Research Article

MMP12 is a Potential Predictive and Prognostic Biomarker of Various Cancers Including Lung Adenocarcinoma

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Abstract

Objective: This study sought to explore the clinical value of matrix metalloproteinases 12 (*MMP12*) in multiple cancers, including lung adenocarcinoma (LUAD).

Methods: Using >10,000 samples, this retrospective study demonstrated the first pan-cancer analysis of *MMP12*. The expression of *MMP12* between cancer groups and their control groups was analyzed using Wilcoxon rank-sum tests. The clinical significance of *MMP12* expression in multiple cancers was assessed using receiver operating characteristic curves, Kaplan–Meier curves, and univariate Cox analysis. A further LUAD-related analysis based on 4565 multi-center and in-house samples was performed to verify the findings regarding MMP12 in pan-cancer analysis partly.

Results: *MMP12* mRNA is highly expressed in 13 cancers compared to their controls, and the MMP12 protein level is elevated in some of these cancers (e.g., colon adenocarcinoma) ($P < .05$). *MMP12* expression makes it feasible to distinguish 21 cancer tissues from normal tissues (AUC = 0.86). A high *MMP12* expression is a prognosis risk factor in eight cancers, such as adrenocortical carcinoma (hazard ratio >1, $P < .05$). The elevated *MMP12* expression is also a prognosis protective factor in breast-invasive carcinoma and colon adenocarcinoma (hazard ratio <1, $P < .05$). Some pan-cancer findings regarding *MMP12* are verified in LUAD—MMP12 expression is upregulated in LUAD at both the mRNA and protein levels ($P < .05$), has the potential to distinguish LUAD with considerable accuracy (AUC = .91), and plays a risk prognosis factor for patients with the disease ($P < .05$).

Conclusions: *MMP12* is highly expressed in most cancers and may serve as a novel biomarker for the prediction and prognosis of numerous cancers.

Keywords

expression, prognosis, immune microenvironment, standardized mean difference, area under the curve

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Data Availability Statement included at the end of the article

Introduction

Cancer has gradually become one of the leading causes of human illness and mortality. International cancer statistics indicate that there were approximately 19 million new cancer cases worldwide and more than 10 million cancer deaths in 2020.[1] Targeted therapy offers greater promise than traditional approaches (e.g., surgery) in treating several cancers.[2] Imatinib provides a noteworthy example of its effectiveness in targeting the BCR-ABL fusion gene, which has led to a significant improvement in the 10-year survival rate for patients with chronic myeloid leukemia (from less than 50% to approximately 80%).[3] Cancer cells expressing PD-L1 can circumvent immune response inhibition by activating the interaction between PD-1/PD-L1, which promotes immune evasion; PD-1 inhibitors, such as nivolumab, can effectively block this interaction and suppress immune evasion and provide benefits to patients with non-small-cell lung cancer.[4] These examples highlight the immense potential value of targeted therapy for treating cancers. However, the presence of drug resistance and the occurrence of side effects associated with current medications underscore the need for researchers to prioritize the exploration of novel cancer markers.[5,6] Furthermore, a lack of valuable markers for many cancers also limits the development of targeted therapy. Therefore, it is crucial to explore markers that potentially play vital roles in multiple cancers.

Among numerous potential tumor markers, matrix metalloproteinase 12 (*MMP12*) has garnered increasing attention from researchers. *MMP12*, a metalloproteinase secreted by macrophages, has been found to be aberrantly expressed in various tumors, affecting tumor progression and prognosis through multiple mechanisms.[7-9] In liver hepatocellular carcinoma (LIHC), upregulation of *MMP12* has been associated with tumor growth and progression by promoting angiogenesis, ultimately resulting in a poorer prognosis for patients.[10] In lung adenocarcinoma (LUAD), MMP12 protein expression levels are significantly higher in tumor tissues than control lung tissues. Knocking down MMP12 or inhibiting its expression can suppress the proliferation and invasion of LUAD cells, possibly by affecting the expression of vascular endothelial growth factor and the epithelial-mesenchymal transition.[11,12] In renal cell carcinoma and esophageal squamous cell carcinoma, patients with increased *MMP12* expression have a significantly worse prognosis compared to those with low *MMP12* expression.[13,14] Furthermore, *MMP12* has been proposed as a target for tumor immunosuppression and immune checkpoints.[15] These studies shed light on the pro-tumorigenic role of *MMP12* and its potential therapeutic value. However, some studies have also suggested anti-tumor effects of *MMP12* in colorectal cancer: knocking out MMP12 leads to the accumulation of M2 macrophages (which predominantly exhibit pro-cancer effects) in the tumor microenvironment, thereby promoting the growth of colorectal tumors.[16] In ovarian cancer, high levels of *MMP12* mRNA have been associated with better overall survival (OS).[17] Therefore, previous research indicates that *MMP12* plays an important role in various tumors, but its clinical significance may not be consistent across different types of cancer. Closing this research gap is needed to identify the pan-cancer clinical significance of *MMP12*.

We analyzed *MMP12*'s cancer cell effects, mRNA and protein expression, immune effects, clinical significance, and potential mechanisms of *MMP12* in 33 cancer tissues and 21 normal tissues by collecting data from the DepMap Portal, Xena database, and Clinical Proteomic Tumor Analysis Consortium database. Additionally, we used data from The Cancer Genome Atlas (TCGA), Genotype-Tissue Expression, ArrayExpress, and Gene Expression Omnibus to analyze *MMP12*'s expression in LUAD and combined those data with our in-house data to explore the role of *MMP12* in LUAD as well as its important pan-cancer role, thereby providing a potential target of cancer immunotherapy.

Materials and Methods

This retrospective study was approved by the medical ethics review committee of the First Affiliated Hospital of Guangxi Medical University (No. 6 Shuangyong Road, Nanning, China) on October 26, 2021, with the approval number 2021(KY-E-246). Informed consent was signed by all patients involved in the in-house data. The personal identification information of the patients included in this study has been removed, and it is not possible to ascertain the identity of the patients through the information provided in this article. The reporting of the study adheres to REMARK guidelines.[18]

Collecting MMP12*-related Expression and Prognosis Data Across Cancers*

The DepMap Portal includes data on numerous cell types. We collected data on RNA interference (n of samples = 494) to analyze the essential roles of *MMP12* in multiple cancer cells. An RNA interference score of less than 0 indicates that *MMP12* is essential for a specific cancer cell.

The Xena database collects data on tumors and their normal samples from various datasets such as TCGA. TCGA data were extracted from the Xena database to analyze *MMP12*'s mRNA expression among 33 tumor tissue types (n of samples = 8305) and 21 normal tissue types (n of samples = 671). The Clinical Proteomic Tumor Analysis Consortium database contains MMP12 protein level data in various cancers from the Proteomic Data Commons. The protein level data from this database were collected to detect the difference in MMP12 protein levels between breast-invasive carcinoma (BRCA), colon adenocarcinoma (COAD), head and neck squamous cell carcinoma (HNSCC), and uterine corpus endometrial carcinoma (UCEC) and their control samples.[19-21]

Clinical parameters were collected from the Xena database, including patients' ages, genders, and cancer stages as defined by the American Joint Committee on Cancer. The cancer patients' prognosis data were also acquired from the Xena database, including OS, disease-specific survival, progression-free interval, and disease-free interval. The define details of clinical endpoints can be seen in previous research.[22]

The Prediction Effect and Prognosis Value of MMP12 *for Cancers*

The area under the curve (AUC) size of receiver operating characteristic (ROC) curves was applied to determine the ability of *MMP12* expression to differentiate tumor tissues and normal tissues. We plotted the summary ROC (sROC) to evaluate the overall ability to discriminate between tumor tissues and normal tissues. Univariate Cox regression and Kaplan–Meier curves revealed differences in prognosis of patients with various expression levels of *MMP12*. The high-*MMP12* and low-*MMP12* groups were identified using a cutoff value determined by the "survminer" software package.

We also examined the role of *MMP12* expression in the immune environment and signaling pathways. Three types of data, including neoantigen count,[23] tumor mutational burden (TMB), and microsatellite instability (MSI), were acquired from Sanger Box (v3.0).[24] We also revealed the regulation of *MMP12* expression on six types of patient's immune cells, including B cells, $CD4^+$ T cells, $CD8^+$ T cells, neutrophils, macrophages, and dendritic cells; the infiltration level data of these cells were obtained from TIMER.[25] Immune environment data based on the ESTIMATE algorithm [26] were acquired from Sanger Box (v3.0); they contained three types of scores, including immune, stromal, and ESTIMATE scores. Using the clusterProfiler package,[27] Kyoto Encyclopedia of Genes and Genomes[28] signaling pathways that *MMP12* may participate in multiple cancers were determined with gene set enrichment analysis; those signaling pathways with the *P*-value <.05 were included.

