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Pan-cancer analysis of the prognostic and immunological role of matrix metalloproteinase 9

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Abstract

Matrix metalloproteinase 9 (MMP9), a zinc ion-dependent endopeptidase, is one of the most complex matrix metalloproteinases in the gelatinase family. During tissue remodeling, MMP9 leads to gelatin and collagen degradation, which in turn promotes tumor invasion and metastasis. However, comprehensive pan-cancer analysis has not been performed for MMP9. In addition, the diagnostic and prognostic value of MMP9 as a cancer biomarker remain poorly understood, as well as the utility of MMP9 expression as a predictor of immunological responses. Based on a comprehensive analysis of bioinformatics information, we investigated MMP9 expression in different cancers, correlations between MMP9 expression and cancer prognosis and gene mutations, and relationships between MMP9 expression and immune cell infiltration. Our results indicated that MMP9 was highly expressed in most malignant cancers. MMP9 expression was significantly positively or negatively associated with the clinical prognoses of patients with different kinds of cancer. Furthermore, MMP9 expression significantly correlated with infiltrating cells and the expression levels of immune checkpoint genes. This pan-cancer analysis provides comprehensive information on the potential value of MMP9 as a theranostic biomarker.

Abbreviations: ACC = adrenocortical carcinoma, BLCA = bladder urothelial carcinoma, BRCA = breast invasive cancer, CHOL = cholangiocarcinoma, COAD = colon adenocarcinoma, DSS = disease-specific survival, ECM = extracellular matrix, ESCA = esophageal carcinoma, GBM = glioblastoma multiforme, HNSC = head and neck squamous cell carcinoma, HPA = The Human Protein Atlas, HRs = hazard ratios, KIRC = kidney renal clear cell carcinoma, KIRP = kidney renal papillary cell carcinoma, LIHC = hepatocellular carcinoma, LUAD = lung adenocarcinoma, LUSC = lung squamous cell carcinoma, MMP9 = matrix metalloproteinase 9, MMPs = matrix metalloproteinases, MSI = microsatellite instability, OS = overall survival, OV = ovarian serous cystadenocarcinoma, PAAD = pancreatic adenocarcinoma, SARC = sarcoma, SKCM = skin cutaneous melanoma, TCGA = The Cancer Genome Atlas, THCA = thyroid carcinoma, TMB = tumor mutation burden, TME = tumor microenvironment, UCEC = uterine corpus endometrial carcinoma, UVM = uveal melanoma.

Keywords: bioinformatics, immune cell infiltration, matrix metalloproteinase 9, pan-cancer, prognosis

1. Introduction

The prevalence of cancer has been increasing over the years, making it a major public health problem.[1] Currently, cancer-treatment strategies primarily include surgery, radiation, chemotherapy, hormone therapy, immunotherapy, and targeted therapy. Despite the success of these treatments, the prognoses and survival rates of patients remains unsatisfactory due to difficulties in detecting tumors at an early stage and high recurrence rates following treatment.[2,3] Recently, great advances have been made in cancer immunotherapy, such as cellular immunotherapy, that is, CD19-targeted chimeric antigen receptor T cell therapy[4,5] and programmed death-1 and programmed death ligand-1 inhibitors.[6] Moreover, the rapid development of cancer gene-information databases, such as The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus, can help researchers analyze genetic changes during tumorigenesis and immune cell infiltration, thereby improving cancer diagnosis and treatment.

Tumorigenesis is a progressive process that includes oncogene activation and the inhibition of tumor-suppressor genes, which cause mutations to accumulate.[7,8] When tumors spread to surrounding and distal areas, they encounter barriers that cannot be

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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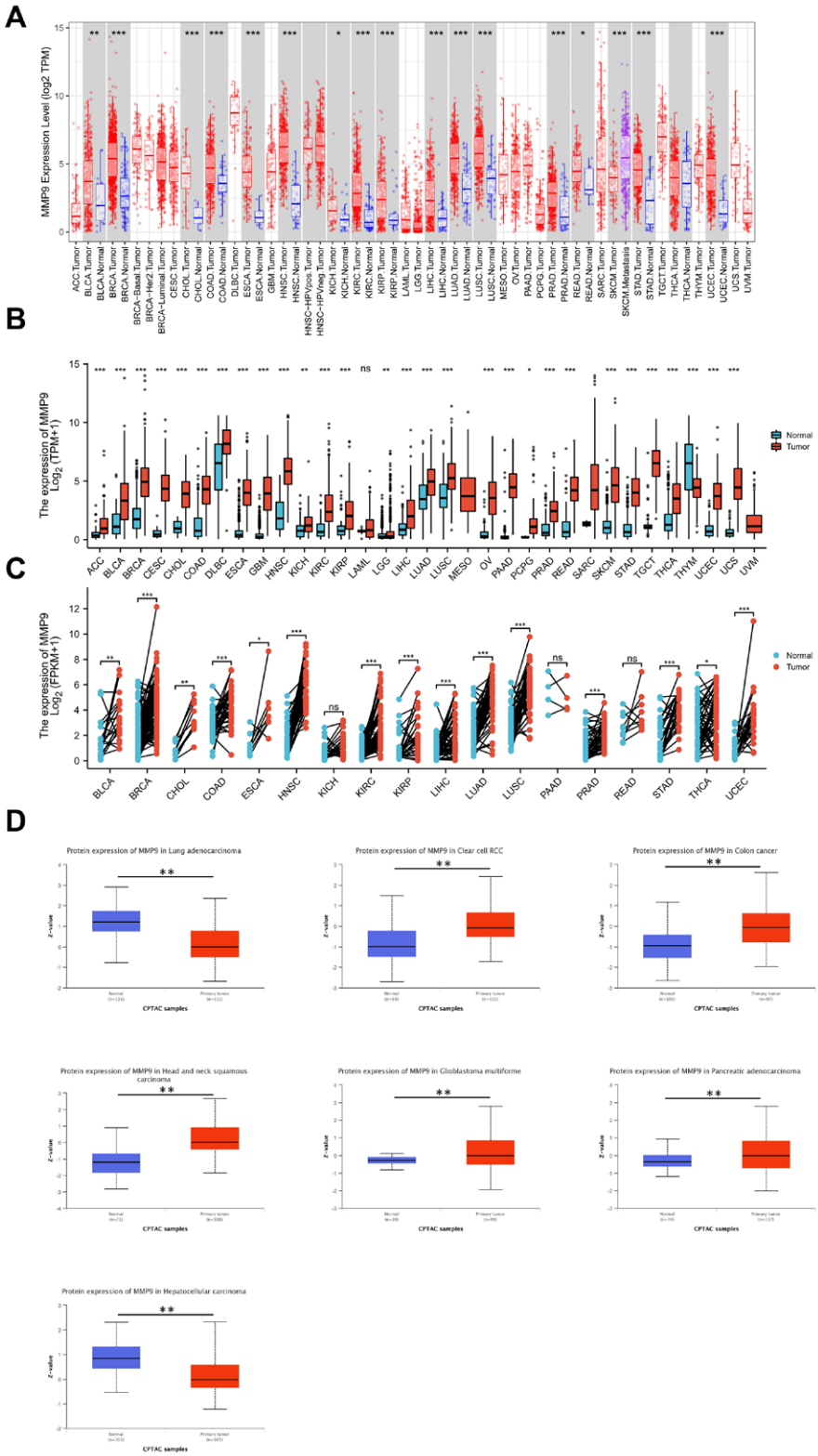

Figure 1. MMP9 expression analysis between tumor and normal samples. (A) Human MMP9 expression levels in different cancer types from TCGA data in TIMER. (B) Pan-cancer expression levels of MMP9 between the 33 cancers and normal tissues in unpaired sample analysis from the TCGA dataset and GTEx datasets. (C) Paired sample analysis of MMP9 mRNA expression between 18 cancers and para-cancerous tissues from the TCGA and GTEx databases. (D) The MMP9 protein expression level in normal tissues and primary tissues of lung adenocarcinoma, clear cell RCC, colon cancer, head and neck squamous carcinoma, glioblastoma, pancreatic adenocarcinoma, and hepatocellular carcinoma were examined using the CPTAC dataset. CPTAC = Clinical Proteomic Tumor Analysis Consortium, MMP-9 = matrix metalloproteinase 9, NS = no significance, TCGA = The Cancer Genome Atlas. *$P < .05$, **$P < .01$, ***$P < .001$.

