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Research article

An integrative pan-cancer bioinformatics analysis of MSRB1 and its association with tumor immune microenvironment, prognosis, and immunotherapy

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ABSTRACT

Methionine sulfoxide reductase B1 (MSRB1) is involved in the development and immune regulation of multiple tumors. However, the role of MSRB1 in the tumor microenvironment and its potential as a therapeutic target remain largely unknown. In this study, MSRB1 expression patterns were evaluated using pan-cancer RNA sequencing data from multiple cell lines, tissues, and single cells. The pan-cancer prognostic role of MSRB1 was assessed and the association between MSRB1 expression and certain cancer characteristics was analyzed. We showed that MSRB1 expression levels were increased in several types of cancer ($P < 0.05$) and in certain cell types (macrophages, dendritic cells, and malignant tumor cells). The upregulation of MSRB1 expression was due to DNA copy number amplification. Furthermore, MSRB1 was significantly associated with the activation of immune pathways ($P < 0.05$, $NES > 0$), immune cell infiltration, and expression of immune checkpoint molecules. In addition, high expression of MSRB1 was found in a series of in vivo and in vitro immunotherapy response models ($P < 0.05$), and showed resistance to most targeted drugs. Our results indicated that MSRB1 may regulate the tumor immune microenvironment through an immunoresponse and potentially influence cancer development. This could make it a promising predictive biomarker and therapeutic target for precise tumor immunotherapy.

1. Introduction

Cancer is the primary cause of premature death and reduces life expectancy worldwide [1]. According to recent studies, there were approximately 4,820,000 and 2,370,000 new cancer cases and 3,210,000 and 640,000 cancer deaths in China and the USA, respectively, in 2022 [2,3]. New diagnostic methods and treatment strategies are urgently required to improve the quality of life and survival of patients with cancer [4]. Immunotherapy has become firmly established as a novel cornerstone of cancer treatment [5,6], which mainly benefits from the discovery of immune checkpoint molecules in the regulation of tumor microenvironment (TME). However, it

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is vital to acknowledge that the existing checkpoint molecules and their targeted drugs may not adequately predict or enhance immunotherapy efficacy. For example, the efficacy of PD-1/PD-L1 inhibitors targeting typical immune checkpoint molecules varies between 10% and 30% in common solid tumors [7]. Therefore, it is important to search for new immune checkpoint molecules and immunotherapeutic targets that are effective in the development and progression of tumor immunotherapy [8].

Methionine sulfoxide reductase B1 (MSRB1) is a selenoprotein that catalyzes the production of methionine (Met) from free and protein-bound R-methionine sulfoxide (*R*-MetO) [9,10]. Selenium participates widely in biological processes, and individual Se status can influence pathological conditions related to the immune system [11]. The discovery of the selenoprotein MSRB1 revealed that it is a novel regulator of macrophage immune function stimulated by LPS, which favors the release of the anti-inflammatory cytokines IL-10 and IL-1RA [12]. Concurrently, the role of MSRB1 has been demonstrated in some cancers, and it has been shown that MSRB1 gene knockdown inhibited the migration and division of human osteosarcoma epithelial (u2os) cells [13] and hepatocellular carcinoma cells [13], as well as the growth of colon cancer cells [9]. Furthermore, we previously demonstrated that MSRB1 promotes the proliferation and invasion of colorectal cancer cells via the GSK-3β/β-catenin signaling axis [9]. However, it remains unclear whether MSRB1 regulates the occurrence and development of cancer by influencing the immune system.

In this study, we examined alterations in MSRB1 expression at the cellular, tissue, and single-cell levels, as well as genomic variations, and its prognostic value in patients with tumors using large-scale RNA sequencing (RNA-seq) data from public databases. Significant associations among MSRB1 expression, immune regulatory-related pathways, and immune cell infiltration in different TMEs were explored. Additionally, the potential predictive value of MSRB1 for immunotherapy response in patients with cancer was evaluated using in vivo and in vitro immunotherapy models. Our results provide insights into immunotherapeutic strategies for MSRB1 targeted therapy.

2. Materials and Methods

2.1. Gene expression analysis

To determine the expression level of MSRB1 in various organ cell lines, RNA-seq data were obtained from the Cancer Cell Line Encyclopedia (CCLE) (https://sites.broadinstitute.org/ccle/) database. Additionally, uniformly normalized pan-cancer samples and normal tissue samples data (TPM matrix) based on The Cancer Genome Atlas (TCGA), Therapeutically Applicable Research to Generate Effective Treatments (TARGET) and Genotype Tissue Expression (GTEx) were downloaded from the UCSC-Xena (https://xenabrowser.net/) database (N = 19131). Simultaneously, the count matrices for each cancer from TCGA database were downloaded for subsequent genetic difference analysis. We further performed a log 2 (TPM+1) transformation of the expression matrix and extracted the expression data for ENSG00000198736 (MSRB1) in each sample. Finally, we excluded cancer species with fewer than three samples from a single cancer species and obtained the expression data for 34 cancer species. The UALCAN tool (https://ualcan.path.uab.edu/analysis-prot.html) was used to perform protein expression analysis based on the Clinical Proteomics Tumor Analysis Consortium (CPTAC) (https://proteomics.cancer.gov/programs/cptac). The GEPIA2 (http://gepia2.cancer-pku.cn/#analysis) tool was used to analyze gene expression at different pathological stages in all TCGA cancers.

2.2. Single-cell expression analysis

To investigate the expression levels of MSRB1 across various cell types, single-cell RNA sequencing (scRNA-seq) data analysis was performed using the TISCH2 (http://tisch.comp-genomics.org/home/) tool [14], which included 190 datasets with a total of 6,297, 320 cells and 47 cancer subtypes. Single-cell expression profile data for MSRB1 were downloaded directly from TISCH2 and visualized using the R package pheatmap. Uniform Manifold Approximation and Projection (UMAP) analysis of individual cancers was performed online using TISCH2.

2.3. Gene prognostic analysis

Gene expression and clinical data from TCGA Pan-Cancer in UCSC-Xena were extracted individually, and samples with a follow-up duration of less than 30 days were excluded. Additionally, cancer species with fewer than ten samples from a single cancer were removed. The coxph function of the R package survival was used to construct a Cox proportional hazards regression model [15] to analyze the effect of MSRB1 expression on patient prognosis for each tumor. The Kaplan–Meier method was used to verify the impact of MSRB1 on the survival of patients with different cancers using the GEPIA2 online database. The median or quartile expression of MSRB1 was set as the expression threshold to divide patients into high- and low-expression cohorts. Statistical tests were performed using the log-rank test to assess the prognostic significance.

2.4. Genome variation analysis

We performed mutation and copy number variation analyses of MSRB1 in all TCGA tumors using the cBioPortal tool (https://www.cbioportal.org/). At the same time, we assessed the survival of patients with or without MSRB1 genome alteration. Log-rank ($P < 0.05$) was considered a significant effect of MSRB1 genome alterations on the survival of patients with cancer.