Validation of MMP12 *mRNA Expression in LUAD*

To validate the *MMP12* expression in LUAD at mRNA levels, LUAD-related public datasets were collected from several public databases: TCGA, Genotype-Tissue Expression, ArrayExpress, and Gene Expression Omnibus. The data retrieval strategy for datasets was "(mRNA or gene) AND lung AND (adenocarcinoma OR [non-small cell])." The inclusion criteria for datasets and their samples were as follows: (1) the samples were sourced from humans; (2) samples from the LUAD group were obtained from pathologically diagnosed LUAD tissues or cells; (3) samples from the control group were obtained from pathologically diagnosed normal lung tissues or cells; (4) the dataset had complete mRNA expression data. The exclusion criteria were as follows: (1) incomplete mRNA expression data; (2) duplicate samples in various datasets. Ultimately, 59 datasets were included in this study and merged to 29 datasets based on the same platform (e.g., GPL10558). Details of the 59 datasets are listed in Supplementary Material 1.

Validation of MMP12 Protein Expression in LUAD

To verify MMP12 expression in LUAD at the level of proteins, in-house tissue microarrays (LUC1021, LUC1502, and LUC481) were purchased from Fanpu Biotech (Guilin, China), including 64 LUAD samples and 24 non-LUAD control samples. The inclusion of samples was as follows: (1) samples were sourced from human LUAD tissues and non-LUAD control lung tissues; (2) samples were pathologically identified; (3) samples were collected from the patients who signed the informed consent. These samples were used in an immunohistochemistry experiment.

We conducted the immunohistochemistry experiment following the instructions of the reagent manufacturer. We used a .01 M citrate buffer solution (pH = 6.0) to wash the dewax and repaired tissue slides to extract the antigen, and we used 3% H_2O_2 to deactivate the endogenous peroxidase. We used the rabbit anti-human MMP12 monoclonal antibody (ab137444, Abcam, UK) in a 1:100 dilution to incubate the prepared tissue slides at 37°C for 30 min. In contrast, we incubated the negative control slides in phosphate buffer overnight. We added a secondary antibody, horseradish peroxidase (D-3004-15, Changdao Biotechnology Co. Ltd., Shanghai, China), to the tissue slides, which were then kept at room temperature (approximately 25°C) for 25 minutes and finally stained with 3,3'-diaminobenzidine for 10 minutes.

The dehydrated and sealed slides were used to assess the degree of MMP12 protein expression under microscopy. Positive and negative anti-MMP12 antibody staining showed diverse colors (brown granules in the nucleus and/or cytoplasm for the positive staining and blue particles for the negative). All specimens of the tissue microarrays were evaluated for positive cells in five randomly selected regions. In the visual field, the anti-MMP12 body staining intensity score was indicated by integers from 0 through 3, representing no staining, light staining, moderate staining, and strong staining. For positive cells, integers from 0 through 4 represented <5%, 5%–25%, 26%–50%, 51%–75%, and >75%, respectively, in the visual field. The final score (i.e., the product of the intensity score and the positive cells score) represents the MMP12 protein level in the LUAD and control tissues.

Validation of the Potential Clinical Value of MMP12 *in LUAD*

The AUC values of the ROC curves and an sROC curve were applied to determine the ability of *MMP12* expression to differentiate LUAD samples and control specimens. We used

the Kaplan–Meier curve to evaluate the prognosis differences between LUAD individuals with a high *MMP12* and those with a low *MMP12* expression. The "survminer" package was employed to determine the high-*MMP12* and low-*MMP12* groups.

Statistical Analysis

We conducted Wilcoxon rank-sum tests to explore the differences in *MMP12* expression between cancer groups and their control groups (e.g., LUAD vs control). The method was also used to detect *MMP12* expression differences in patients with various clinical parameters. Using the "meta" package, differences in *MMP12* expression level between the LUAD group and the non-LUAD group were evaluated with a standardized mean difference (SMD). We used Begg's test to evaluate publication bias, and the *P*-value of less than .1 indicated significant publication bias.[29] All correlation analyses in this study were done with Spearman's rank correlation coefficient. The sROC curve was produced using Stata (v15.0), and the remaining calculations were conducted in R (v4.1.0). Figure 1 shows the overall framework design of this research.

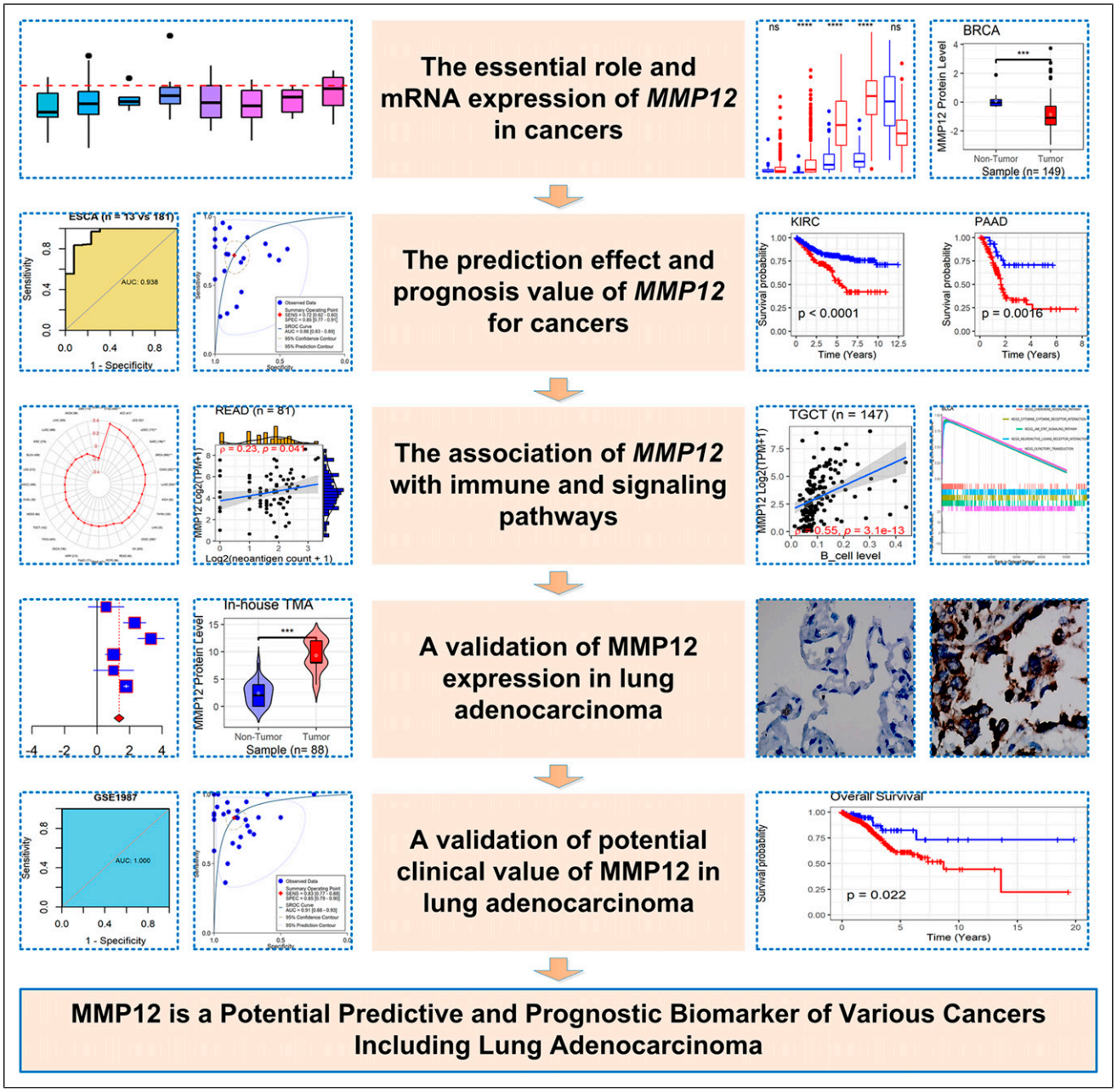

Figure 1. Research overflow of this study.