broken through without enzymes that degrade the barriers and promote metastasis.[9,10] In addition, the hypoxic environment and inflammatory responses in the tumor microenvironment (TME) support tumor cell proliferation and hinder therapeutic progress. Matrix metalloproteinases (MMPs) are proteolytic enzymes that hydrolyze the extracellular matrix (ECM).[11] MMPs can degrade

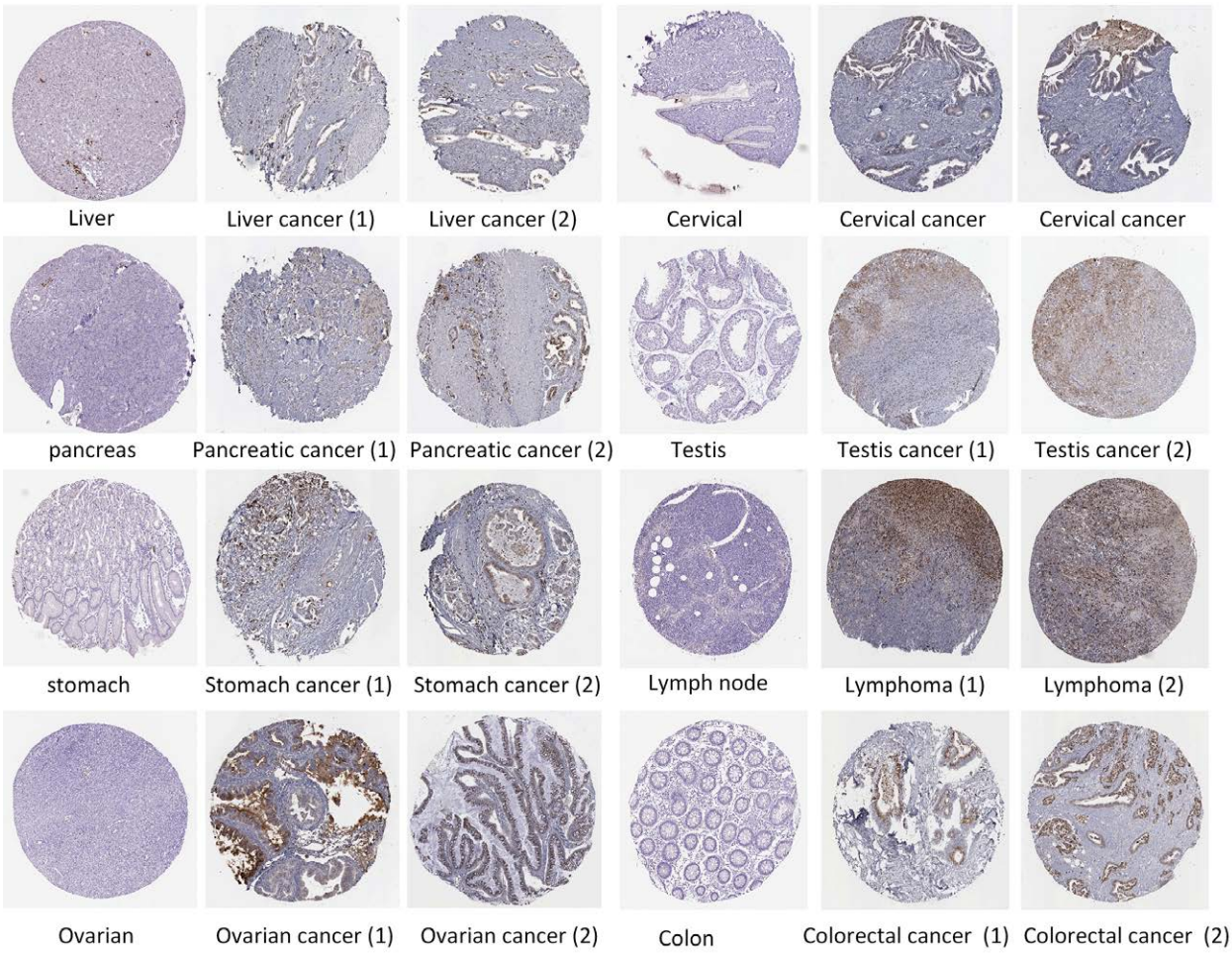

Figure 2. The protein expression of MMP9 in immunohistochemical images of normal and tumor groups. MMP-9 = matrix metalloproteinase 9.

various protein components in the ECM, disrupt barriers against tumor cell invasion, and play key roles in tumor invasion and metastasis; therefore, MMPs are considered the major proteolytic enzymes involved in tumor invasion and metastasis.[11]

Matrix metalloproteinase 9 (MMP9) is a zinc ion-dependent peptide endonuclease that participates in tissue repair, immune regulation, and tumor invasion and metastasis by degrading the ECM.[12,13] Human cells express 23 different MMPs, such as collagenase, gelatinase, hemolysin, strolysin, membrane-type MMPs, and other MMPs.[11,14] Previous data showed that MMP-9 was highly expressed in breast cancer and was associated with the early infiltration and metastasis of breast cancer cells.[15–17] Furthermore, MMP9 was upregulated in prostate cancer tissues or in blood samples from patients with prostate cancer, and elevated urinary MMP-9 levels helped distinguish between prostate cancer and bladder cancer.[18] MMP9 expression was significantly associated with the FIGO stage, degree of differentiation, and lymph node metastasis in ovarian cancer. Thus, MMP9 plays an important role in tumor cell invasion and metastasis.[14,19] However, most studies on the function of MMP9 in cancer have been limited to specific types of cancers.[20] Therefore, it is particularly important to study the regulatory functions and molecular mechanisms of MMP9 in pan-cancer datasets to uncover new research directions and clinical treatment strategies.

In this study, we performed a comprehensive analysis to evaluate the potential value of MMP9 in cancer diagnosis, prognosis, and immune responses, using data from the cBioPortal, GEPIA, Kaplan–Meier, TCGA, TIMER, and UALCAN databases. First, we analyzed the potential oncogenic role of MMP9 in 33 tumors using TCGA datasets. We also studied MMP9 protein expression in normal and tumor tissues using UALCAN datasets. These results suggested that MMP9 was highly expressed in most tumors but weakly expressed in a few tumors. In addition, we assessed the pan-cancer prognostic value of MMP9 expression using different databases (including the GEPIA and Kaplan–Meier databases), suggesting that differences in expression levels correlated significantly with pathological tumor stages patient prognosis. We then explored the potential relationship between MMP9 expression and immune cell infiltration using the TIMER and GEPIA databases. We evaluated potential correlations between MMP9 expression and the tumor mutation burden (TMB), microsatellite instability (MSI), immune cell-infiltration levels, and immune-checkpoint gene-expression levels in various types of cancer. This study comprehensively characterized the multiple roles of MMP9 in different types of tumors. The results of this study suggest that MMP9 may influence the prognosis of patients with cancer through its interactions with infiltrating immune cells.

2. Methods

2.1. Pan-cancer-expression pattern of human MMP9

Pan-cancer MMP9 expression was analyzed using the TIMER database (https://cistrome.shinyapps.io/timer/).[21] Gene-expression levels are represented as $\log_2$ transcript-per-million values. The TCGA and GTEx databases were used to evaluate

