European Review for Medical and Pharmacological Sciences 2020; 24: 6605-6615

High expression of MMP14 is associated with progression and poor short-term prognosis in muscle-invasive bladder cancer

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Abstract. – OBJECTIVE: To evaluate the short-term prognostic value of matrix metalloproteinase 14 (MMP14) in muscle-invasive bladder cancer (MIBC).

PATIENTS AND METHODS: Expression of MMP14 and clinical information from The Cancer Genome Atlas (TCGA) were mined in MIBC patients to analyse expression differences and conduct survival analyses. The mRNA and protein expression levels of MMP14 in other tumours were analysed using Gene Expression Profiling Interactive Analysis (GEPIA) and The Human Protein Atlas. The expression level of MMP14 in bladder cancer (BC) cell lines and clinical samples and its clinical significance were indicated using quantitative Real Time-Polymerase Chain Reaction (qRT-PCR), Western blotting, and immunohistochemistry. The biological functions of MMP14 were investigated by examining cell migration using *in vitro* wound-healing assays and cell invasion using transwell invasion assays. Survival analyses were conducted with the collected clinical follow-up data.

RESULTS: Our study revealed that MMP14 is highly expressed in MIBC based, on both TCGA derived data and our clinical tissues ($p<0.05$). MMP14 is also highly expressed in head and neck cancer, renal cancer, pancreatic cancer and other cancers, as analysed using GEPIA and The Human Protein Atlas ($p<0.05$). Survival analyses of the TCGA data and our clinical follow-up data revealed high expression of MMP14 indicates a poor short-term prognosis in MIBC ($p<0.05$). Furthermore, downregulation of MMP14 suppressed BC cell invasion and migration abilities *in vitro*. MMP14 expression was closely correlated with tumour metastasis ($p<0.05$). T stage [hazard ratio (HR)=1.412, 95% confidence interval (CI)=1.121-1.779, $p=0.003$] and metastasis (HR=2.256, 95% CI=1.242-4.100, $p=0.008$) were unfavourable prognostic factors in BC patients.

CONCLUSIONS: In MIBC, MMP14 expression is upregulated and closely associated with disease progression and poor short-term prognosis.

Key Words:
MMP14, Muscle-invasive bladder cancer, Progression, Prognosis.

Introduction

Bladder cancer (BC) is the most common malignant tumour of the urinary system, and 81,400 new cases are estimated to occur in the United States during 2020[1]. The occurrence of BC is closely related to smoke exposure, and generally has a high rate of mortality[2,3]. BC can be divided into non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC), for which the depth of invasion determines the tendency for distant metastasis[4]. Although approximately 70% of patients present with NMIBC, others will either initially present with MIBC or ultimately develop MIBC[5]. NMIBC is mostly managed by transurethral resection of the bladder with postoperative intravesicular chemotherapeutic/immunological therapy. Regardless, MIBC is always managed by radical cystectomy and has a poorer prognosis than NMIBC[6]. Indeed, half of the patients who receive a radical cystectomy achieve an average survival time of only five years[7]. Despite recent significant

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advances in the treatment of metastatic BC, such as the emergence of drugs targeting programmed cell death-1[8], metastatic BC remains largely incurable.

Metastasis is the leading cause of death among BC patients. Tumour metastasis is a complex and multi-step process involving multiple biological behaviours, including degradation of the extracellular matrix (ECM)[9], and the matrix metalloproteinase (MMP) family plays a key role in this process. In addition to regulating the degradation of the ECM, MMPs also promote the adhesive movement of tumour cells[10]. Matrix metalloproteinase 14 (MMP14), an important member of the MMP family, is involved in various biological behaviours during physiological and cancerous processes[11]. MMP14 participates in tumour invasion and metastasis by activating MMP2 to mediate ECM degradation, and by stimulating tumour neovascularisation[12,13]. Studies[14-16] in recent years have reported high MMP14 expression in several tumour types. Besides, increased MMP14 expression has been associated with poor survival and metastatic disease in oesophageal squamous cell carcinoma, gastric cancer, and epithelial ovarian cancer. Downregulation of MMP14 activity inhibits the growth of the primary tumour in breast carcinomas and gastric cancer cells[17,18]. However, the prognostic value of MMP14 in BC has not been fully elucidated.

In this study, we investigated the prognostic value of MMP14 in MIBC. Bioinformatic data mining and analysis were performed on MMP14's clinical significance from public databases, and cell behaviour experiments were performed *in vitro*. MMP14 expression and survival outcomes were verified in clinical samples with follow-up. Our study demonstrated that high MMP14 expression might serve as a marker of progression and poor short-term prognosis in MIBC.

Patients and Methods

Bioinformatic Mining and Analysis

Data of genomic alteration and survival information for patients with MIBC were downloaded from UCSC Xena[19] (http://xena.ucsc.edu/), which is a functional genomics browser for analysing public data hubs containing The Cancer Genome Atlas (TCGA). Data for a total of 374 primary tumour and 19 normal tissue samples are included in the dataset **(Supplementary File 1)**. Patients were divided into high or low MMP14 expression groups by the median value. Correlations between MMP14 expression and disease severity were analysed, and survival analysis was performed for cases with complete follow-up information.

mRNA expression of MMP14 in other tumours was analysed by using the online tool Gene Expression Profiling Interactive Analysis (GEPIA)[20] (http://gepia.cancer-pku.cn/), an interactive web server for cancer expression profile data containing RNA sequencing (RNA-Seq) data from TCGA and the Genotype-Tissue Expression (GTEx) project. The protein expression of MMP14 in other tumours was analysed using The Human Protein ATLAS[21] (https://www.proteinatlas.org/), which is a map of the tissue and cell distributions of the human proteome.