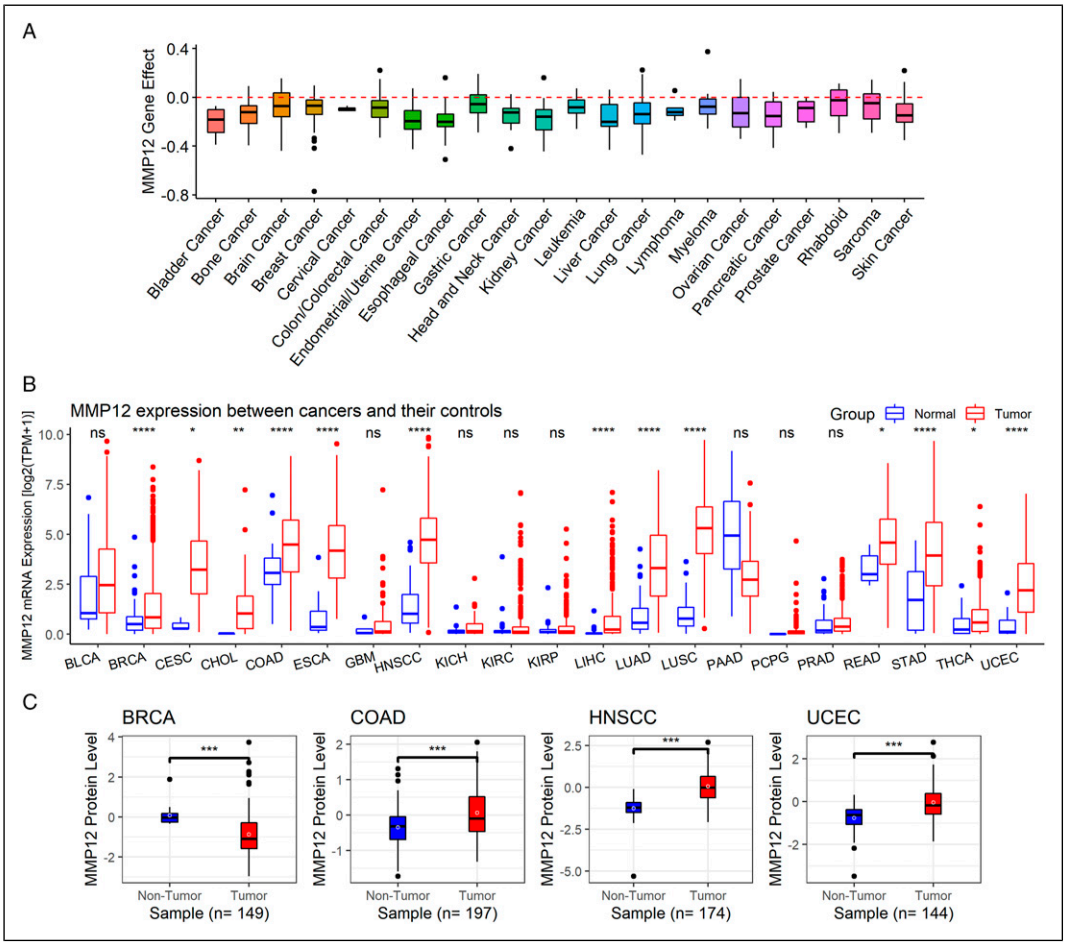

Figure 2. Essential role of *MMP12*, *MMP12* mRNA expression, and MMP12 protein levels in cancers. Panel A: Identification of essential roles of *MMP12* for multiple cancers. Panel B: The differential expression of *MMP12* mRNA between cancers and controls; *P*-value was based on the Wilcoxon rank-sum test with a false discovery rate. Panel C: The differential levels of MMP12 protein between cancers and controls. $^{ns}P \geq .05$; $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$; $^{****}P < .0001$.

Results

Pan-Cancer MMP12 Expression Level

MMP12 is essential for various cancers, particularly esophageal cancer (ESCA) and liver cancer (Figure 2A). *MMP12* mRNA overexpression compared to normal tissues was observed in the cancer tissues of 13 cancer types ($P < .05$), including BRCA, COAD, ESCA, HNSCC, LIHC, LUAD, UCEC, cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), cholangiocarcinoma (CHOL), lung squamous cell carcinoma (LUSC), rectum adenocarcinoma (READ), stomach adenocarcinoma (STAD), and thyroid carcinoma (THCA) (Figure 2B). Furthermore, at the protein level, MMP12 expression was higher in COAD, HNSCC, and UCEC than in normal tissues, while the opposite result was found in BRCA ($P < .05$) (Figure 2C).

Clinical Significance of Pan-Cancer MMP12 Expression

We drew ROC curves to identify the pan-cancer clinical significance of *MMP12*. The results show that 13 of 21 cancer types have an AUC value above .7 (Figure 3A), indicating that *MMP12* has a conspicuous ability to distinguish these cancer tissues from normal tissues. In addition, the AUC in sROC was .86, indicating that *MMP12* has a good ability to distinguish 21 cancer tissues from normal tissues (Figure 3B).

Univariate Cox analysis showed that high *MMP12* expression predicts a poor OS (hazard ratio [HR] > 1, $P < .05$) in adrenocortical carcinoma (ACC), kidney renal clear cell carcinoma (KIRC), and pancreatic adenocarcinoma (PAAD) and a favorable OS in BRCA (HR < 1, $P < .05$) (Table 1). For disease-specific survival, high *MMP12* expression was associated with poor clinical outcomes in ACC, ESCA,

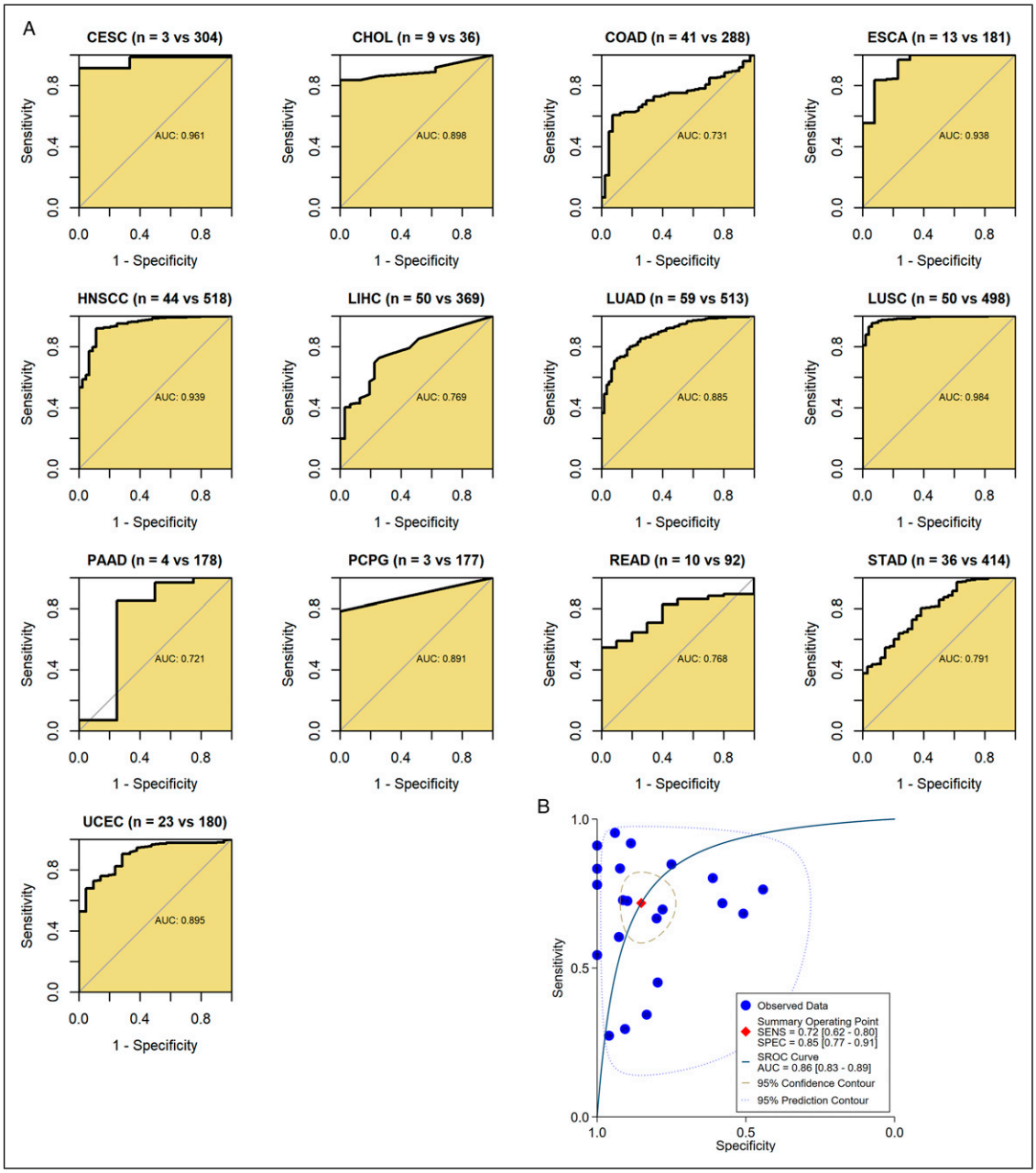

Figure 3. Ability of *MMP12* to differentiate the tumor tissue from the control tissue. Panel A: *MMP12* can accurately distinguish cancer tissues from control tissues in some cancers. Panel B: *MMP12* can well distinguish cancers from controls in 21 cancer types.

kidney chromophobe (KICH), KIRC, and PAAD (HR > 1, $P <$ .05) (Table 1). In addition, high expression of *MMP12* was associated with a poor progression-free interval in ESCA, KIRC, PAAD, sarcoma (SARC), and thymoma (THYM) (HR > 1, $P <$ .05) and favorable progression-free interval in STAD (HR < 1, $P <$ .05) (Table 2). Upregulation of *MMP12* in LUAD, PAAD, and SARC was associated with a decreased disease-free interval (HR > 1, $P <$ .05), while elevated expression of *MMP12* was relevant to an increased disease-free interval in COAD and STAD (HR < 1, $P <$ .05) (Table 2). Finally, we used Kaplan–Meier curves to test *MMP12* expression and prognosis in cancer patients, which validated the above results ($P <$ .05) (Figure 4).