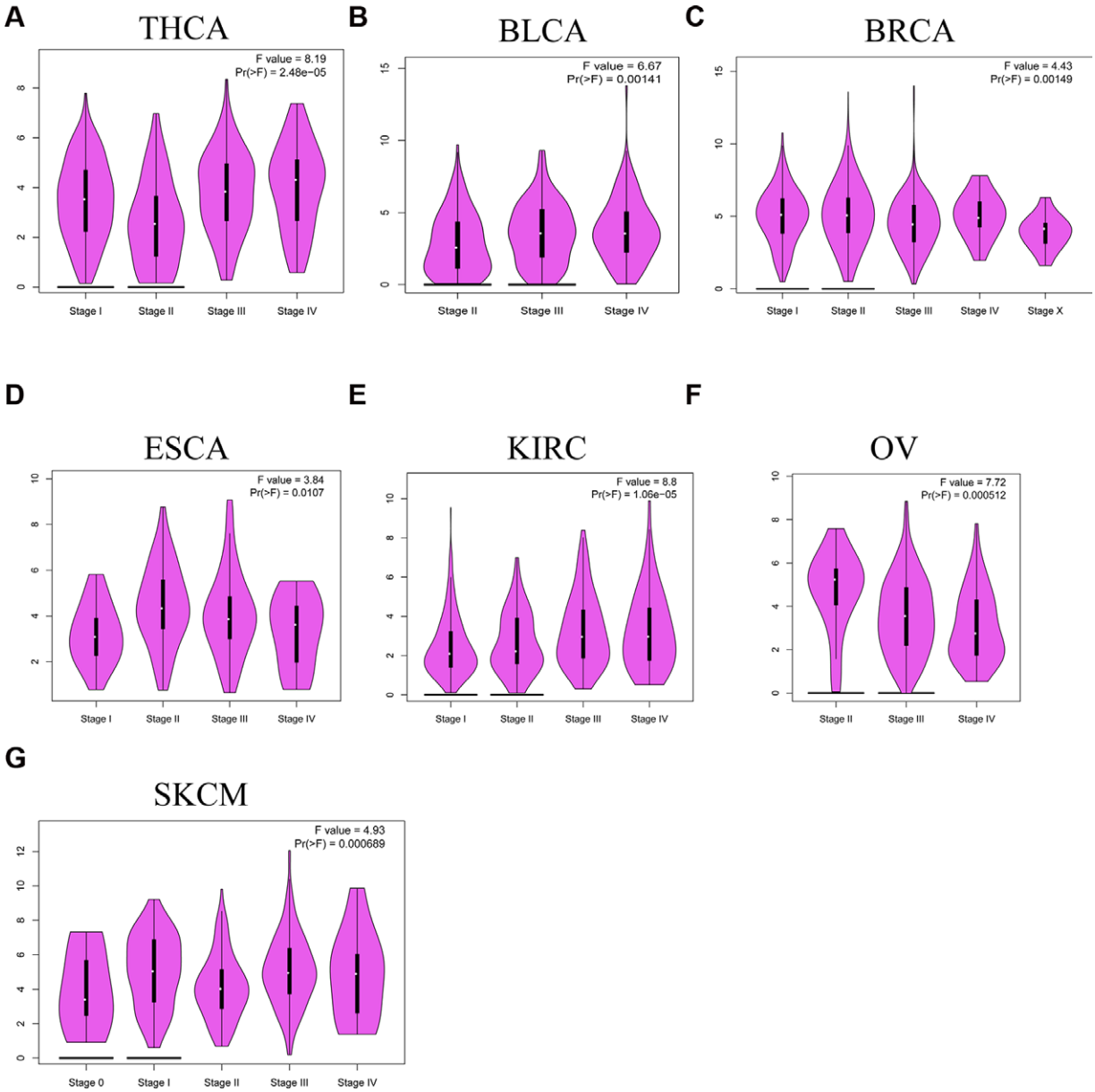

Figure 3. The correlation between MMP9 expression and cancer stages, including stage I, stage II, stage III, and stage IV of THCA, BLCA, BRCA, ESCA, KIRC, OV, and SKCM were investigated based on the TCGA data. BLCA = bladder urothelial carcinoma, BRCA = breast invasive cancer, ESCA = esophageal carcinoma, KIRC = kidney renal clear cell carcinoma, MMP-9 = matrix metalloproteinase 9, OV = ovarian serous cystadenocarcinoma, SKCM = skin cutaneous melanoma, THCA = thyroid carcinoma.

the mRNA-expression levels of MMP9. The thresholds were set at a *P*-value of .001 and fold-change of 1.5. The Clinical Proteomic Tumor Analysis Consortium dataset, which is from UALCAN database (http://ualcan.path.uab.edu/analysis.html), allowed us to investigate MMP9 protein-expression analysis of the levels in different cancers and normal tissues.[22] Herein, we explored the expression level of the total protein of MMP9 between primary tumor and normal tissues. Seven cancer types of the available datasets were selected, lung adenocarcinoma (LUAD), clear cell RCC, colon cancer, head and neck squamous carcinoma, glioblastoma, pancreatic adenocarcinoma (PAAD), and hepatocellular carcinoma (LIHC). Differences were evaluated using Student's *t*-test, and $P < .05$ was considered to reflect a statistically significant difference. The association between MMP9 and the cancer stage was analyzed using pathological stage plots with the GEPIA database (http://gepia2.cancer-pku.cn/#index/).[23] And 7 tumors of the MMP9 expression in different pathological stages were investigated based on the database.

2.2. Immunohistochemical staining data for MMP9

The Human Protein Atlas (HPA, https://www.proteinatlas.org/) database is a human proteome database that contains information based on immunohistochemical analyses of human tissues and cells.[24] To study differential MMP9 protein expression, we downloaded immunohistochemical images for 8 types of tumor tissues (liver, testicular, lymphoma, ovarian, stomach, pancreatic, cervical, and colorectal cancer) and corresponding normal tissues from the HPA database.

2.3. Diagnostic and prognostic analyses

Connections between MMP9 expression and patient prognosis, including overall survival (OS) and disease-specific survival (DSS), for patients with different types of cancer were examined using TCGA data. Specifically, we analyzed relationships between MMP9 expression and OS and DSS rates for patients with adrenocortical carcinoma (ACC), bladder urothelial carcinoma (BLCA),

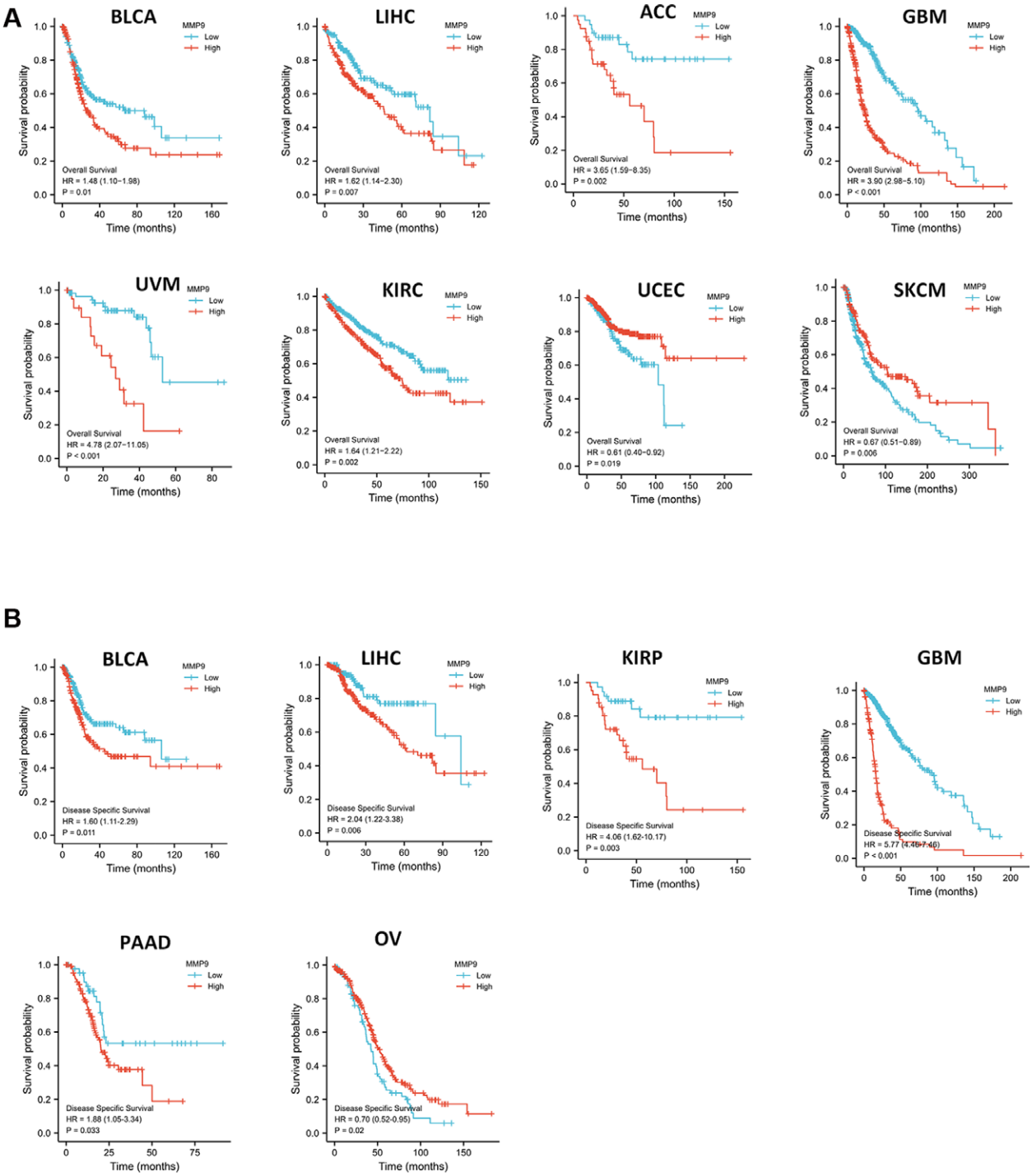

Figure 4. The prognosis value of MMP9 in pan-cancer. (A) Kaplan–Meier analysis of the association between MMP9 expression and OS of cancer patients. (B) Kaplan–Meier analysis of the association between MMP9 expression and DSS of cancer patients. DSS = disease-specific survival, OS = overall survival, MMP-9 = matrix metalloproteinase 9.

glioblastoma multiforme (GBM), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), LIHC, ovarian serous cystadenocarcinoma (OV), PAAD, skin cutaneous melanoma (SKCM), uterine corpus endometrial carcinoma (UCEC), and uveal melanoma (UVM). Hazard ratios and 95% confidence intervals were calculated by performing univariate survival analysis.