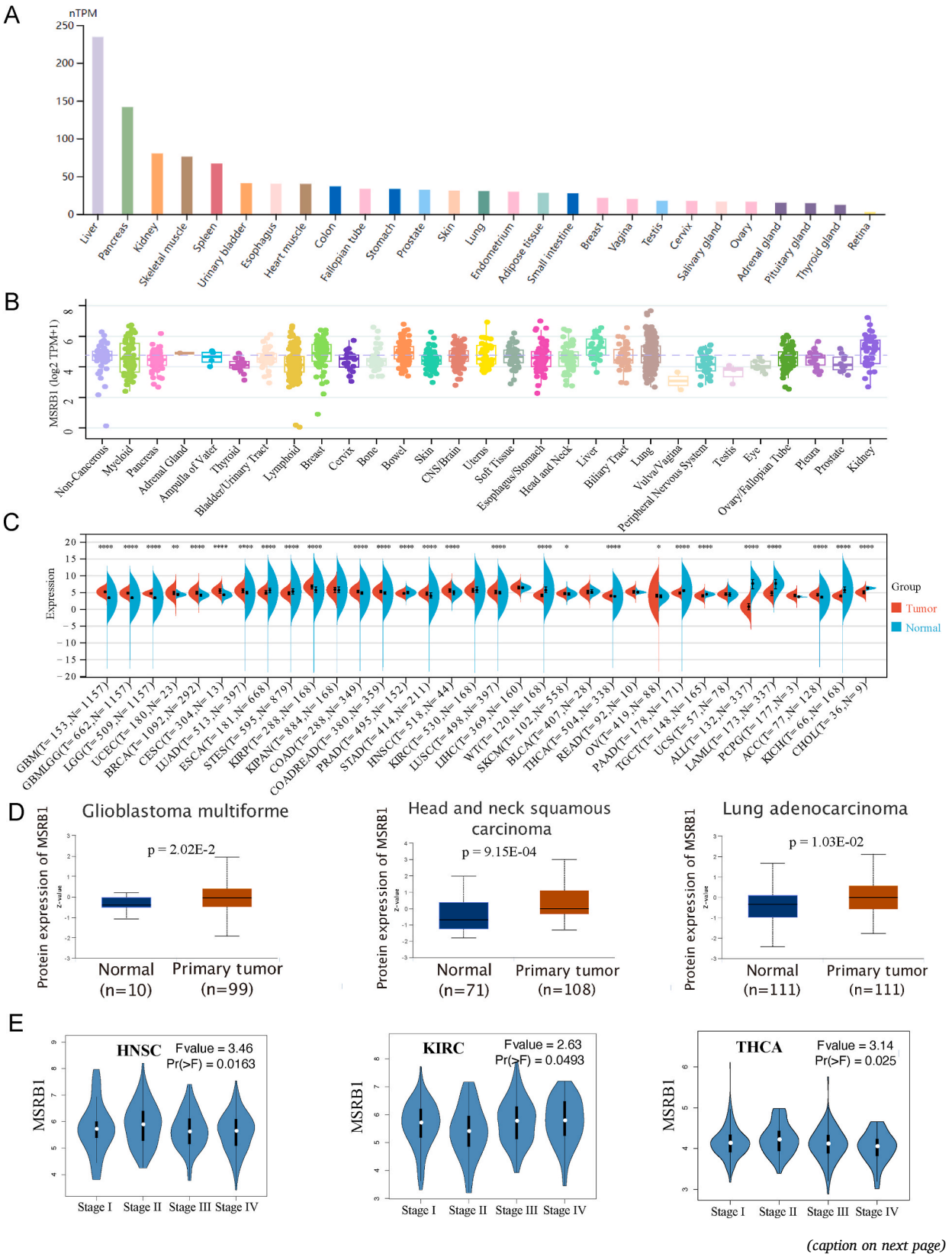

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Fig. 1. Differential expression of methionine sulfoxide reductase B1 (MSRB1) in normal and tumor tissues. **(A)** Expression Levels of MSRB1 in 27 human tissues (GTEx). **(B)** Expression Levels of MSRB1 in non-tumor and 28 tumor cell lines (CCLE). **(C)** The expression levels of MSRB1 were analyzed in both normal and tumorous tissues of each cancer type using the combined data from The Cancer Genome Atlas (TCGA) and GTEx datasets. **(D)** The protein expression level of MSRB1 in glioblastoma multiforme (GBM), lung adenocarcinoma (LUAD), and head and neck squamous cell carcinoma (HNSC) tumor tissues was compared to that in control tissues. The analysis was performed using the UALCAN database. **(E)** The correlation between the differential expression of MSRB1 and the pathological stages of HNSC, kidney renal clear cell carcinoma (KIRC), and thyroid carcinoma (THCA) was analyzed using GEPIA2. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

2.5. Gene set enrichment analysis

Gene set enrichment analysis (GSEA) was performed using the R package cluster Profiler [16]. First, each cancer sample was grouped according to median MSRB1 expression. Differential analysis (based on the count matrix) was conducted using the edgeR package, in which all the genes were sorted by logFC and used as the input gene set for GSEA. Fifty cancer hallmark gene sets were obtained from the MsigDB database (https://www.gsea-msigdb.org/gsea/msigdb) as background gene sets (H: hallmark gene sets) for the GSEA analysis. Finally, the “GSEA" function of clusterProfiler was used for analysis, and pathways with a false discovery rate (FDR) < 0.05 were considered significantly enriched. We extracted the normalized enrichment score (*NES*) values and FDR calculated for each hallmark of each cancer for heat mapping.

2.6. Analysis of immune cell infiltration

The R package IOBR [17] was used to assess the infiltration of immune cells into the cancer samples. Five algorithms, including TIMER [18], QUANTIS-EQ [19], EPIC [20], MCPcounter [21] and Cibersort [22] were used to calculate the correlation between MSRB1 expression and immune cell infiltration, and $P < 0.05$ was considered significant.

2.7. Correlation analysis

The expression data for 60 immune checkpoint-related genes [23] in individual tumor samples were extracted. Next, we obtained the microsatellite instability (MSI) score for each tumor from a previous study [24] and single-nucleotide variant data for level 4 of all TCGA samples processed using Mu-Tect2 software [25] were downloaded from GDC (https://portal.gdc.cancer.gov/). The “tmb" function of the R package maftools was used to calculate the tumor mutation burden (TMB) for each patient. Spearman’s correlations were then calculated between MSRB1 and the immune checkpoint genes, MSI, and TMB ($P < 0.05$ was considered significant).

2.8. Evaluation of immune response and targeted drugs

TISMO (http://tismo.cistrome.org) was used to evaluate the therapeutic efficacy of MSRB1 in vivo mouse models receiving immune checkpoint blockade (ICB) treatment and in vitro cell models treated with cytokines. Drug-gene association analysis based on GDSC [26] was performed using the RNAactDrug (http://bio-bigdata.hrbmu.edu.cn/RNAactDrug/) tool employing Spearman correlation analysis and drugs with FDR <0.01 were chosen. The strong correlation coefficient (r) suggests that heightened MSRB1 expression typically implies increased tolerance to the drug.

2.9. RNA isolation and quantitative real time PCR (qRT-PCR) analysis

Total RNA was isolated from cultured cells using TRIzol reagent (AG21101, Accurate Biology, China), according to the manufacturer’s instructions. RNA was reverse-transcribed using the ABScript II cDNA First-Strand Synthesis Kit (RK20400, ABclonal, Wuhan, China) to generate first-strand cDNA. qRT-PCR experiments were performed using DNA Engine Opticon 2 (MJ Research, Watertown, MA, USA). 2X Universal SYBR Green Fast qPCR Mix kit (RK21203; ABclonal, Wuhan, China) was used for PCRs. The following primers were used: GAPDH forward 5′-CAGTGCCAGCCTCGTCCCGTAGA-3′ and reverse 5′-CTGCAAATGGCAGCCCTGGTGAC-3′; MSRB1 forward 5′-GACGTTACACCCTCACCTT-3′ and reverse 5′-AGCTACTTCCGCACAGATT-3′. The $2^{-\Delta\Delta Ct}$ method was used to analyze the mRNA expression levels of target genes in the control and experimental groups.