MMP12 expressed variously among the cancer staging levels of 21 cancer types. *MMP12* expression was at high levels in the advanced stages of several cancers, including ACC, ESCA, KIRC, LIHC, and THCA ($P <$ .05) (Figure 5A). In contrast, it expressed at low levels in terminal cancers, including BRCA and COAD ($P <$ .05) (Figure 5A). Other cancers, including bladder urothelial carcinoma (BLCA), CHOL, HNSCC, KICH, kidney renal papillary cell carcinoma

Table 1. Relation of *MMP12* Expression With Overall Survival (OS) and Disease-specific Survival (DSS) of Cancer Patients.

Cancer (sample)	OS HR[a]	*P*-value	Cancer (sample)	DSS HR	*P*-value
ACC (41)	1.555	.002	ACC (39)	6.396	<.001
BLCA (416)	.975	.455	BLCA (401)	.972	.496
BRCA (1143)	.879	.031	BRCA (1115)	.926	.324
CESC (273)	1.072	.253	CESC (272)	1.062	.380
CHOL (40)	.988	.949	CHOL (38)	1.045	.809
COAD (317)	.920	.184	COAD (302)	.920	.339
DLBC (44)	1.035	.865	DLBC (44)	1.210	.488
ESCA (188)	1.099	.119	ESCA (185)	1.217	.007
GBM (114)	.888	.373	GBM (106)	.895	.429
HNSCC (554)	.974	.437	HNSCC (526)	.959	.347
KICH (77)	2.440	.055	KICH (77)	3.341	.017
KIRC (500)	1.282	<.001	KIRC (486)	1.303	<.001
KIRP (240)	1.007	.979	KIRP (238)	1.147	.576
LGG (205)	1.118	.721	LGG (201)	1.175	.606
LIHC (326)	1.121	.086	LIHC (317)	1.078	.390
LUAD (549)	1.042	.230	LUAD (515)	1.005	.913
LUSC (515)	1.000	.989	LUSC (457)	.957	.396
MESO (67)	1.001	.993	MESO (49)	.913	.528
OV (382)	.947	.337	OV (354)	.946	.369
PAAD (176)	1.146	.034	PAAD (170)	1.159	.037
PCPG (93)	1.408	.425	PCPG (93)	1.240	.718
PRAD (518)	.615	.410	PRAD (516)	.961	.951
READ (101)	.939	.664	READ (95)	.885	.596
SARC (219)	1.063	.336	SARC (214)	1.082	.240
SKCM (94)	1.118	.521	SKCM (94)	1.212	.317
STAD (406)	.944	.111	STAD (384)	.915	.065
TGCT (127)	1.647	.123	TGCT (127)	1.670	.129
THCA (503)	1.273	.226	THCA (497)	.255	.127
THYM (106)	1.180	.842	THYM (106)	2.488	.301
UCEC (178)	.915	.472	UCEC (176)	1.066	.657
UCS (54)	1.225	.106	UCS (52)	1.203	.164
UVM (33)	1.417	.082	UVM (33)	1.480	.054

[a]Notes: hazard ratio.

(KIRP), LUAD, LUSC, mesothelioma (MESO), PAAD, READ, skin cutaneous melanoma (SKCM), STAD, testicular germ cell tumor (TGCT), and uveal melanoma (UVM), showed little difference in *MMP12* expression between various stages (Figure 5A). Furthermore, we observed that *MMP12* expression did not differ significantly by age and gender in most cancers (Supplementary Materials 2 and 3).

Relationship Between the Expression of Immune Gene MMP12 *and Genomic Heterogeneity*

TMB is related to the number of tumor cell mutations; a high level of TMB can induce the body to produce neoantigens and cause more immune cells to play a role in immune recognition.[30] *MMP12* expression was positively correlated with TMB ($P < .05$) in STAD, ACC, uterine carcinosarcoma (UCS), UCEC, SARC, BRCA, COAD, LUAD, and CESC (Figure 5B).

Microsatellites, because they are short-chain repetitive DNA sequences, are prone to MSI when they are affected by mismatch repair.[31] The expression of *MMP12* was positively correlated with COAD and STAD but negatively correlated with prostate adenocarcinoma, HNSCC, LUSC, and TGCT ($P < .05$) (Figure 5C).

DNA damage increases neoantigens on the surface of tumor cells, which benefits immune cells in recognizing and killing tumor cells.[32] Our research found a weak correlation between *MMP12* expression and cancer neoantigens in READ and COAD ($P < .05$) (Figure 5D).

Correlation Assessment of MMP12 *Expression and the Immune Microenvironment*

MMP12 expression was correlated with the degree of six types of immune cell infiltration in various cancers (Figure 6A and Supplementary Material 4). Notably, *MMP12* expression was

Table 2. Relation of *MMP12* Expression With Progression-free Interval (PFI) and Disease-free Interval (DFI) of Cancer Patients.

Cancer (sample)	PFI HR[a]	*P*-value	Cancer (sample)	DFI HR	*P*-value
ACC (40)	1.008	.965	ACC (20)	165.846	.223
BLCA (415)	1.003	.928	BLCA (189)	.999	.995
BRCA (1142)	.973	.632	BRCA (975)	.988	.873
CESC (276)	.960	.507	CESC (173)	.858	.147
CHOL (40)	.884	.450	CHOL (29)	1.019	.910
COAD (314)	.934	.247	COAD (121)	.684	.020
DLBC (43)	.930	.697	DLBC (26)	.366	.119
ESCA (185)	1.189	.002	ESCA (91)	1.219	.055
GBM (113)	.991	.947	HNSCC (134)	1.202	.072
HNSCC (553)	.993	.839	KICH (36)	.027	.538
KICH (77)	2.011	.089	KIRC (112)	.764	.582
KIRC (489)	1.202	.009	KIRP (133)	1.279	.463
KIRP (237)	1.048	.810	LGG (52)	1.083	.894
LGG (204)	.660	.207	LIHC (274)	1.056	.532
LIHC (326)	1.061	.328	LUAD (331)	1.160	.003
LUAD (545)	1.037	.259	LUSC (316)	.950	.423
LUSC (515)	.981	.638	MESO (11)	1.987	.085
MESO (65)	.966	.783	OV (191)	.967	.632
OV (382)	.916	.087	PAAD (71)	1.382	.001
PAAD (175)	1.127	.047	PCPG (77)	2.092	.469
PCPG (92)	1.444	.084	PRAD (367)	.534	.125
PRAD (518)	1.155	.330	READ (32)	1.185	.620
READ (100)	.962	.760	SARC (129)	1.223	.002
SARC (216)	1.160	.003	STAD (252)	.864	.033
SKCM (93)	1.125	.455	TGCT (101)	1.004	.967
STAD (409)	.903	.009	THCA (357)	1.409	.110
TGCT (125)	.992	.923	UCEC (126)	.729	.077
THCA (502)	1.086	.575	UCS (26)	.894	.766
THYM (106)	2.175	.029			
UCEC (178)	.899	.313			
UCS (54)	1.211	.103			
UVM (32)	1.186	.389			

[a]Notes: hazard ratio.

significantly associated with the infiltration levels of B cells and dendritic cells in CHOL, TGCT, and READ ($P < .05$) (Figure 6A). Moreover, the expression levels of *MMP12* were relevant to the immune microenvironment (Figure 6B and Supplementary Material 5). Among THCA, COAD, READ, and UVM, *MMP12* expression had the strongest relationship with the stromal score in COAD, and the relationship of *MMP12* expression with immune and ESTIMATE scores was conspicuous in READ and COAD (Figure 6B).

MMP12 Expression and Potential Signaling Pathways

The results of gene enrichment analysis show that *MMP12* may participate in 31 potential molecular mechanisms in 33 cancers. The analysis results of 12 cancers (BLCA, BRCA, CHOL, COAD, HNSCC, KIRP, LIHC, LUSC, MESO, pheochromocytoma and paraganglioma, READ, and THCA) suggest that *MMP12* is associated with at least five signaling pathways, indicating that *MMP12* is likely to regulate the occurrence and development of cancer through these pathways, such as the cytokine–cytokine receptor interaction and the chemokine signaling pathway (Figure 7 and Supplementary Material 6).

