [40], and PIAS3 silencing reduced metastasis in melanoma models [11]. Our findings provide further support for the critical involvement of PIAS genes in promoting metastasis, suggesting that they could be potential targets for therapeutic intervention to limit cancer spread.

Conclusion

In summary, our study provides a detailed characterization of the PIAS family genes in various cancers, highlighting their overexpression, prognostic value, limited genetic alterations, and significant roles in modulating the tumor microenvironment, immune responses, and drug sensitivity. These findings contribute to the growing body of evidence supporting PIAS genes as crucial players in cancer biology and potential targets for novel therapeutic strategies. Future research should focus on elucidating the precise mechanisms by which PIAS proteins regulate these processes and exploring their potential as biomarkers and targets in cancer treatment.

Disclosure of conflict of interest

None.

Address correspondence to: Zhen Sun, Traditional Chinese Medicine Department, People's Liberation Army Air Force Hangzhou Special Service Recuperation Center, Nanjing 210000, Jiangsu, China. E-mail: 17768612617@163.com

References

[1] Weth FR, Hoggarth GB, Weth AF, Paterson E, White MPJ, Tan ST, Peng L and Gray C. Unlocking hidden potential: advancements, approaches, and obstacles in repurposing drugs for cancer therapy. Br J Cancer 2024; 130: 703-715.

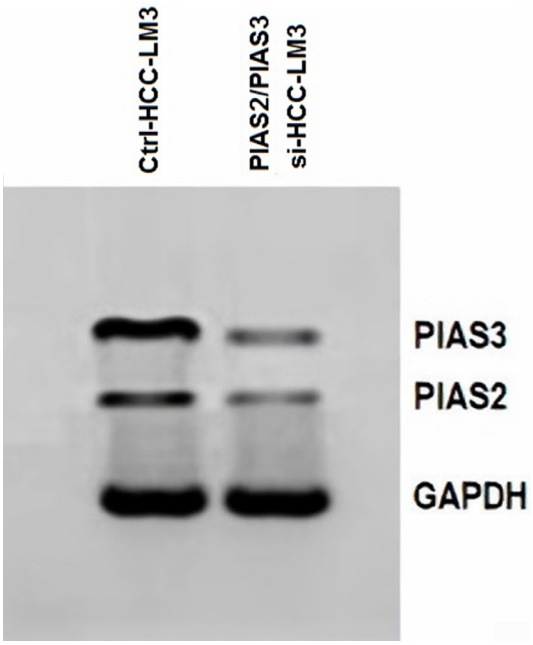
[2] Usman M, Okla MK, Asif HM, AbdElgayed G, Muccee F, Ghazanfar S, Ahmad M, Iqbal MJ, Sahar AM, Khaliq G, Shoaib R, Zaheer H and Hameed Y. A pan-cancer analysis of GINS complex subunit 4 to identify its potential role as a biomarker in multiple human cancers. Am J Cancer Res 2022; 12: 986-1008.

[3] Zhang L, Sahar AM, Li C, Chaudhary A, Yousaf I, Saeedah MA, Mubarak A, Haris M, Nawaz M, Reem MA, Ramadan FA, Mostafa AAM, Feng W and Hameed Y. A detailed multi-omics analysis of GNB2 gene in human cancers. Braz J Biol 2022; 84: e260169.

[4] Xu W, Li H, Hameed Y, Abdel-Maksoud MA, Almutairi SM, Mubarak A, Aufy M, Alturaiki W, Alshalani AJ, Mahmoud AM and Li C. Elucidating the clinical and immunological value of m6A regulator-mediated methylation modification patterns in adrenocortical carcinoma. Oncol Res 2023; 31: 819-831.