2.10. Immunohistochemistry (IHC)

Colorectal cancer tissue microarray slides (BC05118c) were purchased from Alenabio Bioscience Corporation (Xi’an, China). Anti-MSRB1 (sc-135558; Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used for staining.

2.11. Statistical analysis

Statistical analysis and data visualization were performed using R (version 4.1.1), GraphPad softwares and Sangerbox [27]. Comparisons between the two groups were performed using the Wilcoxon rank-sum test [28] and Student’s t-test. Statistical significance was set at $P < 0.05$, and is indicated as follows: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$. *P*-values were adjusted using the Benjamin–Hochberg method.

3. Results

3.1. Differences in the expression of MSRB1 in samples from normal and cancer tissue

First, to provide a complete description of MSRB1 expression levels in both normal and cancerous samples, RNA-seq data from three publicly available samples were analyzed. Tissue RNA-seq analysis revealed that MSRB1 was expressed in 27 normal tissues, with the top five tissues showing high MSRB1 expression in the liver, pancreas, kidney, skeletal muscle, and spleen (Fig. 1A). Cell line RNA-seq analysis showed differential expression profiles of MSRB1 in non-tumor and 28 tumor cell lines (Fig. 1B), with the highest expression of MSRB1 in hepatocytes and renal tumor cell lines. In addition, qRT-PCR experiments showed that MSRB1 expression was higher in colorectal tumor cells than in colorectal epithelial cells. This result is consistent with that of our previous RNA-seq analysis and further supports the differential expression of MSRB1 in tumor cells and normal cells (Fig. S1). Comparison on MSRB1 transcriptome level in tumor and normal tissue samples shows significant upregulation (Wilcoxon rank-sum test, $P < 0.05$) was observed in 17 tumors including glioblastoma multiforme low-level glioma (GBMLGG), uterine corpus endometrial carcinoma (UCEC), breast cancer (BRCA), cervical and endocervical cancer (CESC), lung adenocarcinoma (LUAD), kidney papillary cell carcinoma (KIRP), colon adenocarcinoma/rectum adenocarcinoma esophageal carcinoma (COADREAD), stomach adenocarcinoma (STAD), head and neck squamous cell carcinoma (HNSC), lung squamous cell carcinoma (LUSC), skin cutaneous melanoma (SKCM), thyroid carcinoma (THCA), ovarian cancer (OV), adenoid cystic carcinoma (ACC), colon adenocarcinoma (COAD), glioblastoma multiforme (GBM), lower grade glioma (LGG) and a significant downregulation (Wilcoxon rank-sum test, $P < 0.05$) was observed in 10 tumors such as esophageal carcinoma (ESCA), stomach and esophageal carcinoma (STES), prostate adenocarcinoma (PRAD), Wilms tumor (WT), pancreatic adenocarcinoma (PAAD), testicular germ cell tumors (TGCT), acute lymphocytic leukemia (ALL), acute myeloid leukemia (LAML), kidney chromophobe (KICH), cholangiocarcinoma (CHOL) (Fig. 1C). The protein expression level of MSRB1 was significantly higher in the primary tumor tissues of GBM, HNSC, and LUAD than in normal tissues (Wilcoxon rank-sum test, $P < 0.05$), which was

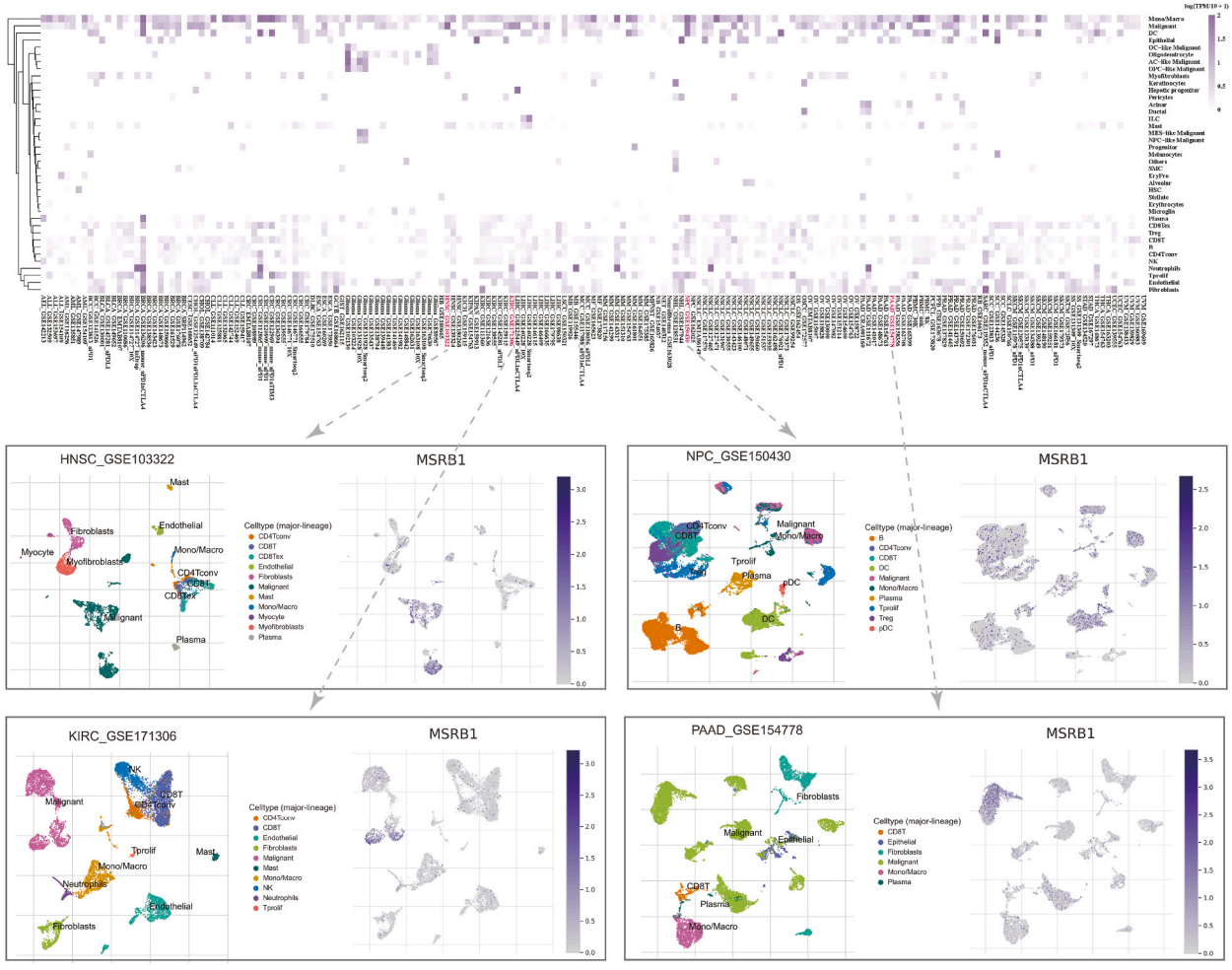

Fig. 2. Single-cell expression of MSRB1 in multiple cancer cohorts. **(A)** Heatmap showing the expression levels of MSRB1 in different cell types in multiple cancer cohorts. **(B)** UMAP plots showing significant MSRB1 expression in malignant cells from the selected cancer cohorts.

consistent with the mRNA expression level of MSRB1 (Fig. 1D). Due to the lack of data related to colorectal cancer in the database, we performed IHC experiments on a colorectal cancer tissue microarray slide (containing tumor and adjacent normal tissue). Moreover, we observed elevated expression levels of the MSRB1 protein in colorectal tumor tissues by IHC assay (Fig. S2). This result consolidated the trend of high expression of MSRB1 in colorectal cancer. In addition, there was a significant association between MSRB1 expression and different pathological stages of HNSC, kidney renal clear cell carcinoma (KIRC), THCA [ANOVA, Pr (>F) < 0.05] (Fig. 1E).