Overall Expression Level of MMP12 in LUAD

To further explore the findings regarding *MMP12* in pan-cancer analysis, we examined the comprehensive expression level of *MMP12* in LUAD. Among the 29 collected datasets, *MMP12* mRNA was upregulated in LUAD (SMD = 1.35; 95% CI [1.04–1.66]) (Figure 8A), and no significant publication bias was found using Begg's test ($P > .1$, Supplementary Material 7). A Wilcoxon rank-sum test also revealed

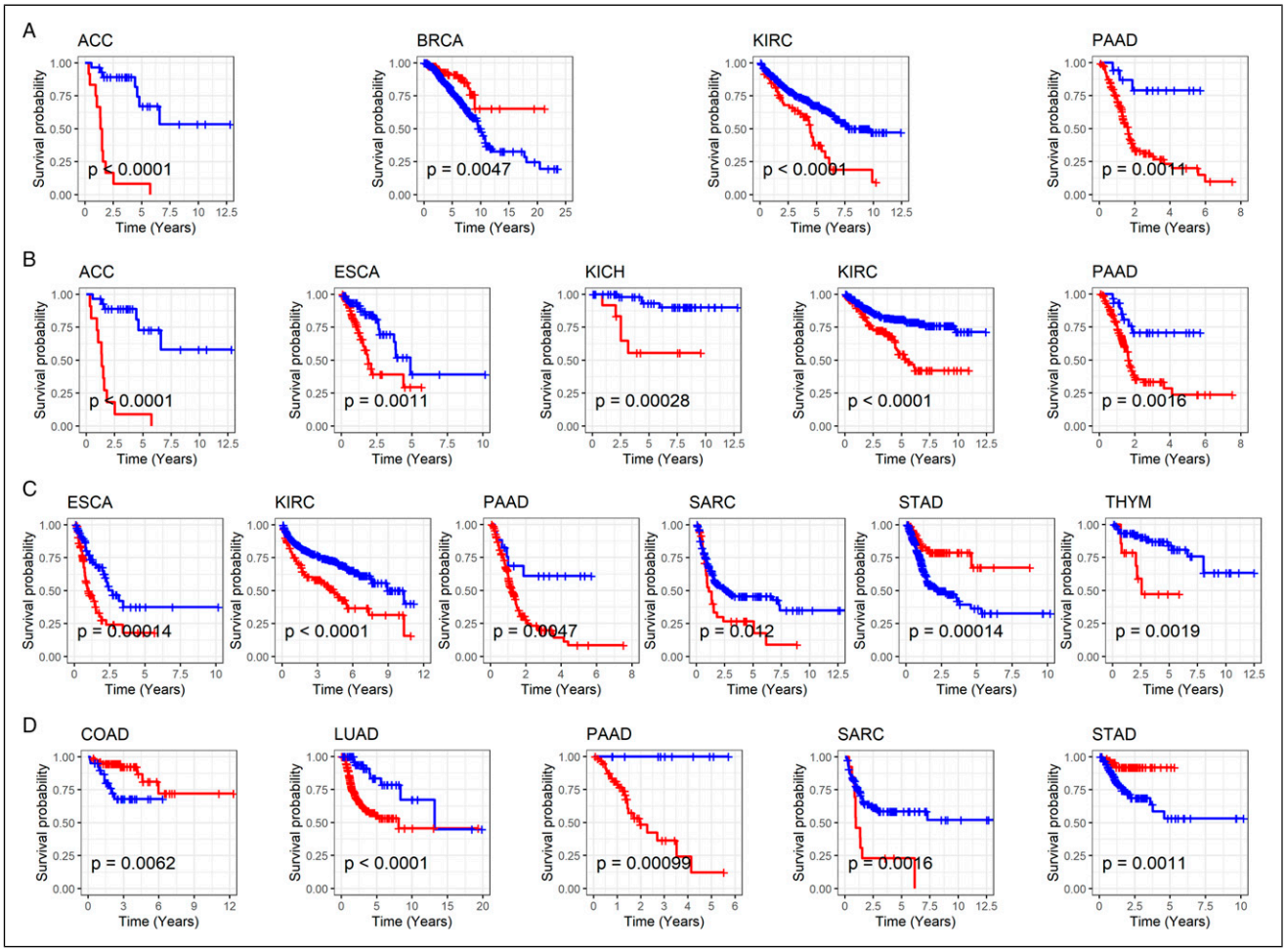

Figure 4. Relation of *MMP12* expression with overall survival (A), disease-specific survival (B), progression-free interval (C), and disease-free interval (D) of cancer patients. The red and blue curves represent the high-*MMP12* expression group and low-*MMP12* expression group, respectively.

overexpression of *MMP12* mRNA in LUAD ($P < .05$) (Figure 8B). In addition, using in-house tissue microarrays, no positive MMP12 protein staging was found in the alveoli and bronchi of the control tissue (Figures 9A and C). However, a high level of MMP12 protein was observed in the LUAD tissue (Figures 9B and D), which was confirmed by the further Wilcoxon rank-sum test ($P < .05$) (Figure 8C).

Clinical Value of MMP12 in LUAD

Among the 29 included datasets, ROC curves showed that *MMP12* mRNA expression in LUAD exceeded moderate accuracy in 19 datasets (AUC >.75) (Figure 10A). The sROC analysis revealed that *MMP12* mRNA expression accurately distinguished LUAD from non-LUAD (sensitivity = .83, specificity = .85; AUC = .91) (Figure 10B). Furthermore, using OS curves, a lower *MMP12* expression in LUAD patients tended to predict a good prognosis ($P = .022$) (Figure 10C).

Discussion

Cancer seriously threatens human health as one of the leading causes of death, so it is meaningful to explore novel markers for identifying the disease status and prognosis of cancer patients.[33] As an immune gene, *MMP12* can regulate inflammatory responses and play an essential role in specific cancers.[34] However, there was a dearth of a comprehensive pan-cancer analysis of *MMP12*.

This study used numerous multi-center samples and multiple approaches to explore the pan-cancer expression, clinicopathology, potential mechanisms, and clinical significance of MMP12. *MMP12* was differently expressed in various cancers, and its expression level was related to TMB, MSI, neoantigen counts, and immune microenvironments in some cancers. The relationship between *MMP12* expression and the potential molecular mechanism, prognosis, and clinical significance was also investigated in various cancers. Furthermore, we analyzed the expression of *MMP12* in LUAD to support pan-cancer research by using public database

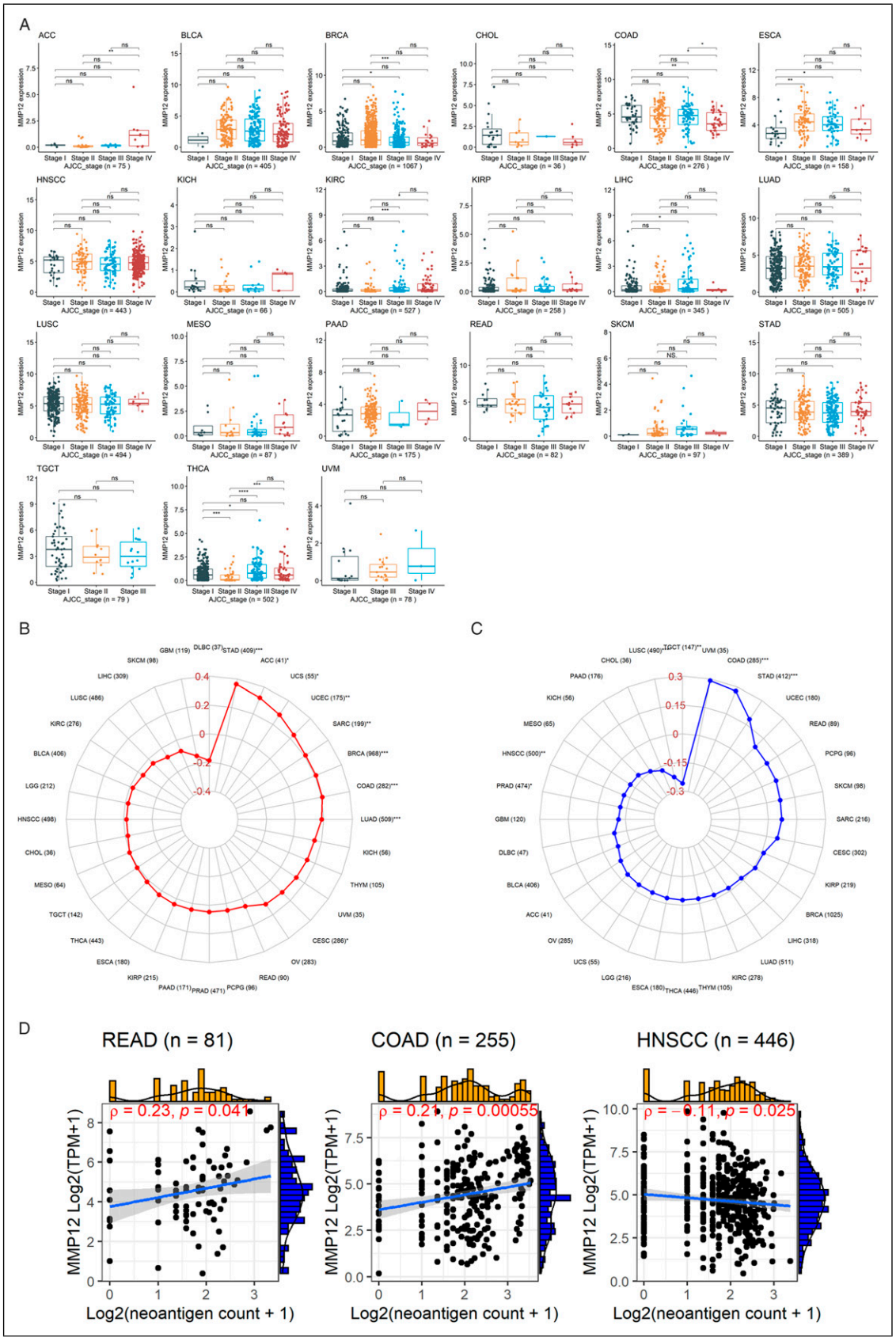

Figure 5. Relation of *MMP12* expression with tumor stages (panel A), tumor mutational burden (panel B), microsatellite instability (panel C), and neoantigen (panel D) of cancer patients. *P < .05; **P< .01; ***P < .001.