2.4. Genetic-alteration analysis

Mutational signatures (mutations, amplification, and deletions) of MMP9 in 30 different types of tumors were analyzed using the cBioPortal database (http://www.cBioPortal.org/).[25] The alteration frequencies and mutation types in all TCGA tumors were supplied in the "Cancer Types Summary" module.

2.5. Immune-cell infiltration and immune-checkpoint analysis

RNA sequencing-based expression profiles and corresponding clinical information for MMP9 were downloaded from the TCGA database (https://portal.gdc.com). To assess the reliability of the immune score evaluations, we used the TIMER and xCell databases to analyze the infiltration levels of immune cells in 32 different types of cancer. Moreover, we also assessed the

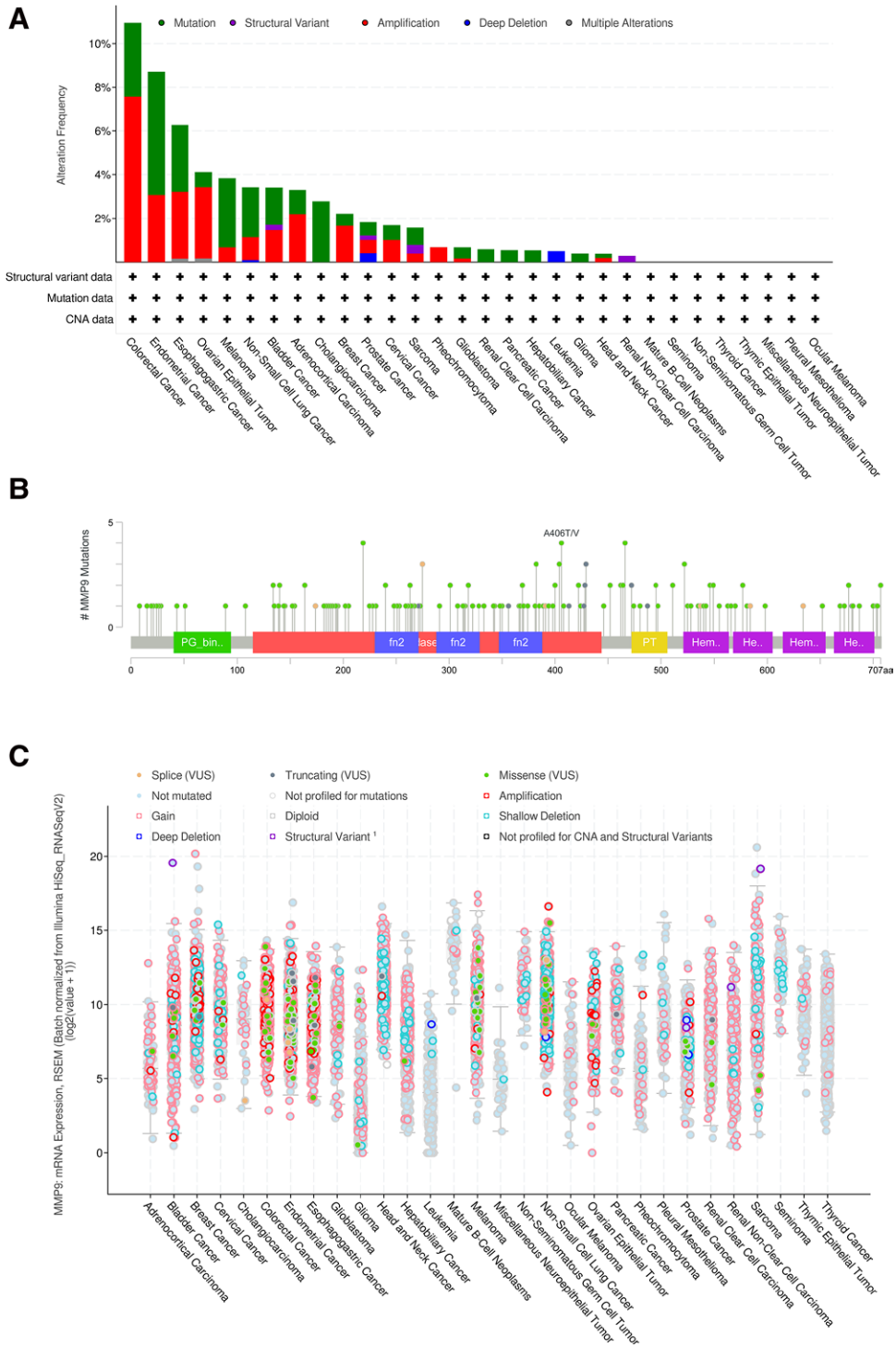

Figure 5. The mutation character of MMP9 in pan-cancer. (A) Alteration frequency of MMP9 in pan-cancer. (B) Mutation diagram of MMP9 across protein domains. (C) Mutation counts and types of MMP9 in 30 cancers. MMP-9 = matrix metalloproteinase 9.

expression levels of immune checkpoint-related genes in different types of cancer. *CD274*, *CTLA4*, *HAVCR2*, *LAG3*, *PDCD1*, *PDCD1LG2*, *SIGLEC15*, and *TIGIT* mRNA transcripts are associated with immune checkpoints. Statistical differences between the 2 groups were compared using the Wilcoxon test.

2.6. TMB and MSI analyses

Furthermore, TMB and MSI are considered predictive of immune checkpoint inhibitor sensitivity.[26] So, TMB and MSI analysis were obtained from the TCGA database. Correlation analysis between MMP9-expression levels and TMB or MSI was performed using Spearman method.

3. Results

3.1. MMP9 was upregulated in multiple human cancers

To determine the potential role of MMP9 in tumors, we analyzed MMP9 mRNA-expression levels using the TCGA and GTEx databases. The results in Figure 1A and B indicate