Further analysis of the expression of MSRB1 in various cell types obtained from cancer samples using scRNA-seq showed that MSRB1 was highly expressed in monocytes/macrophages, malignant cells, and dendritic cells (DCs), indicating that MSRB1 might participate in cell-mediated TME regulation. In contrast, the significant upregulation and expression of MSRB1 in malignant cells of

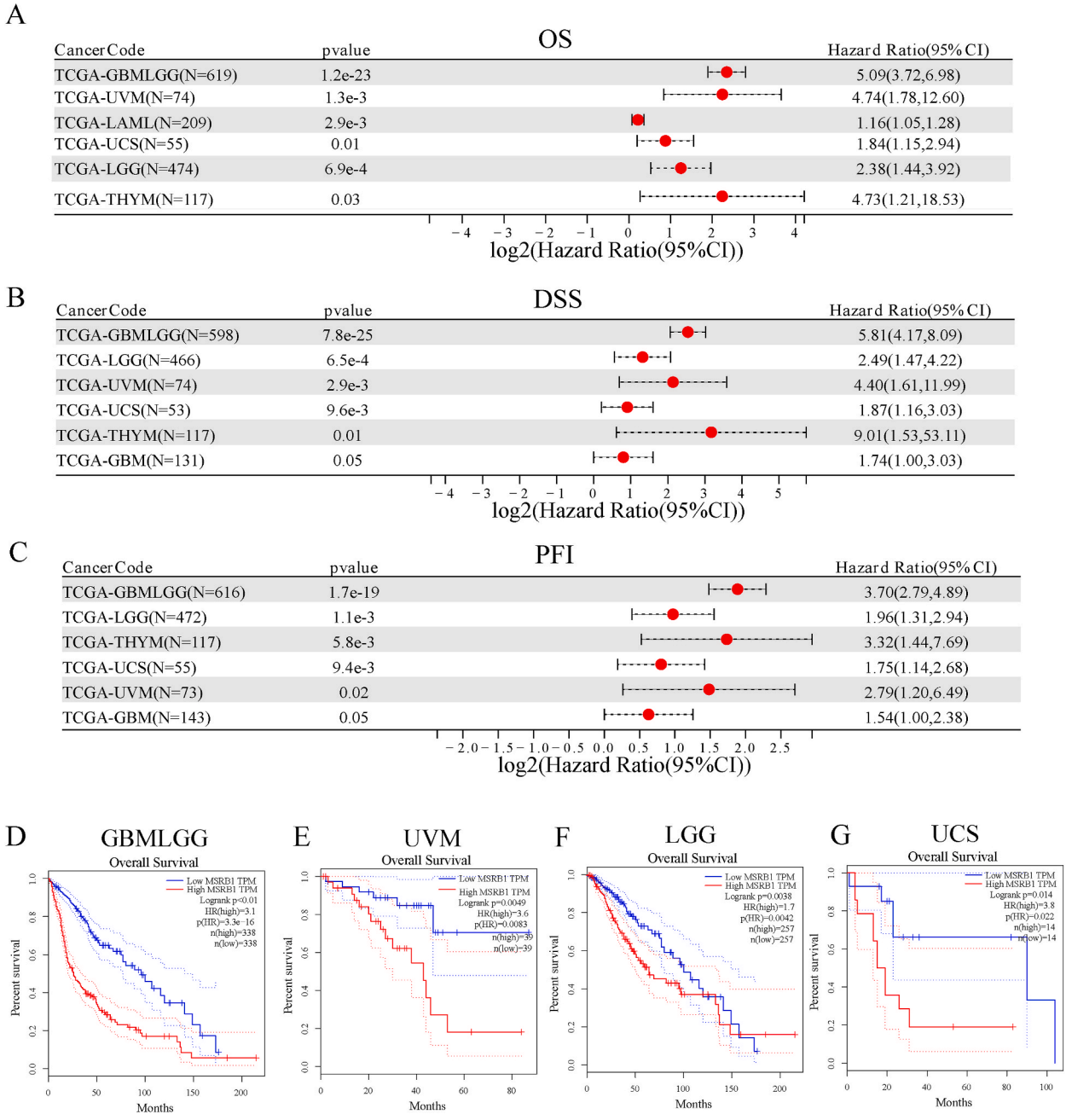

Fig. 3. Correlation analysis between MSRB1 expression level and survival in patients with cancer. **(A–C)** Forest plots show the prognostic effect of MSRB1 on **(A)** overall survival (OS), **(B)** disease-specific survival (DSS), **(C)** progression-free interval (PFI). **(D–G)** Kaplan–Meier overall survival curves of MSRB1 in **(D)** glioblastoma multiforme low-level glioma (GBMLGG), **(E)** uveal melanoma (UVM), **(F)** lower grade glioma (LGG), and **(G)** uterine carcinosarcoma (UCS). Only *P*-values <0.05 are shown.

HNSC, nasopharyngeal carcinoma (NPC), KIRC, and PAAD suggested that MSRB1 may play an important role in the development of these tumors (Fig. 2).

3.2. High MSRB1 expression predicts worse survival in cancer patients

Survival and univariate Cox analyses were performed to evaluate the prognostic significance of MSRB1 expression in tumors. The results showed that high MSRB1 expression was usually associated with worse overall survival (OS) in patients with GBMLGG, LGG, uveal melanoma (UVM), uterine carcinosarcoma, LAML, and thymoma (THYM) (Cox $P < 0.05$, $HR > 1$) (Fig. 3A). Moreover, the predictive role of MSRB1 expression in poor disease-specific survival (DSS) (Cox $P < 0.05$, $HR > 1$) (Fig. 3B) and progression-free survival (PFS) (Cox $P < 0.05$, $HR > 1$) (Fig. 3C) was confirmed in patients with cancer, suggesting the impact of MSRB1 in the development of specific cancers. The association between OS and MSRB1 expression in patients with GBMLGG (Log-rank $P < 0.001$), UVM (Log-rank $P = 0.0049$), LGG (Log-rank $P = 0.0038$), and uterine carcinosarcoma (UCS) (Log-rank $P = 0.012$) was further validated using the GEPIA2 online database (Fig. 3D, E, 3F, 3G).