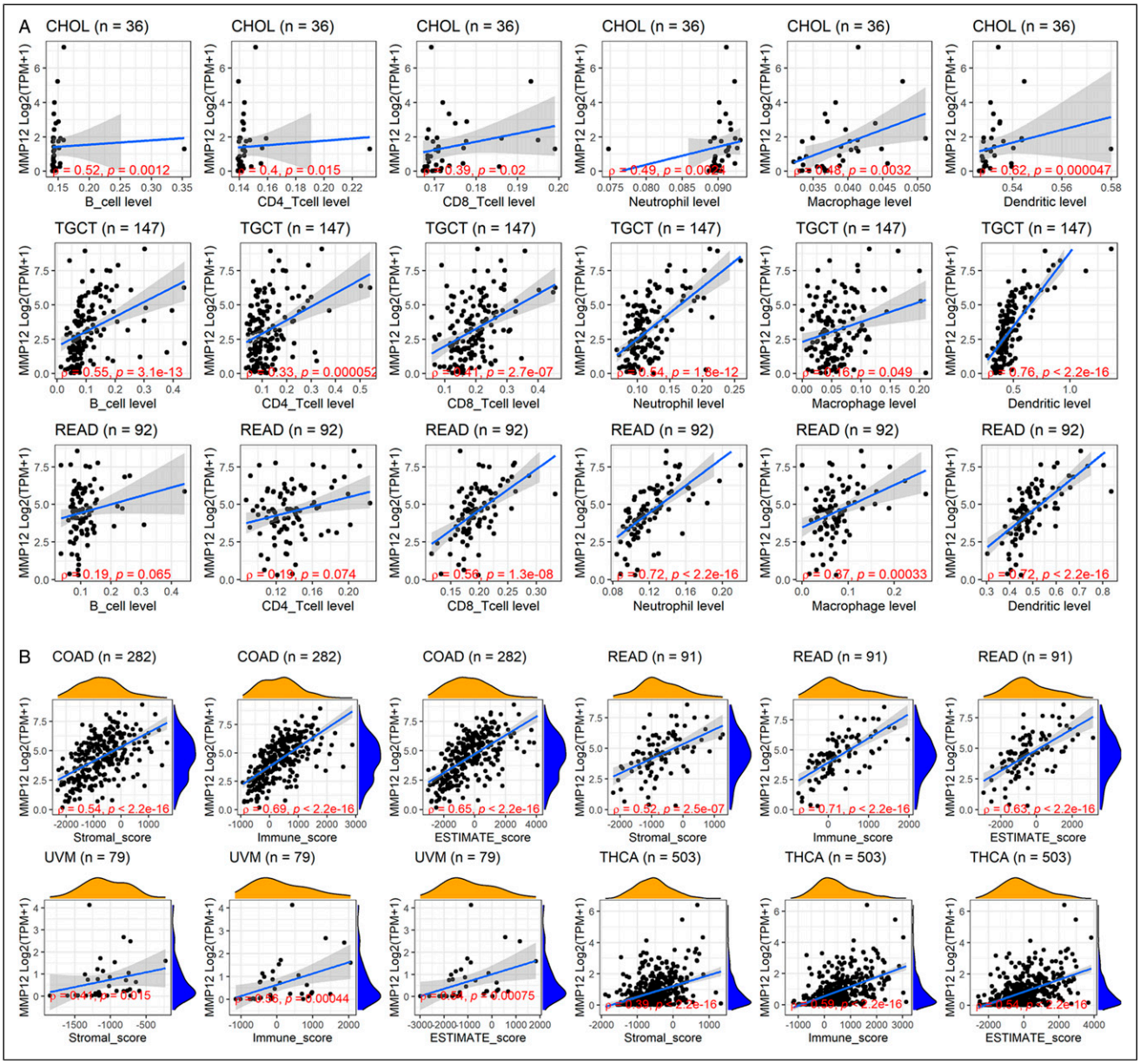

Figure 6. Relation of *MMP12* expression and infiltration levels of immune cells. Panel A: TIMER algorithm; B: ESTIMATE algorithm.

datasets and in-house tissue microarrays to comprehensively determine that MMP12 mRNA and protein expression are upregulated in LUAD.

MMP12 is highly expressed in various cancers and plays a critical role in these diseases. Studies report that elevated *MMP12* expression represents a poor prognosis for BRCA and that the gene was positively correlated with neutrophils and dendritic cells.[35] In terms of COAD, overexpressed *MMP12* promotes tumors and is associated with a poor OS of patients.[36] In HNSCC, increasing *MMP12* expression promotes cancer cell proliferation, migration, invasion, and the metastasis of cancer cells.[37] Overexpressed *MMP12* mRNA may promote the progression and metastasis of ESCA, LIHC, and LUSC and is associated with a poor prognosis in cancer patients.[25,38,39] Additionally, a high expression of *MMP12* mRNA in LUAD promotes lymph node metastasis.[12] Similarly, our study detected that *MMP12* is differently expressed in various cancers and that *MMP12* is associated with patients' cancer status and prognosis in several cancers. Briefly, in regard to expression between cancer and normal tissues, *MMP12* mRNA is overexpressed in 13 cancers (CESC, etc.), and higher MMP12 protein levels are observed in COAD, HNSCC, and UCEC. Some of these findings have not previously been reported. For instance, our study for the first time

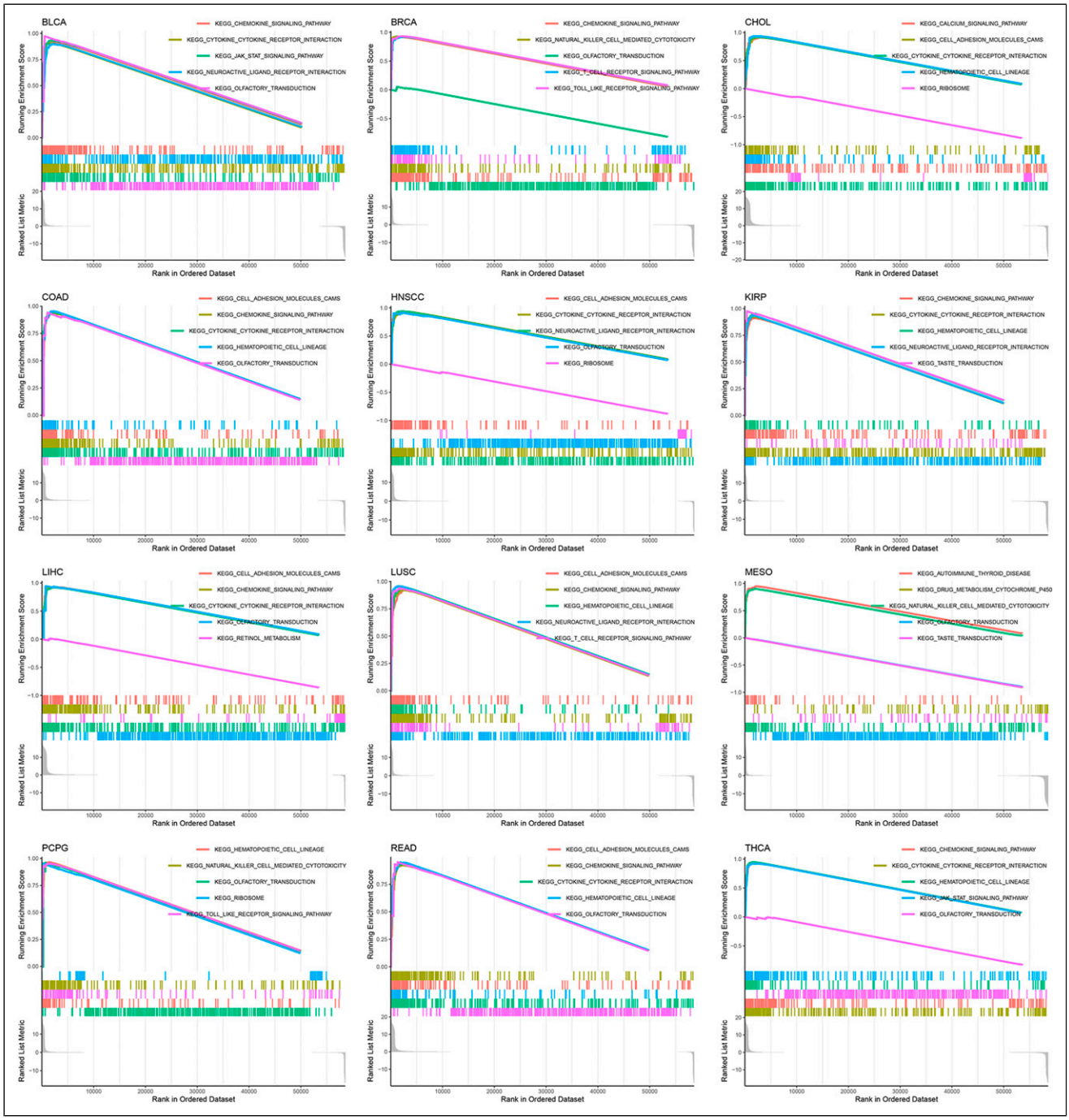

Figure 7. Potential signaling pathways of *MMP12* may affect multiple cancers.