[5] Chhikara BS and Parang K. Global cancer statistics 2022: the trends projection analysis. Chem Biol Lett 2023; 10: 451.
[6] Batis N, Brooks JM, Payne K, Sharma N, Nankivell P and Mehanna H. Lack of predictive tools for conventional and targeted cancer therapy: barriers to biomarker development and clinical translation. Adv Drug Deliv Rev 2021; 176: 113854.
[7] Pal M, Muinao T, Boruah HPD and Mahindroo N. Current advances in prognostic and diagnostic biomarkers for solid cancers: detection techniques and future challenges. Biomed Pharmacother 2022; 146: 112488.
[8] Park H, Lee S, Lee J, Moon H and Ro SW. Exploring the JAK/STAT signaling pathway in hepatocellular carcinoma: unraveling signaling complexity and therapeutic implications. Int J Mol Sci 2023; 24: 13764.
[9] Chuang KT, Chiou SS and Hsu SH. Recent advances in transcription factors biomarkers and targeted therapies focusing on epithelial-mesenchymal transition. Cancers (Basel) 2023; 15: 3338.
[10] Lao M, Shi M, Zou Y, Huang M, Ye Y, Qiu Q, Xiao Y, Zeng S, Liang L and Yang X. Protein inhibitor of activated STAT3 regulates migration, invasion, and activation of fibroblast-like synoviocytes in rheumatoid arthritis. J Immunol 2016; 196: 596-606.
[11] Li X, Rasul A, Sharif F and Hassan M. PIAS family in cancer: from basic mechanisms to clinical applications. Front Oncol 2024; 14: 1376633.
[12] Zheng X, Wang L, Zhang Z and Tang H. The emerging roles of SUMOylation in pulmonary diseases. Mol Med 2023; 29: 119.
[13] Robinson NJ, Miyagi M, Scarborough JA, Scott JG, Taylor DJ and Schiemann WP. SLX4IP promotes RAP1 SUMOylation by PIAS1 to coordinate telomere maintenance through NF-κB and Notch signaling. Sci Signal 2021; 14: eabe9613.
[14] Zhang G, Hou S, Li S, Wang Y and Cui W. Role of STAT3 in cancer cell epithelial-mesenchymal transition. Int J Oncol 2024; 64: 1-19.
[15] Liu Y, Li Y and Lu X. Regulators in the DNA damage response. Arch Biochem Biophys 2016; 594: 18-25.
[16] Hu H, Umair M, Khan SA, Sani AI, Iqbal S, Khalid F, Sultan R, Abdel-Maksoud MA, Mubarak A, Dawoud TM, Malik A, Saleh IA, Al Amri AA, Algarzae NK, Kodous AS and Hameed Y. CDCA8, a mitosis-related gene, as a prospective pancancer biomarker: implications for survival prognosis and oncogenic immunology. Am J Transl Res 2024; 16: 432-445.
[17] Sial N, Rehman JU, Saeed S, Ahmad M, Hameed Y, Atif M, Rehman A, Asif R, Ahmed H, Hussain MS, Khan MR, Ambreen A and Ambreen A. Integrative analysis reveals methylenetetrahydrofolate dehydrogenase 1-like as an independent shared diagnostic and prognostic biomarker in five different human cancers. Biosci Rep 2022; 42: BSR20211783.
[18] Rabellino A, Andreani C and Scaglioni PP. The role of PIAS SUMO E3-ligases in cancer. Cancer Res 2017; 77: 1542-1547.
[19] Brantley EC, Nabors LB, Gillespie GY, Choi YH, Palmer CA, Harrison K, Roarty K and Benveniste EN. Loss of protein inhibitors of activated STAT-3 expression in glioblastoma multiforme tumors: implications for STAT-3 activation and gene expression. Clin Cancer Res 2008; 14: 4694-4704.
[20] Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi BVSK and Varambally S. UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia 2017; 19: 649-658.
[21] Bartha Á and Győrffy B. TNMplot.com: a web tool for the comparison of gene expression in normal, tumor and metastatic tissues. Int J Mol Sci 2021; 22: 2622.
[22] Lánczky A and Győrffy B. Web-based survival analysis tool tailored for medical research (KMplot): development and implementation. J Med Internet Res 2021; 23: e27633.
[23] Liu CJ, Hu FF, Xie GY, Miao YR, Li XW, Zeng Y and Guo AY. GSCA: an integrated platform for gene set cancer analysis at genomic, pharmacogenomic and immunogenomic levels. Brief Bioinform 2023; 24: bbac558.
[24] Ru B, Wong CN, Tong Y, Zhong JY, Zhong SSW, Wu WC, Chu KC, Wong CY, Lau CY, Chen I, Chan NW and Zhang J. TISIDB: an integrated repository portal for tumor-immune system interactions. Bioinformatics 2019; 35: 4200-4202.
[25] Han Y, Wang Y, Dong X, Sun D, Liu Z, Yue J, Wang H, Li T and Wang C. TISCH2: expanded datasets and new tools for single-cell transcriptome analyses of the tumor microenvironment. Nucleic Acids Res 2023; 51: D1425-D1431.
[26] Sherman BT, Hao M, Qiu J, Jiao X, Baseler MW, Lane HC, Imamichi T and Chang W. DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Res 2022; 50: W216-W221.
[27] Nairuz T, Mahmud Z, Manik RK and Kabir Y. Cancer stem cells: an insight into the development of metastatic tumors and therapy resistance. Stem Cell Rev Rep 2023; 19: 1577-1595.
[28] Liu J, Luo F, Wen L, Zhao Z and Sun H. Current understanding of microbiomes in cancer metastasis. Cancers (Basel) 2023; 15: 1893.