3.3. Genome variation affects the expression of MSRB1

Genomic variation in MSRB1 may be responsible for the changes in its expression. To understand which cancers may be affected by

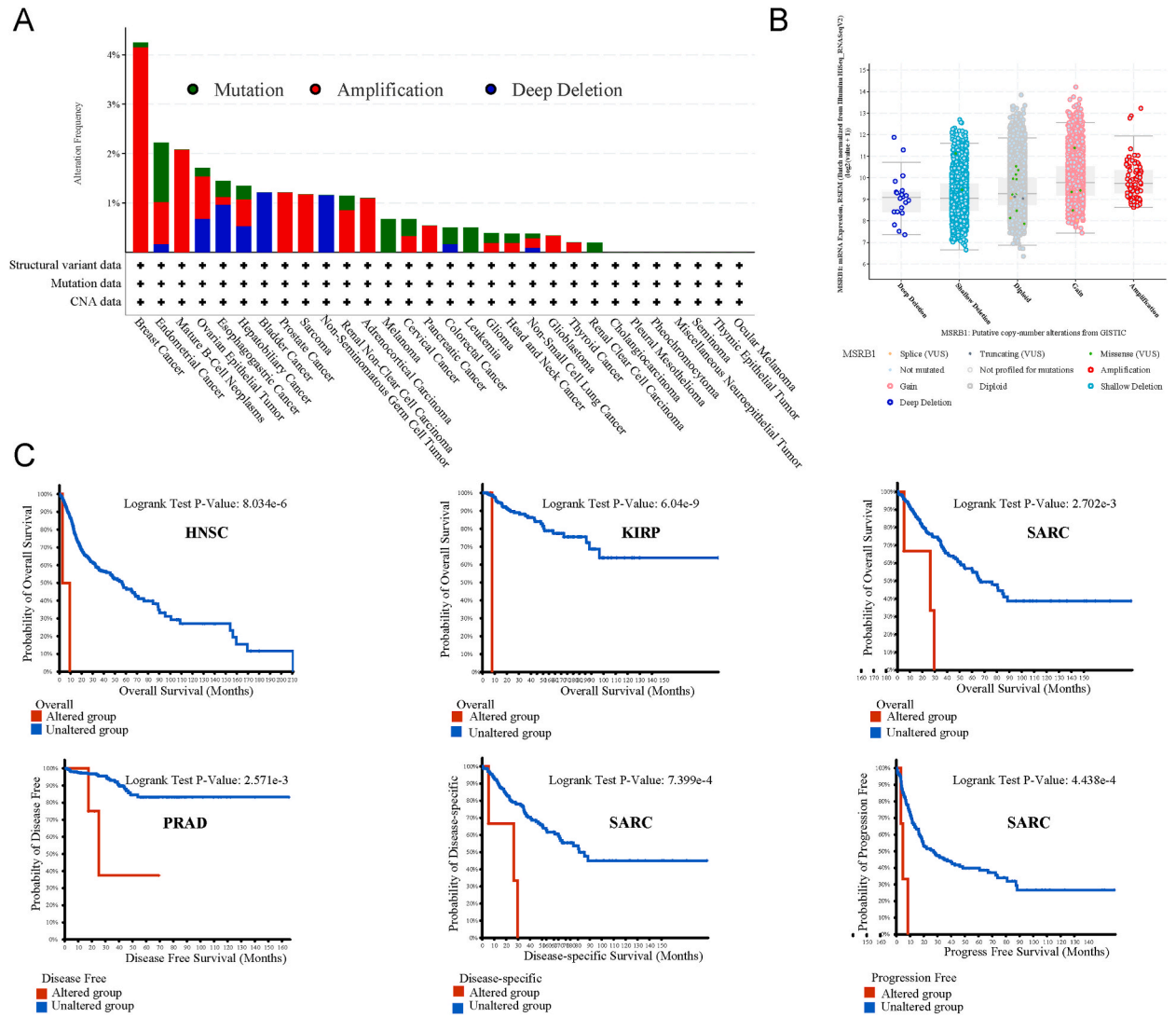

Fig. 4. Genome variation analysis of MSRB1 using the cBioPortal. **(A)** Summary of the alteration frequencies for different mutation types in various cancers. **(B)** Relationship between copy number changes in MSRB1 and mRNA expression levels. **(C)** The prognostic value of MSRB1 genome variation was analyzed by the Kaplan–Meier curves of OS, DFS, DSS, and PFI. Only *P*-values <0.05 are shown.

genomic variations in MSRB1, we further analyzed the frequency of mutations, amplifications, and deep deletions of MSRB1 in different cancers. The results showed that BRCA had the highest amplification frequency of MSRB1 (approximately 4%), bladder carcinoma (BLCA) had the highest deep deletion frequency (1.2%), and endometrial cancer (EC) had the highest mutation frequency (1.2%) (Fig. 4A). Meanwhile, the analysis revealed that MSRB1 expression gradually increased with increasing copy number, suggesting that copy number amplification is the main cause of the elevated expression of MSRB1 in most cancers (Fig. 4B).

Additionally, potential correlations between MSRB1 alterations and prognosis of all TCGA cancer types were identified. The results showed that patients with sarcoma with MSRB1 variations showed worse OS (Log-rank $P = 2.702e\text{-}3$), DSS (Log-rank $P = 7.399e\text{-}4$), and PFS (Log-rank $P = 4.538e\text{-}4$) than those in the unaltered group. MSRB1 genome-altered HNSC (Log-rank $P = 8.034e\text{-}6$) and KIRP (Log-rank $P = 6.04e\text{-}9$) patients showed poorer survival in terms of OS, and PRAD (Log-rank $P = 2.571e\text{-}3$) patients showed poorer clinical outcomes in terms of disease free survival. These results indicated that the genomic variation of MSRB1 was different in predicting the survival of different cancer patients, suggesting its predictive value at different cancer progression levels (Fig. 4C).

3.4. *MSRB1 activates pathways related to the immune response*

To uncover the biological processes associated with MSRB1 in cancer, GSEA was conducted on 33 cancer types to assess MSRB1

Fig. 5. Hallmark gene set enrichment analysis (GSEA) of MSRB1 in pan-cancer. The size of the circle represents the false discovery rate (FDR) value of each cancer enrichment item, the square represents the FDR value < 0.05, and the color represents the normalized enrichment score (*NES*) of each enrichment item.

related cancer features. The results indicated that immune-related pathways, including inflammatory response, TNFA signaling via NF-κB, interferon-α and interferon-γ responses, and allograft rejection, were significantly enriched in a variety of tumors (FDR <0.05), especially ACC, BLCA, CESC, lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), LGG, and mesothelioma (MESO). High MSRB1 expression in these cancers promoted the activation of these pathways (*NES* > 0) (Fig. 5), suggesting that MSRB1 may promote the occurrence and development of cancer by affecting the TME. Additionally, we observed that MSRB1 promoted the activation of the epithelial-mesenchymal transition pathway in ACC, ESCA, LGG, and OV, while inhibiting the activation of this pathway in KIRP, LAML, and liver hepatocellular carcinoma (LIHC), suggesting that MSRB1 may play different roles in the invasion and migration of different tumors. These results provided evidence that abnormal MSRB1 expression is involved in immune dysregulation and