describes an elevated MMP12 expression in UCEC, including mRNA and protein levels, indicating the novelty of our findings. Notably, our study found that, in contrast to *MMP12* mRNA expression, MMP12 protein levels are lower in BRCA than in control tissues; this may be related to various factors, such as translation-level regulation and post-translational modification regulation,[40-42] which requires further experimental verification. Such a phenomenon also indicates that investigations of gene expression should at least focus on both mRNA and protein levels rather than on only one of them. Regarding cancer status prediction, our study indicates that *MMP12* makes it feasible to distinguish 21 cancer tissues from normal tissues. For prognosis, *MMP12* expression is a prognosis risk factor in multiple cancers, including ACC,

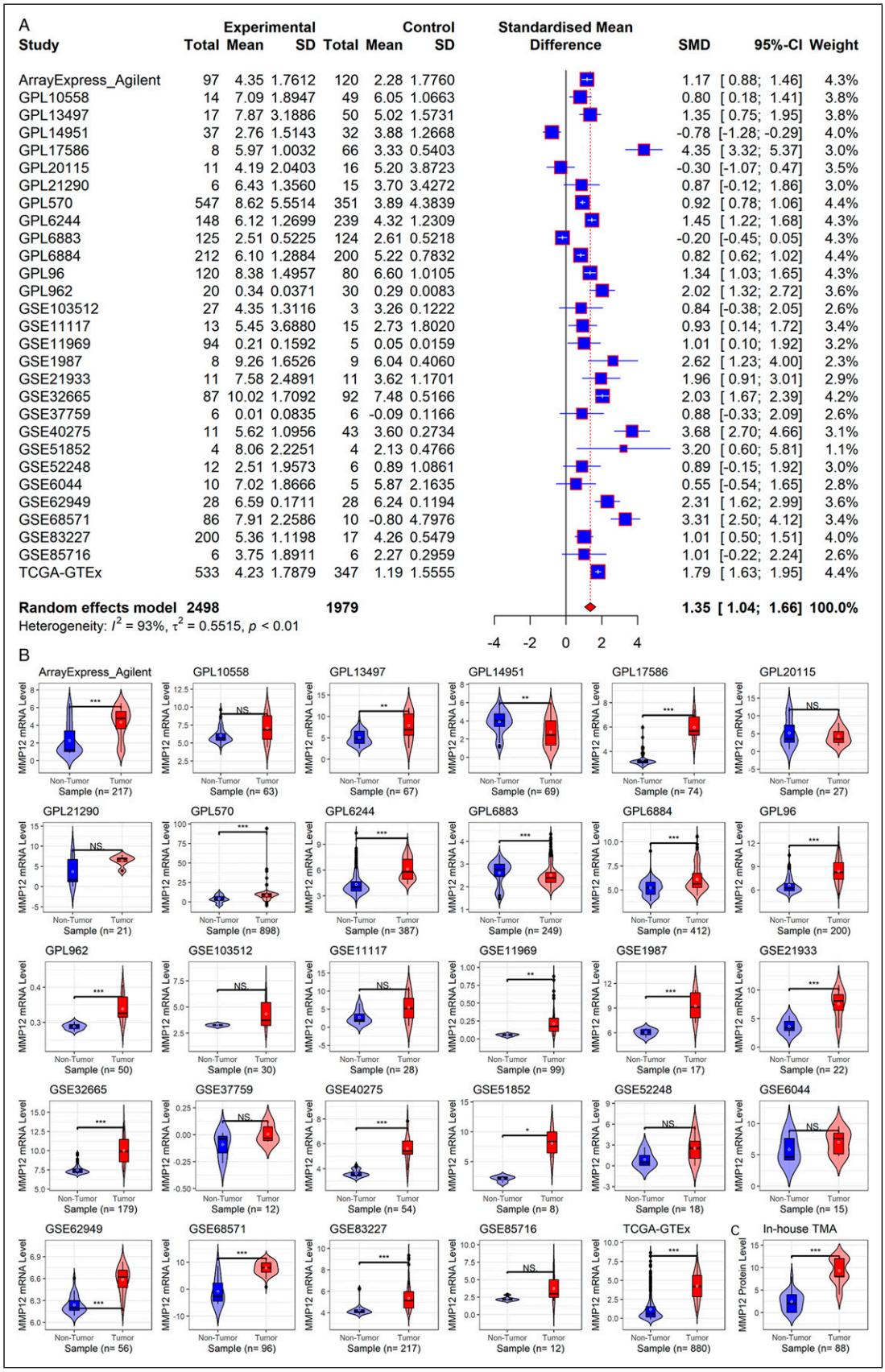

Figure 8. MMP12 mRNA and protein levels in LUAD. Panel A: *MMP12* mRNA expression forest plot in LUAD and control tissues. Panel B: Violin plots of *MMP12* mRNA expression in each dataset. Panel C: The violin plot of MMP12 protein levels. The *P*-value is calculated based on the Wilcoxon rank-sum test. $^{NS}P \geq .05$; $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$.

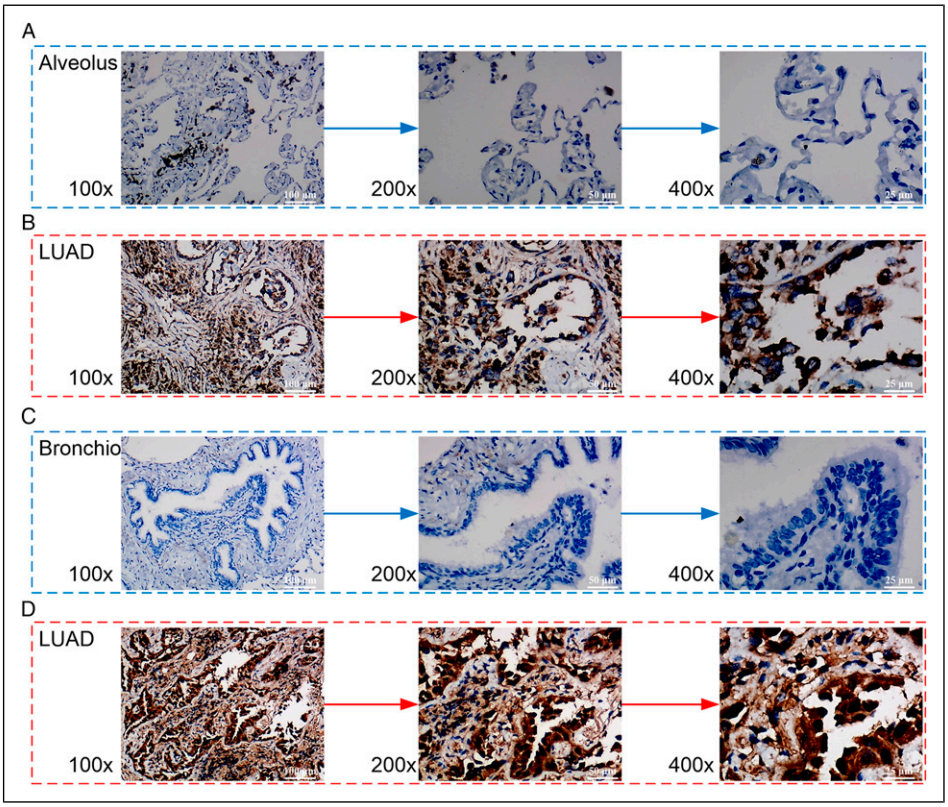

Figure 9. Microscopic images of MMP12 protein levels in control tissues (panels A and C) and LUAD tissues (panels B and D). The numerical value in the bottom left corner of each image represents the magnification of the microscope. The white numerical value in the lower right corner of each image represents the scale.

ESCA, KICH, KIRC, LUAD, PAAD, SARC, and THYM. Notably, *MMP12* expression may be a prognosis protective factor for patients with BRCA and STAD; this may be attributed to the beneficial effects of *MMP12* on macrophage development and suppression of angiogenesis in these two types of cancer, leading to its anti-tumor function.[16,43] This indicates that the clinical significance may not be consistent across different types of cancer. Altogether, elevated *MMP12* expression may serve as a potential predictive and prognostic biomarker for various cancers.

MMP12 may act as a potential target gene for immunotherapy in cancers. Previously, highly expressed *MMP12* has been identified as an immune gene promoting the proliferation of immune cells (e.g., B cells and dendritic cells), and the gene stimulates the host immune system to cause immune responsiveness.[44,45] TMB and MSI are considered predictive biomarkers for immunotherapy, as they may contribute to the generation of neoantigens and thus stimulate the host immune system to recognize and clear neoantigens in the immune microenvironment.[32,46,47] In our study, *MMP12* expression is related to TMB, MSI, and neoantigen numbers in various cancers (e.g., STAD and COAD), implying its participation in the immune microenvironment. Such a conclusion is also supported by the correlation (mainly positive) of *MMP12* expression with several immune cells (e.g., B cells and dendritic cells) and immune scores (e.g., ESTIMATE algorithm scores). Moreover, *MMP12* may participate in the occurrence and development of cancers through molecular signaling pathways, such as the cytokine–cytokine receptor interaction and the chemokine signaling pathway as has been verified in colorectal cancer and breast cancer.[48,49] The abovementioned findings suggest that *MMP12* may have the potential to act as a tumor marker in tumor immunotherapy.