[29] Li C, Boutet A, Pascariu CM, Nelson T, Courcelles M, Wu Z, Comtois-Marotte S, Emery G and Thibault P. SUMO proteomics analyses identify protein inhibitor of activated STAT-mediated regulatory networks involved in cell cycle and cell proliferation. J Proteome Res 2023; 22: 812-825.
[30] Constanzo JD, Tang KJ, Rindhe S, Melegari M, Liu H, Tang X, Rodriguez-Canales J, Wistuba I and Scaglioni PP. PIAS1-FAK interaction promotes the survival and progression of non-small cell lung cancer. Neoplasia 2016; 18: 282-293.
[31] Ogata Y, Osaki T, Naka T, Iwahori K, Furukawa M, Nagatomo I, Kijima T, Kumagai T, Yoshida M, Tachibana I and Kawase I. Overexpression of PIAS3 suppresses cell growth and restores the drug sensitivity of human lung cancer cells in association with PI3-K/Akt inactivation. Neoplasia 2006; 8: 817-825.
[32] Yang SF, Hou MF, Chen FM, Ou-Yang F, Wu YC, Chai CY and Yeh YT. Prognostic value of protein inhibitor of activated STAT3 in breast cancer patients receiving hormone therapy. BMC Cancer 2016; 16: 20.
[33] Puhr M, Hoefer J, Eigentler A, Dietrich D, Van Leenders G, Uhl B, Hoogland M, Handle F, Schlick B, Neuwirt H, Sailer V, Kristiansen G, Klocker H and Culig Z. PIAS1 is a determinant of poor survival and acts as a positive feedback regulator of AR signaling through enhanced AR stabilization in prostate cancer. Oncogene 2016; 35: 2322-2332.
[34] Li X and Meng Y. SUMOylation regulator-related molecules can be used as prognostic biomarkers for glioblastoma. Front Cell Dev Biol 2021; 9: 658856.
[35] Rabellino A and Khanna KK. The implication of the SUMOylation pathway in breast cancer pathogenesis and treatment. Crit Rev Biochem Mol Biol 2020; 55: 54-70.
[36] Sharrocks AD. PIAS proteins and transcriptional regulation--more than just SUMO E3 ligases? Genes Dev 2006; 20: 754-758.
[37] Pan X, Feng J, Zhu Z, Yao L, Ma S, Hao B and Zhang G. A positive feedback loop between miR-181b and STAT3 that affects Warburg effect in colon cancer via regulating PIAS3 expression. J Cell Mol Med 2018; 22: 5040-5049.
[38] Zhang Q, Zhang J, Lan T, He J, Lei B, Wang H, Mei Z and Lv C. Integrative analysis revealed a correlation of PIAS family genes expression with prognosis, immunomodulation and chemotherapy. Eur J Med Res 2024; 29: 195.
[39] Zhang FL, Yang SY, Liao L, Zhang TM, Zhang YL, Hu SY, Deng L, Huang MY, Andriani L, Ma XY, Shao ZM and Li DQ. Dynamic SUMOylation of MORC2 orchestrates chromatin remodelling and DNA repair in response to DNA damage and drives chemoresistance in breast cancer. Theranostics 2023; 13: 973-990.
[40] Xiao Y, Huang W, Huang H, Wang L, Wang M, Zhang T, Fang X and Xia X. miR-182-5p and miR-96-5p target PIAS1 and mediate the negative feedback regulatory loop between PIAS1 and STAT3 in endometrial cancer. DNA Cell Biol 2021; 40: 618-628.



Supplementary Figure 1. Representative uncut western blot analysis bands showing the expression levels of PIAS2 and PIAS3 proteins in HCC-LM3 cells. The Ctrl-HCC-LM3 lane represents control HCC-LM3 cells. The PIAS2-HCC-LM3 and PIAS3-HCC-LM3 lanes represent transfected HCC-LM3 cells. GAPDH was used as a loading control.