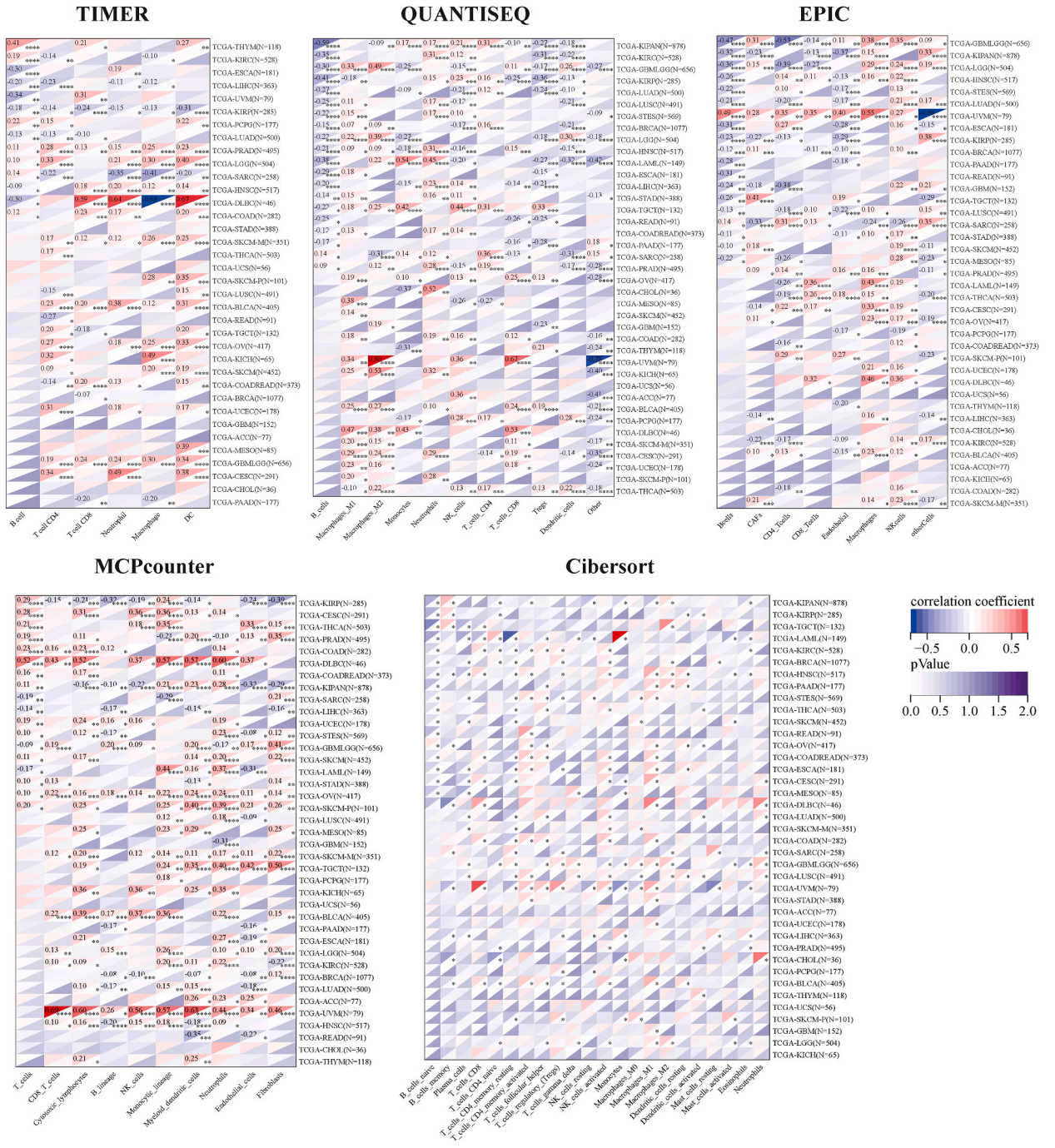

Fig. 6. Correlation analysis between the MSRB1 expression and immune cell infiltration in cancers. Positive correlation is shown in red and negative correlation is shown in blue. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

tumorigenesis.

3.5. MSRB1 positively correlated with the immune infiltration levels

Based on the aforementioned results, we analyzed the correlation between MSRB1 and immune cell infiltration using five immune cell infiltration inference algorithms based on bulk RNA-seq. The results indicated a positive correlation between MSRB1 and the infiltration levels of numerous immune cells, including macrophages, natural killer (NK) cells, neutrophils, $CD8^+$ T cells, and $CD4^+$ T cells, in most cancers, particularly UVM, BLCA, and HNSC. This aligns with the results of single-cell analysis. Notably, the expression level of MSRB1 was negatively correlated with B cells in ESCA, LIHC, KIRP, and HNSC, indicating a potential regulatory association