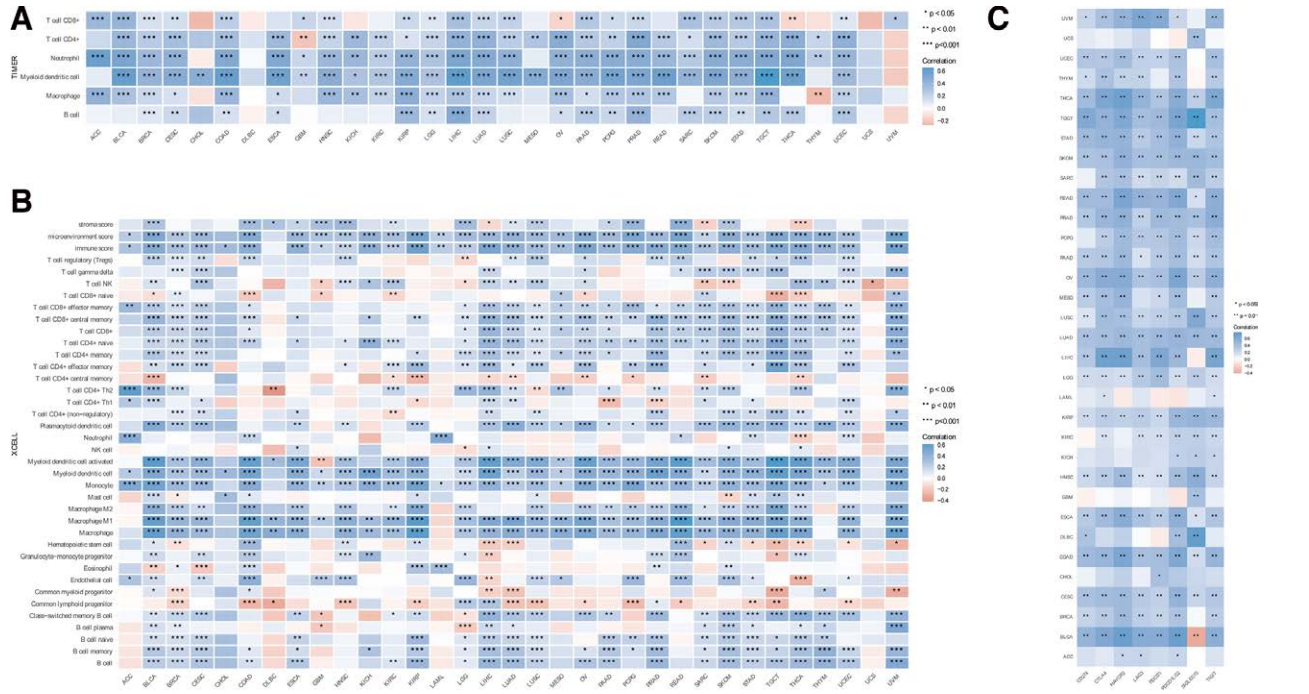

Figure 6. The MMP9 expression correlated with immune infiltration and immune checkpoint genes. (A) The MMP9 expression significantly correlated with the infiltration levels of various immune cells in the TIMER database. (B) The MMP9 expression significantly correlated with the infiltration levels of various immune cells based on xCell. (C) Correlation analyses of the MMP9 expression with immune checkpoint genes in pan-cancer. MMP-9 = matrix metalloproteinase 9. $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$.

that MMP9 was upregulated in BLCA, breast invasive cancer (BRCA), cervical and endocervical cancer, cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), lymphoid neoplasm diffuse large B-cell lymphomas, esophageal carcinoma (ESCA), GBM, head and neck squamous cell carcinoma (HNSC), KIRC, KIRP, brain lower grade glioma, LIHC, LUAD, lung squamous cell carcinoma (LUSC), OV, PAAD, pheochromocytoma and paraganglioma, prostate adenocarcinoma, rectum adenocarcinoma, sarcoma (SARC), SKCM, stomach adenocarcinoma, testicular germ cell tumors, thyroid carcinoma (THCA), UCEC, and uterine carcinosarcoma. However, MMP9 expression did not differ between acute myeloid leukemia tumor tissues and unpaired tissues. Conversely, MMP9 was expressed at much lower levels in THYM. To further assess MMP9 expression in pan-cancer, we also analyzed the differences in MMP9 expression in cancerous tissues and their adjacent tissues using the TCGA database (Fig. 1C). As shown in Figure 1C, MMP9 expression was significantly higher in BLCA, BRCA, CHOL, COAD, ESCA, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, rectum adenocarcinoma, THCA, and UCEC, but not in KICH, PAAD, and RAED.

Further, we found that MMP9 protein expression was significantly higher in advanced tumor tissues in RCC, COAD, HNSC, GBM, and PAAD while lower in LUAD and LIHC (Fig. 1D) by using the Clinical Proteomic Tumor Analysis Consortium database.

We further investigated MMP9 protein-expression levels using the HPA database. Inspection of Figure 2 shows MMP9 protein expression was significantly higher in eight different cancers than in normal tissues.

In addition, we analyzed the association between MMP9 expression and pan-cancer stages using the GEPIA2 website. MMP9 expression was associated with different stages of BLCA, THCA, BRCA, ESCA, KIRC, OV, and SKCM (Fig. 3A–G). These results suggest that MMP9 functions as an oncogene in several cancers.

3.2. Survival analysis related to MMP9 expression in pan-cancer

To investigate the prognostic value of MMP9, we downloaded TCGA clinical data and estimated associations between MMP9 expression and patient prognoses. Figure 4A shows that MMP9 expression in 8 cancers was significantly associated with OS. Specifically, our results showed that increased MMP9 expression was linked to poor OS in people with BLCA, LIHC, ACC, GBM, UVM, or KIRC. However, high MMP9 expression was associated with a significantly better OS rate than low MMP9 expression in people with UCEC or SKCM. The DSS results suggested that high MMP9 expression posed a prognostic risk for people with BLCA, LIHC, KIRP, GBM, and PAAD while protected against OV (Fig. 4B).

3.3. The characteristics of MMP9 mutations in the TCGA pan-cancer cohort

To understand the mutation status of MMP9 in various tumors, we evaluated MMP9 alterations in different tumor samples using the cBioPortal database. All TCGA Pan-Cancer Atlas Studies, comprising 32 studies and 10,967 samples, were included. As shown in Figure 5A, the highest MMP9-alteration frequency was approximately 13% in patients with CRC. Amplification was the main type of genetic alteration in the samples. Furthermore, MMP9 was the most frequently mutated gene in endometrial cancer. The sites, types, and numbers of MMP alterations in different cancers are shown in Figure 5B. Among the 32 cancers studied, amplifications and shallow deletions in MMP9 most commonly affected MMP9 mRNA expression (Fig. 5C).

3.4. MMP9 was related to immune infiltration in pan-cancer

We performed pan-cancer analysis of the association between MMP9 expression and immune cell-infiltration levels based on

information in the TCGA database. We used immunedeconv (an R software package) to reliably assess immune cell correlations. MMP9 expression was significantly associated with the abundance of infiltrating immune cells (Fig. 6A and B). To further investigate the potential role of MMP9 in immune checkpoints, we downloaded expression data for 8 genes associated with immune-checkpoint proteins, and observed that MMP9 expression was significantly associated with the expression of immune-checkpoint-related genes (Fig. 6C) such as *CD274*, *CTLA4*, *HAVCR2*, *LAG3*, *PDCD1*, *PDCD1LG2*, *SIGLEC15*, and *TIGIT*.

We also explored the role of MMP9 in immune cell infiltration by LIHC and BRCA cells. The results suggested that B cell, T cell, neutrophil, dendritic cell, microphage, and T regulatory cell (Tregs) infiltration were significantly and positively correlated with MMP9 expression in both LIHC and BRCA (Fig. 7A and B) but not in NK.

TMB and MSI are 2 emerging biomarkers associated with immunotherapy responses. Thus, we investigated relationships between MMP9 expression and TMBs. MMP9 expression correlated with the TMB in several tumors, including LAML,