Some pan-cancer findings regarding *MMP12* are verified in LUAD. With relatively small samples (n = 52), Lv et al. determined that MMP12 protein levels are higher in LUAD tissues than in normal tissues, and they report that MMP12 can promote the proliferation and growth of cancer cells and increase their invasiveness.[12] Employing a large sample (n = 4565) from multiple centers and in house, we identified the upregulation of MMP12 expression in LUAD at both the mRNA and protein levels. We also show for the first time that *MMP12* mRNA expression has the potential to distinguish LUAD with considerable accuracy. Furthermore, *MMP12* expression serves as a risk prognosis factor for patients with LUAD. Thus, *MMP12* may play an

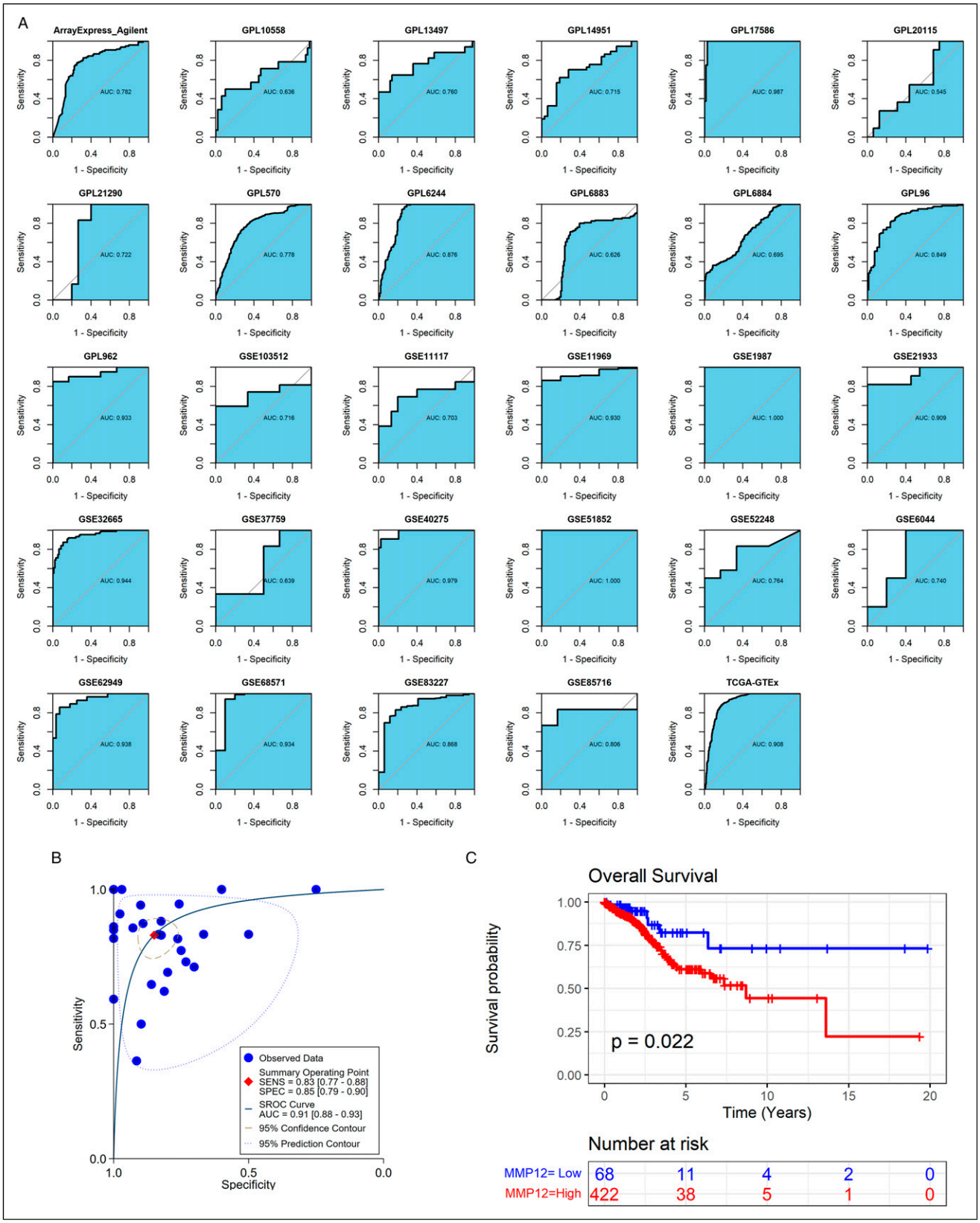

Figure 10. Clinical value of *MMP12* in LUAD. Panels A–B: *MMP12* can well distinguish LUAD from controls. Panel C: Kaplan–Meier curves of the relation of *MMP12* expression with overall survival of LUAD patients.

important role in LUAD as a predictive and prognostic biomarker. Based on this, a promising application of *MMP12* in LUAD involves detecting *MMP12* mRNA expression levels during pathological diagnosis of potential patients with LUAD; this approach may contribute to the evaluation of patient prognosis.

This study has a few limitations. For example, for various cancers, we need to collect more samples to determine MMP12 expression at the protein level. We also need to include more clinicopathological parameters to explore whether other clinicopathological variables may impact our results. More in vivo and in vitro experiments are needed to investigate the pan-cancer mechanisms of *MMP12*. In addition, the pan-cancer examination of the relationship between prognosis and *MMP12* expression was based on retrospective data; thus, prospective studies are needed for further validation.

Conclusion

This study comprehensively explores *MMP12* in multiple cancers. *MMP12* is highly expressed in most cancers. The gene may serve as a novel biomarker for the prediction and prognosis of numerous cancers.

Appendix

Abbreviations

ACC	adrenocortical carcinoma
AUC	area under the curve
BLCA	bladder urothelial carcinoma
BRCA	breast-invasive carcinoma
CESC	cervical squamous cell carcinoma and endocervical adenocarcinoma
CHOL	cholangiocarcinoma
COAD	colon adenocarcinoma
DLBC	lymphoid neoplasm diffuse large b-cell lymphoma
ESCA	esophageal carcinoma
GBM	glioblastoma multiforme
HNSCC	head and neck squamous cell carcinoma
HR	hazard ratio
KEGG	Kyoto Encyclopedia of Genes and Genomes
KICH	kidney chromophobe
KIRC	kidney renal clear cell carcinoma
KIRP	kidney renal papillary cell carcinoma
LGG	brain lower grade glioma
LIHC	liver hepatocellular carcinoma
LUAD	lung adenocarcinoma
LUSC	lung squamous cell carcinoma
MESO	mesothelioma
MMP12	matrix metalloproteinase 12
OS	overall survival
OV	ovarian serous cystadenocarcinoma
PAAD	pancreatic adenocarcinoma
PCPG	pheochromocytoma and paraganglioma
PRAD	prostate adenocarcinoma
READ	rectum adenocarcinoma
ROC	receiver operating characteristic
SARC	sarcoma
SKCM	skin cutaneous melanoma
sROC	summary receiver operating characteristic
SMD	standardized mean difference
STAD	stomach adenocarcinoma
OS	overall survival
TCGA	The Cancer Genome Atlas
TGCT	testicular germ cell tumors
THCA	thyroid carcinoma
THYM	thymoma
TMB	tumor mutation burden
MSI	microsatellite instability
UCEC	uterine corpus endometrial carcinoma
UCS	uterine carcinosarcoma
UVM	uveal melanoma.

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Authors' Contributions

Study design and draft writing: Guo-Sheng Li, Yu-Xing Tang, Jin-Liang Kong, Hua-Fu Zhou, and Gang Chen. Data acquisition: Guo-Sheng Li, Yu-Xing Tang, Wei Zhang, Jian-Di Li, and He-Qing Huang. Data analysis and interpretation: Guo-Sheng Li, Yu-Xing Tang, Wei Zhang, Jian-Di Li, He-Qing Huang, Jun Liu, Zong-Wang Fu, and Rong-Quan He. All authors were involved in reading and revising the draft and approved the final version for publication.

Declaration of Conflicting Interests

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Ethical Statement

Ethical Approval

This study was approved by the medical ethics review committee of the First Affiliated Hospital of Guangxi Medical University (No. 6 Shuangyong Road, Nanning, China) on October 26, 2021, with the approval number 2021(KY-E-246). Informed consent was signed by all patients involved in the in-house data.

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Data Availability Statement

The data that support the findings of pan-cancer analyses are available in public databases, including DepMap Portal (https://depmap.org/portal/), Xena database (https://xenabrowser.net/datapages), Proteomic Data Commons (https://pdc.cancer.gov), Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/gds/), and TCGA Research Network (www.cancer.gov/tcga). Data on in-house tissue samples used during the current study are available from the corresponding author upon reasonable request.

Supplemental Material

Supplemental material for this article is available online.

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