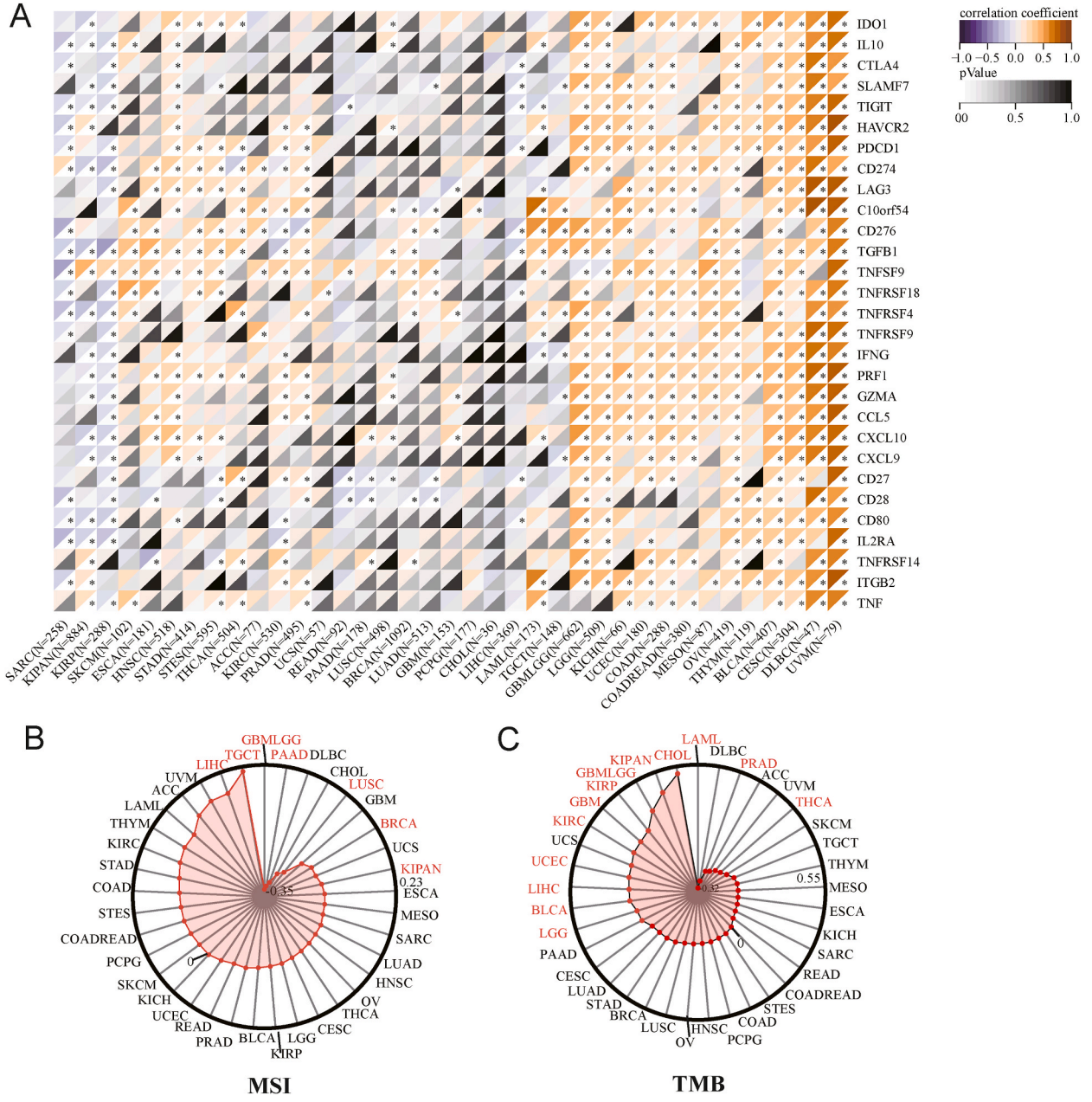

Fig. 7. Correlation analysis between MSRB1 expression levels and immune checkpoint genes, microsatellite instability (MSI) and tumor mutation burden (TMB). **(A)** The Spearman correlation heatmap shows the correlation between the expression levels of MSRB1 and immune checkpoint genes in pan-cancer. **(B)** Correlation analysis between the MSRB1 expression levels and MSI in pan-cancer. **(C)** Correlation analysis between the MSRB1 expression levels and TMB in pan-cancer. For cancers shown in red, $P < 0.05$.

between MSRB1 and B cells in TME (Fig. 6).

3.6. Significant association between MSRB1 and immune checkpoint genes, MSI and TMB

Based on these findings, MSRB1 expression may affect the efficacy of immunotherapy. Therefore, a correlation analysis was conducted, and the results revealed that MSRB1 was positively correlated with most immune checkpoint molecules in GBMLGG, KICH, UCEC, colon adenocarcinoma (COAD), COADREAD, MESO, OV, THYM, BLCA, CESC, DLBC, and UVM. This suggested that patients with high MSRB1 expression responded better to immunotherapy for specific cancers (Fig. 7A). To enhance our understanding of the influence of MSRB1 on the prediction of the effectiveness of immune checkpoint inhibitor therapy (ICI), we evaluated the correlation between MSRB1 expression and TMB and MSI, two widely recognized predictive biomarkers of immunotherapy. High TMB and MSI have been found to predict better immunotherapeutic outcomes in patients with certain tumors [29,30]. The results showed that MSRB1 expression positively correlated with MSI in LIHC and TGCT (Fig. 7B). The expression of MSRB1 was positively correlated with TMB in LGG, BLCA, LIHC, UCEC, KIRC, GBM, KIRP, GBMLGG, pan-kidney cohort (KIPAN), and CHOL (Fig. 7C). The heterogeneous presentation of MSRB1 in different cancers allows for personalized drug treatment strategies for patients.

3.7. High expression of MSRB1 promotes tumor response to immunotherapy

We first analyzed the guiding value of MSRB1 for pan-cancer immunotherapy based on in vivo and in vitro models, and found that the models responding to ICB treatment usually had higher MSRB1 expression in the in vivo mouse models of SKCM, COADREAD, BRCA, LUAD, and STAD (Fig. 8A). Moreover, MSRB1 expression was associated with cytokines in the in vitro BRCA, PAAD, and LUAD cell models (Fig. 8B). Additionally, we analyzed the correlation between MSRB1 expression and the IC50 values of 198 targeted drugs in GDSC and found a significant positive correlation between MSRB1 expression and 53 targeted drugs (Fig. 8C), suggesting that high

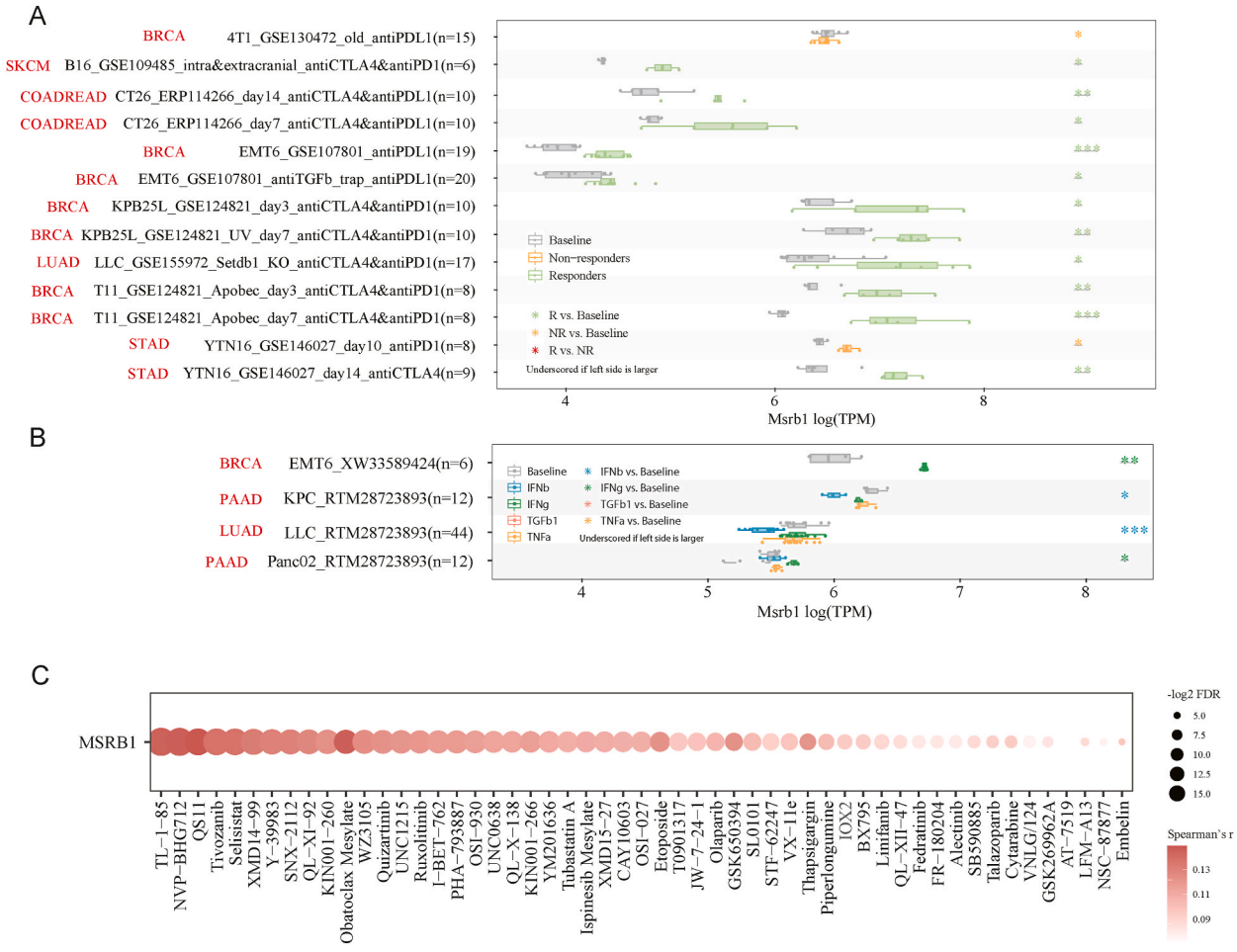

Fig. 8. Immunotherapy value of MSRB1 in pan-cancer. **(A)** Immunotherapy response of MSRB1 in immunotherapy cohorts based on in vivo studies. **(B)** Immunotherapy response of MSRB1 in immunotherapy cohorts based on in vitro studies. **(C)** Correlation between MSRB1 expression and targeted drugs from RNAactDrug datasets.