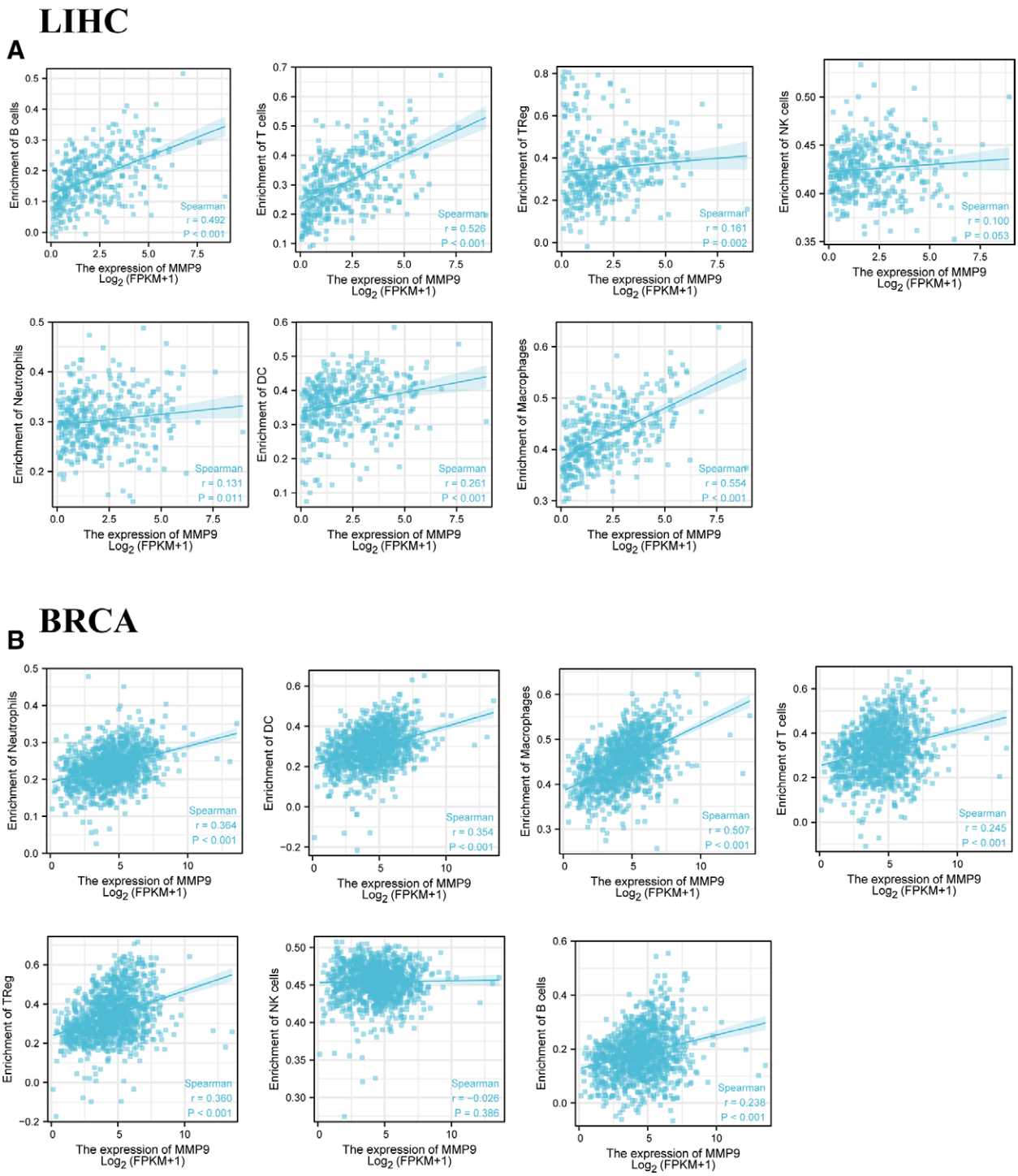

Figure 7. Correlation between MMP9 expression and the immune infiltration of cancer-associated cells. Scatter plots of the relationship between the expression of MMP9 and the infiltration level of cancer-associated cells in LIHC (A) and BRCA (B) cancer were explored via different algorithms. BRCA = breast invasive cancer, LIHC = hepatocellular carcinoma, MMP-9 = matrix metalloproteinase 9.

ACC, SARC, UCEC, testicular germ cell tumor, uterine carcinosarcoma, and CHOL (Fig. 8A). We also investigated correlations between MMP9 expression and MSI in 33 types of cancer; ACC, lower grade glioma, UCEC, and SARC exhibited positive correlations, and LUSC exhibited a negative correlation (Fig. 8B).

3.5. Functional states of MMP9 in single-cell RNA-sequencing (scRNA-Seq) datasets

We further explored the functional state of MMP9 in various cancer types using scRNA-Seq data from the cancer SEA database[27] (Fig. 9A). We explored correlations between MMP9-expression levels and the functional statuses of specific cancers.

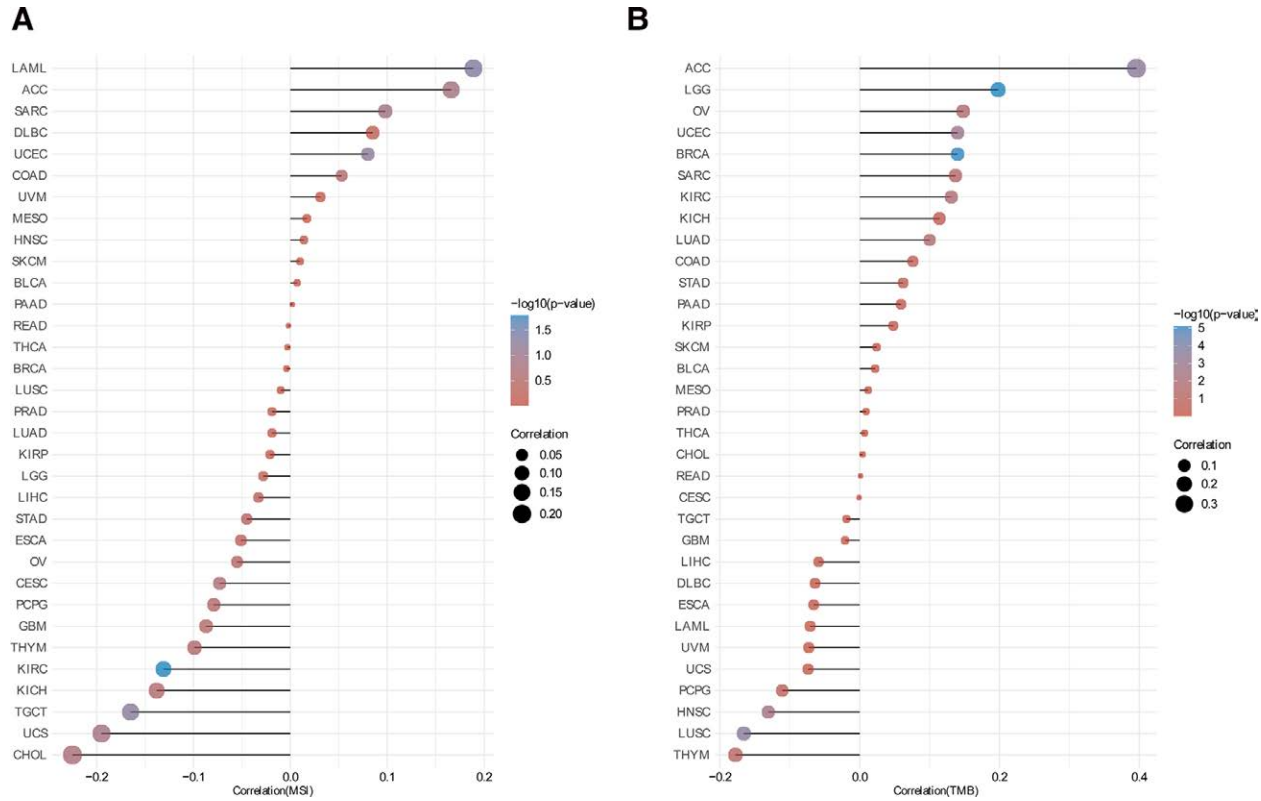

Figure 8. Correlation between the MMP9 gene expression and TMB and MSI in pan-cancer. (A) A stick chart shows the relationship between the MMP9 gene expression and TMB in diverse tumors. The red curve represents the correlation coefficient, and the blue value represents the range. (B) A stick chart shows the association between the MMP9 gene expression and MSI in diverse tumors. MMP-9 = matrix metalloproteinase 9, MSI = microsatellite instability, TMB = tumor mutation burden.

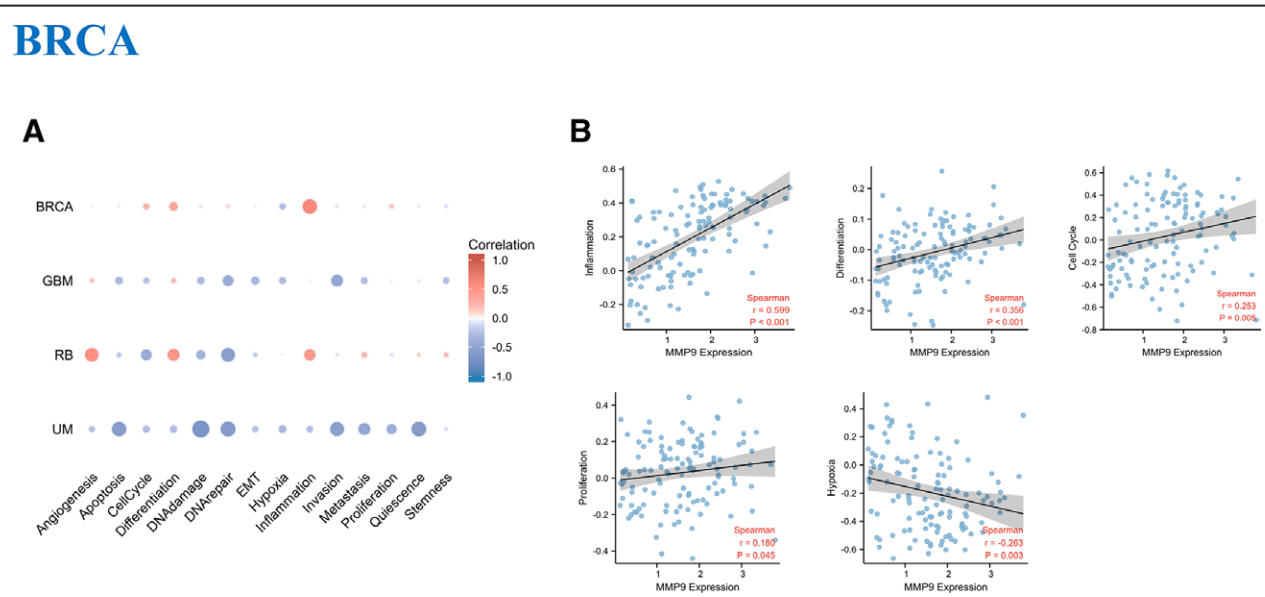

Figure 9. The correlation between MMP9 expression and 4 cancer functional states using single-cell sequence data from the Cancer SEA database. (A) The correlation between MMP9 expression and 4 cancer functional states in pan-cancer. (B) The expression of MMP9 is positively correlated to the inflammation, differentiation, cell cycle, proliferation, and hypoxia of BRCA. BRCA = breast invasive cancer, MMP-9 = matrix metalloproteinase 9.