MSRB1 expression may predict higher targeted drug resistance. In most cancers, patients exhibiting high MSRB1 expression may be better suited for immunotherapy, but respond poorly to targeted therapy.

A comprehensive summary of the pan-cancer characterization of MSRB1 is presented in Supplementary Table S1. A more detailed summary of the bioinformatic tools and sources used in our work is presented in Supplementary Table S2.

4. Discussion

In this study, a comprehensive pan-cancer bioinformatic analysis of MSRB1 was performed to explore its role of MSRB1 in cancer development and prognosis. MSRB1 was overexpressed in multiple cancer types based on tissue samples and aggregated in macrophages, DCs, and malignant tumor cells based on single-cell data. MSRB1 has prognostic predictive abilities in several cancers. In addition, MSRB1 was significantly associated with the activation of immune pathways, immune cell infiltration, and the expression of immune checkpoint molecules. Furthermore, MSRB1 was highly expressed in a series of in vivo and in vitro immunotherapy response models. However, high MSRB1 expression results in resistance to most targeted drugs. By regulating the TME, MSRB1 seems to play a role as both a predictive biomarker and a therapeutic target for tumor immunotherapy. Our findings provide persuasive indications for subsequent research on the plausible function of MSRB1 in tumor immunity and immunotherapy.

First, the expression of MSRB1 was analyzed in 27 normal tissues, non-tumor tissues, and 28 tumor cell lines. In addition, 19131 samples from both normal and tumor tissue samples from TCGA were analyzed. The results revealed that MSRB1 was significantly upregulated in 17 cancer types, downregulated in 10 cancer types, and more likely to be highly expressed in LIHC, KIRP, BRCA, and COAD. This bidirectional trend implies that MSRB1 may function either as an oncogene or as an antitumor gene depending on the specific cancer type. Previous studies have corroborated the oncogene-related function of MSRB1, which has been reported that MSRB1 can promote proliferation and invasion of colon cancer, hepatocellular carcinoma, and U2OS cells [9]. In addition, GSEA results showed that reactive oxygen species and oxidative phosphorylation pathways were correlated with MSRB1 expression, suggesting that MSRB1 may be involved in cancer development by influencing the state of oxidative stress [31]. The expression of MSRB1 in patients with different cancer types was investigated using scRNA-seq. MSRB1 was found to be highly expressed in monocytes/macrophages, malignant cells, and DCs throughout the analysis in most cancers.

High levels of MSRB1 expression have been observed in various immunocytes (monocytes/macrophages, DCs) and malignant cells, indicating that MSRB1 participates in immune responses during tumorigenesis and the malignant progression of cancer. This analysis is consistent with the results of several previous studies. MSRB1 has been reported to act as a novel regulator of immune function in LPS-stimulated macrophages, promoting the secretion of the anti-inflammatory cytokines IL-10 and IL-1RA [12]. Another study reported in DCs, MSRB1 may regulate adaptive immune responses by regulating antigen presentation and influencing the production of co-stimulatory molecules and cytokines [32]. Based on these findings, along with our results, it appears that MSRB1 has a strong influence on immunocytes. This influence affects immune cells, which subsequently impact the immune system and participate in tumorigenesis and the malignant development of cancer.

Gene structure, expression, and their relationship with clinical situations will be analyzed in future studies. The gene structure of MSRB1 was barely altered, and gene expression increased significantly as the copy number increased, suggesting that copy number amplification of MSRB1 contributes to its elevated expression. Genome alterations in MSRB1, although with a lower probability, were associated with worse prognosis. An analysis based on TNM stage also reflects a similar point, as MSRB1 expression correlates with TNM stage. In the survival analysis based on bulk RNA-seq, we found that patients with high MSRB1 expression in GBMLGG, UVM, LGG, and UCS had a poorer clinical prognosis, suggesting that MSRB1 is a powerful prognostic biomarker for these cancer patients. A similar report revealed that high expression of MSRB1 in tumor tissues, and increased in expression were associated with worse OS in patients with hepatocellular carcinoma after hepatectomy. An in vitro study also reported that the knockdown of MSRB1 inhibits HCC cell growth [33]. Moreover, MSRB1 may also affect the phosphorylation of p53, the most critical antitumor gene, thereby affecting cancer [33]. Regarding p53, our GSEA analysis suggested that MSRB1 acts as a hallmark of the p53 pathway. GSEA results also predicted that subgroups with high MSRB1 expression were positively associated with the activation of immune reaction-related processes. Previous reports also hold the same point that MSRB1 has the potential to be a hallmark of the inflammatory response, TNFA signaling via NF-κB, interferon-α response, and interferon-γ response, all of which are closely associated with immunotherapy efficacy and prognosis [34,35].

The correlation between MSRB1 expression and immune checkpoints was also analyzed. The results showed that MSRB1 expression was positively correlated with most immune checkpoint genes in GBMLGG, KICH, UCEC, COAD, COADREAD, MESO, OV, THYM, BLCA, CESC, DLBC, and UVM. Immune checkpoint proteins not only help tumor cells escape host immune surveillance [36] but also cause immunological agent resistance [37]. MSRB1 expression is positively correlated with immune checkpoint genes in these cancers, indicating that MSRB1 is a potential immunotherapy target.

From an exogenous perspective, tumor immunity is regulated by infiltrating inflammatory cells in the TME, which are crucial for tumorigenesis and metastasis [38,39]. A positive correlation was observed between MSRB1 and the infiltration levels of multiple immune cells such as macrophages, NK cells, neutrophils, $CD8^+$ T cells, and $CD4^+$ T cells, particularly in UVM, BLCA, and HNSC. These results suggest that by affecting the composition of immune cells in the TME, MSRB1 may influence tumor development while elevating immunotherapy efficacy. With reference to clinical applications, we evaluated the therapeutic potential of MSRB1 in pan-cancer using in vivo and in vitro models. A high MSRB1 expression generally results in a more sensitive immune response. According to previous guidelines [40], we believe that, in most cancers, patients with high MSRB1 expression may be more suitable for immunotherapy. It should be noted that more cancer-specific large cohort data and further phenotypic and functional experiments are needed to confirm the results of bioinformatics analysis.

5. Conclusions

In conclusion, by systematically analyzing pan-cancer MSRB1 expression, possible associations between MSRB1 expression and clinical prognosis, pathological stage, TMB/MSI, immune cell infiltration, immune checkpoints, and immunotherapy efficacy were determined. Based on this analysis, MSRB1 can be considered as a potential pan-cancer biomarker for prognosis and immunotherapy.

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Ethics approval and consent to participate

Not applicable.

Data availability statement

The datasets we analyzed during the current study are available from the public repositories, as described in the "Materials and Methods" section. And also all data related to this study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Shanshan Jiang: Writing – original draft, Methodology, Investigation. **Shengyong Yang:** Writing – review & editing, Supervision, Funding acquisition. **Zhengdan Gao:** Resources. **Chuan Yin:** Investigation. **Mengmeng Zhang:** Software. **Qian Wu:** Resources. **Yi Li:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: YI Li, Shengyong Yang reports financial support was provided by The National Natural Science Foundation of China, the Natural Science Foundation of Chongqing grants, The Science and Technology Planning Project of Yuzhong District, Chongqing City. If there are other authors, they 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

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