Interestingly, the results showed that MMP9 expression correlated positively with inflammation ($P < .001$), differentiation ($P < .001$), cell-cycle progression ($P = .005$), proliferation ($P = .045$), and hypoxia ($P = .003$) in BRCA (Fig. 9B).

4. Discussion

Cancer is characterized by the uncontrolled growth and division of abnormal cells due to imbalanced expression between tumor-suppressor genes and proto-oncogene genes and the loss of normal regulatory mechanisms.[28] Cancer poses serious threats to human health owing to high morbidity and mortality rates. According to the American Association for Research on Cancer, 1,918,030 new cases of cancer and 609,360 cancer-related deaths were expected to occur in the US in 2022.[1] Although several clinical options are available for treating cancer, including surgical resection, radiotherapy, and adjuvant chemotherapy, their effectiveness remains limited.[29] Early detection and effective treatment are important prerequisites for improving the treatment outcomes for patients with cancer.[30] Pan-cancer analysis can reveal similarities and differences among different cancers, providing profound insights into the design of cancer-prevention and personalized-treatment strategies.[24]

Members of the MMP family play major roles in cell matrix degradation, inflammation, tumor invasion, metastasis, and TME.[31] Among these, MMP9 has received attention for its involvement in tumor development, progression, and treatment.[32–35] In this study, we revealed the role of MMP9 in pan-cancer using bioinformatics methods. Here, we evaluated MMP9 mRNA-expression levels in human normal and tumor tissues and cell lines and found that MMP9 was significantly upregulated and associated with various cancers. Prognostic analysis showed that MMP9 expression was inversely associated with OS and DSS rates in various cancers. Furthermore, we evaluated the most common mutation types and mutation sites in MMP9. MMP9-expression levels were also evaluated in different subtypes of infiltrating immune cells in various cancers, revealing correlations between MMP9-expression levels and the levels of infiltrating immune cells and regulators in various cancers. Finally, scRNA-Seq data analysis also indicated that MMP9 expression correlated positively with cell proliferation, inflammation, differentiation, hypoxia, and cell-cycle progression in BRCA.

Previous findings have shown that MMP9 is closely associated with a poor prognosis in various cancers. For example, MMP-9 can mediate the protein kinase C pathway and promote the development of colon cancer.[36] Elevated MMP-9 expression has been associated with increased risks of recurrence and decreased survival in patients with breast and colon cancer.[37] In addition, MMP-9 overexpression has been strongly correlated with a poor prognosis of patients with lung cancer, vascular endothelial growth factor can induce MMP-9 expression during lung tumor metastasis, and inhibiting MMP-9 can significantly reduce the occurrence of metastasis.[38] Further data showed that MMP9 expression in plasma and tumor tissues from patients with lung cancer can be used as an independent prognostic factor to determine the prognosis of patients with pathological stage IA non-small cell lung cancer.[39] In this study, we assessed the prognostic value of MMP9 in cancer in terms of OS and DSS rates. Regarding OS, MMP9 expression is a risk factor for various cancers, including BLCA, LIHC, ACC, GBM, UVM, and KIRC, but is a protective factor for cancers such as UCEC and SKCM. In addition, we performed DSS analysis of MMP9 in cancer, finding that high MMP9 expression was a risk factor for BLCA, LIHC, KIRP, GBM, and PAAD but was a protective factor in OV. These findings suggest that MMP9 expression is a good prognostic indicator for these cancers. The above findings suggest that MMP9 is not only influences disease prognosis, but also a possible target for tumor therapy.

Previous results have shown that MMPs may help tumor cells metastasize by cleaving receptors and chemokines that promote the development, proliferation, and chemotaxis of antitumor T lymphocytes, natural killer cells, neutrophils, and macrophages.[40] For example, MMP9 cleaves and inactivates a positive regulator of T cell development, proliferation, and interleukin 2 receptor α.[41–43] MMP9 is mainly involved in the PI3K/AKT signaling pathway, ECM–receptor interactions, and other inflammatory signaling pathways (such as the IL-17 signaling pathway, ligand–ligand receptor interactions, the TNF signaling pathway, and chemokine signaling pathways).[44,45] Previous data showed that MMP9 played an important role in inflammatory signaling pathways associated with osteoarthritis.[46] MMP9 can suppress immune responses by reducing quiescent CD4 memory T cells and activated NK cells, and MMP9 can increases M0 macrophages to induce inflammation during osteoarthritis.[47] Consistent with previous findings, our current analysis showed that MMP9 expression was positively associated with inflammatory responses in tumors such as breast cancer. In addition, high MMP9 expression can effectively regulate tumor-specific Treg survival and inhibit tumor-specific CD8+ T cell function. Notably, MMP9 can regulate immune cell migration and can endogenously regulate T-cell activity.[48] Consistent with previous studies, our immune cell-infiltration analyses revealed a complex role for MMP9 in regulating immunity.

The TME is a complex structure composed of tumor cells, tumor-associated cells, blood vessels, ECM, and other substances that plays important roles in cancer recurrence and drug resistance.[49] Increasing evidence suggests that immune cells in the TME, including CD8+ T cells, B cells, macrophages, neutrophils, and NK cells, are associated with the efficacy of immunotherapy.[50] Tumor proliferation, recurrence, and metastasis remain as health risks due to the complexity of the TME. Although some breakthroughs have been made in cancer immunotherapy, its successful application still faces several challenges. Therefore, identifying new targets and biomarkers is key for further improving the efficacy of immunotherapy. Importantly, the function of MMP9 and its effect on the tumor immune microenvironment have not been fully investigated. Here, we revealed a relationship between MMP9 and tumor immune cells, and we investigated the immune status of patients with cancer by analyzing MMP9-expression levels. We found that, in many cancers, MMP9 was significantly associated with the infiltration levels of CD8+ T cells, CD4+ T cells, macrophages, and other immune cells. Additionally, MMP9 expression was significantly positively correlated with immune checkpoint-related genes. In LIHC and BCRA, MMP9 expression was positively correlated with CD8+ T cell, B cell, macrophage, and neutrophil infiltration, but negatively correlated with NK cell infiltration. In addition, scRNA-Seq data indicated that MMP9 expression was positively correlated with inflammation levels in BRCA. Therefore, our findings indicate that MMP9 is associated with immune cell-infiltration levels and may function as a potential immunotherapy-related biomarker in tumors.

5. Conclusion

In multiple cancers, MMP9 upregulation corresponds to poor patient prognosis and correlates with the infiltration levels of CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells. Furthermore, MMP9 expression correlated significantly with the expression of immune-checkpoint markers. Based on these findings, further insights could be provided into tumor-treatment strategies by analyzing MMP9 expression and immune cell infiltration in different populations to improve the therapeutic effects of cancer immunotherapy. Nonetheless, the results of this study were limited by several limitations. Firstly, further research in vivo and in vitro is necessary to validate our conclusions. A second reason is that the etiology of tumors is very complicated, involving gene–gene interactions and gene–environment interactions. Therefore, it is unlikely that a single gene polymorphism could significantly affect its development. So, more experiments are needed to further explore the regulatory relationships between MMP9 and other genes. In

addition, since our data are from a database, we were unable to assess some aspects like patients' sex, age or complications, which suggest that more clinical investigations are needed.

Author contributions

Conceptualization: Jie Zhang, Hairong Liu.
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Funding acquisition: Jie Zhang.
Data curation: Lei Xu.
Methodology: Ying Liu, Xiang Li, Hairong Liu.
Resources: Jingjun Zhang, Xiang Li, Tao Ren.
Supervision: Tao Ren, Hairong Liu.
Validation: Tao Ren.
Writing – original draft: Jie Zhang, Ying Liu.
Writing – review & editing: Lei Xu, Tao Ren.

References

[1] Siegel RL, Miller KD, Fuchs HE, et al. Cancer statistics, 2022. CA Cancer J Clin. 2022;72:7–33.
[2] Batlle E, Clevers H. Cancer stem cells revisited. Nat Med. 2017;23:1124–34.
[3] Williams JB, Li S, Higgs EF, et al. Tumor heterogeneity and clonal cooperation influence the immune selection of IFN-gamma-signaling mutant cancer cells. Nat Commun. 2020;11:602.
[4] Sadelain M. CAR therapy: the CD19 paradigm. J Clin Invest. 2015;125:3392–400.
[5] Davila ML, Brentjens RJ. CD19-Targeted CAR T cells as novel cancer immunotherapy for relapsed or refractory B-cell acute lymphoblastic leukemia. Clin Adv Hematol Oncol. 2016;14:802–8.
[6] Alsaab HO, Sau S, Alzhrani R, et al. PD-1 and PD-L1 checkpoint signaling inhibition for cancer immunotherapy: mechanism, combinations, and clinical outcome. Front Pharmacol. 2017;8:561.
[7] Mano H. Discovery and clinical application of mutations in the cancer genome. Nihon Rinsho. 2015;73:1251–5.
[8] Croce CM. Oncogenes and cancer. N Engl J Med. 2008;358:502–11.
[9] Kalluri R. Basement membranes: structure, assembly and role in tumour angiogenesis. Nat Rev Cancer. 2003;3:422–33.
[10] Lamar JM, Pumiglia KM, DiPersio CM. An immortalization-dependent switch in integrin function up-regulates MMP-9 to enhance tumor cell invasion. Cancer Res. 2008;68:7371–9.
[11] Brinckerhoff CE, Matrisian LM. Matrix metalloproteinases: a tail of a frog that became a prince. Nat Rev Mol Cell Biol. 2002;3:207–14.
[12] Robert S, Gicquel T, Victoni T, et al. Involvement of matrix metalloproteinases (MMPs) and inflammasome pathway in molecular mechanisms of fibrosis. Biosci Rep. 2016;36:e00360.
[13] Van den Steen PE, Dubois B, Nelissen I, et al. Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9). Crit Rev Biochem Mol Biol. 2002;37:375–536.
[14] Galliera E, Tacchini L, Corsi Romanelli MM. Matrix metalloproteinases as biomarkers of disease: updates and new insights. Clin Chem Lab Med. 2015;53:349–55.
[15] Ren F, Tang R, Zhang X, et al. Overexpression of MMP family members functions as prognostic biomarker for breast cancer patients: a systematic review and meta-analysis. PLoS One. 2015;10:e0135544.
[16] Klassen LMB, Chequin A, Manica GCM, et al. MMP9 gene expression regulation by intragenic epigenetic modifications in breast cancer. Gene. 2018;642:461–6.
[17] Chiranjeevi P, Spurthi KM, Rani NS, et al. Gelatinase B (-1562C/T) polymorphism in tumor progression and invasion of breast cancer. Tumour Biol. 2014;35:1351–6.
[18] Roy R, Louis G, Loughlin KR, et al. Tumor-specific urinary matrix metalloproteinase fingerprinting: identification of high molecular weight urinary matrix metalloproteinase species. Clin Cancer Res. 2008;14:6610–7.
[19] Turpeenniemi-Hujanen T. Gelatinases (MMP-2 and -9) and their natural inhibitors as prognostic indicators in solid cancers. Biochimie. 2005;87:287–97.
[20] Davidson B, Goldberg I, Gotlieb WH, et al. The prognostic value of metalloproteinases and angiogenic factors in ovarian carcinoma. Mol Cell Endocrinol. 2002;187:39–45.
[21] Li T, Fan J, Wang B, et al. IMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. Cancer Res. 2017;77:e108–10.
[22] Chandrashekar DS, Bashel B, Balasubramanya SAH, et al. UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia. 2017;19:649–58.
[23] Tang Z, Kang B, Li C, et al. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Res. 2019;47:W556–60.
[24] Uhlen M, Fagerberg L, Hallstrom BM, et al. Proteomics. Tissue-based map of the human proteome. Science. 2015;347:1260419.
[25] Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2:401–4.
[26] Passaro A, Stenzinger A, Peters S. Tumor mutational burden as a pan-cancer biomarker for immunotherapy: the limits and potential for convergence. Cancer Cell. 2020;38:624–5.
[27] Yuan H, Yan M, Zhang G, et al. CancerSEA: a cancer single-cell state atlas. Nucleic Acids Res. 2019;47:D900–8.
[28] Zhang J, Chen YH, Lu Q. Pro-oncogenic and anti-oncogenic pathways: opportunities and challenges of cancer therapy. Future Oncol. 2010;6:587–603.
[29] Gotwals P, Cameron S, Cipolletta D, et al. Prospects for combining targeted and conventional cancer therapy with immunotherapy. Nat Rev Cancer. 2017;17:286–301.
[30] Overman MJ, Modak J, Kopetz S, et al. Use of research biopsies in clinical trials: are risks and benefits adequately discussed? J Clin Oncol. 2013;31:17–22.
[31] Kessenbrock K, Plaks V, Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. Cell. 2010;141:52–67.
[32] Huang H, Xiang W, Tao Y, et al. Matrix Metalloproteinase-9 (MMP-9) as a cancer biomarker and MMP-9 biosensors: recent advances. Sensors (Basel). 2018;18:4417.
[33] Bai XY, Li S, Wang M, et al. Kruppel-like factor 9 down-regulates matrix metalloproteinase 9 transcription and suppresses human breast cancer invasion. Cancer Lett. 2018;412:224–35.
[34] Xue Q, Cao L, Chen XY, et al. High expression of MMP9 in glioma affects cell proliferation and is associated with patient survival rates. Oncol Lett. 2017;13:1325–30.
[35] Chang C, Werb Z. The many faces of metalloproteases: cell growth, invasion, angiogenesis and metastasis. Trends Cell Biol. 2001;11:S37–43.
[36] Agrez M, Gu X, Turton J, et al. The alpha v beta 6 integrin induces gelatinase B secretion in colon cancer cells. Int J Cancer. 1999;81:90–7.
[37] Bjorklund M, Koivunen E. Gelatinase-mediated migration and invasion of cancer cells. Biochim Biophys Acta. 2005;1755:37–69.
[38] Hiratsuka S, Nakamura K, Iwai S, et al. MMP9 induction by vascular endothelial growth factor receptor-1 is involved in lung-specific metastasis. Cancer Cell. 2002;2:289–300.
[39] Lee CY, Shim HS, Lee S, et al. Prognostic effect of matrix metalloproteinase-9 in patients with resected non small cell lung cancer. J Cardiothorac Surg. 2015;10:44.
[40] Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer. 2002;2:161–74.
[41] Bergers G, Brekken R, McMahon G, et al. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol. 2000;2:737–44.
[42] Shao L, Zhang B, Wang L, et al. MMP-9-cleaved osteopontin isoform mediates tumor immune escape by inducing expansion of myeloid-derived suppressor cells. Biochem Biophys Res Commun. 2017;493:1478–84.
[43] Sheu BC, Hsu SM, Ho HN, et al. A novel role of metalloproteinase in cancer-mediated immunosuppression. Cancer Res. 2001;61:237–42.
[44] Hu J, Van den Steen PE, Sang QX, et al. Matrix metalloproteinase inhibitors as therapy for inflammatory and vascular diseases. Nat Rev Drug Discov. 2007;6:480–98.
[45] Gao F, Zhou C, Qiu W, et al. Total flavonoids from Semen Cuscutae target MMP9 and promote invasion of EVT cells via Notch/AKT/MAPK signaling pathways. Sci Rep. 2018;8:17342.
[46] Li S, Wang H, Zhang Y, et al. COL3A1 and MMP9 serve as potential diagnostic biomarkers of osteoarthritis and are associated with immune cell infiltration. Front Genet. 7212;12:58.
[47] Milaras C, Lepetsos P, Dafou D, et al. Association of matrix metalloproteinase (MMP) gene polymorphisms with knee osteoarthritis: a review of the literature. Cureus. 2021;13:e18607.
[48] Wang BQ, Zhang CM, Gao W, et al. Cancer-derived matrix metalloproteinase-9 contributes to tumor tolerance. J Cancer Res Clin Oncol. 2011;137:1525–33.
[49] Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med. 2013;19:1423–37.
[50] Bellone M, Elia AR. Constitutive and acquired mechanisms of resistance to immune checkpoint blockade in human cancer. Cytokine Growth Factor Rev. 2017;36:17–24.