Human Tissue Samples

A total of 113 patients from Peking University First Hospital, between July 25, 2006, and December 21, 2012, with pathologically confirmed BC, were included in this study. Tumour and adjacent normal bladder tissues of the patients were immediately snap-frozen in liquid nitrogen following surgical resection. The detailed clinicopathological information and follow-up data of the patients are summarised in **Supplementary File 2**. This study was approved by the Ethics Committee of Peking University First Hospital. Written informed consent was obtained from all patients.

Immunohistochemistry

Differences in protein expression of MMP14 between tumour tissue and normal tissue was evaluated by immunohistochemistry. Based on standard procedures, the tissue microarray was incubated with a primary antibody against MMP14 (No. ab51074; Abcam, Cambridge, Cambridgeshire, UK) overnight at 4°C. Subsequently, the tissue microarray was incubated with a secondary antibody (No. ab205718; Abcam, Cambridge, Cambridgeshire, UK), and then, stained with 3,3′-diaminobenzidine (DAB; No. ZLI-9018; ZSGB-BIO, Xicheng, Beijing, China). The immunohistochemical results for MMP14 expression were evaluated by the semi-quantitative scoring method according to protocols described in a previous study[14]. This method comprehensively judges the staining intensity and positive cell rate scores.

Cell Culture

Immortalized human bladder cells (SV-HUC-1) and human BC cells (5637, T24, J82, BIU-87, and UMUC-3) were obtained from American Type

Culture Collection (ATCC; Manassas, VA, USA). All cell lines were cultured in Dulbecco's Modified Eagle's Medium (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Gibco, Carlsbad, CA, USA), 1% antibiotics (100 UI/mL penicillin and 100 μg/mL streptomycin sulphate) and 1% glutamate (Gibco, Carlsbad, CA, USA) under conditions detailed in the ATCC recommendations.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Total RNA was isolated from cell lines using TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. RNA samples with 260/280 ratios of 1.8-2.0 were used for further experiments. cDNA was produced using reverse transcription (TansGen, Xicheng, Beijing, China). qRT-PCR was performed using an ABI PRISM 7000 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. The primers used in this study are shown in **Supplementary File 3**. All reactions were performed at least three times.

Western Blot Analysis

Cells were lysed in 1% NP-40 lysis buffer (No. FNN0021; Thermo Fisher Scientific, Waltham, MA, USA) containing 1 mM phenylmethylsulphonyl fluoride (No. ab141032; Abcam, Cambridge, Cambridgeshire, UK). Protein samples were separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Immunoreactive bands were visualised using Immobilon Western Kit (Millipore, Billerica, MA, USA) according to the manufacturer's instructions with a Syngene G: BOX imaging system (Syngene, Frederick, MD, USA). The membrane containing the proteins was blocked and incubated with anti-human MMP14 (No. ab51074; Abcam, Cambridge, Cambridgeshire, UK) and GAPDH (sc-25778; Santa Cruz Biotechnology, Santa Cruz, NM, USA) antibodies overnight at 4°C, followed by a horseradish peroxidase-labelled secondary antibody incubation (sc-2004; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Signals were detected by enhanced chemiluminescence (ECL; Western Blotting Detection Reagents; GE Healthcare, Milwaukee, WI, USA) and visualized using the G:BOX Chemi gel documentation system (Syngene, Frederick, MD, USA).

SiRNAs and Transfection

We knocked down MMP14 expression by transfecting predesigned and functionally verified siRNAs directed against human MMP14 (siMMP14#1, SI3488-3510-S, oligo sequence 5′-AAUAUACAUUAAACAAAACAA-3′; and siMMP14#2, SI3488-3510-A, oligo sequence 5′-GUUUUGUUUAAUGUAUAUUUU-3′; TSINGKE, Changping, Beijing, China) using Lipofectamine™ 3000 transfection reagent (Thermo Fisher Scientific, Waltham, MA, USA) and following the manufacturer's instructions. Cells treated with non-targeting siRNAs and without these siRNAs were used as controls. The knockdown efficiency of MMP14 was assessed by qRT-PCR after 24 h.

Transwell Cell Invasion Assay

A cell invasion assay was conducted in a 24-well transwell chamber (CoStar, Alexandria, VA, USA). The medium containing 60 μL of Matrigel (Corning Inc., Chemung County, NY, USA) and approximately 2000 cells were added to the transwell inserts (Corning Inc., Chemung County, NY, USA). Adherent cells on the bottom surface were stained with 0.5% crystal violet after 72 h. A microscope (Leica DM IL; Leica Microsystems Inc., Wetzlar, Hesse-Darmstadt, Germany) equipped with a digital camera (Leica DFC300FX; Leica Microsystems Inc., Wetzlar, Hesse-Darmstadt, Germany) was used to photograph and count the number of cells on the bottom surface.

Wound-Healing Assay

Cell migration capacity was assessed *via* a wound-healing assay. Approximately 3×10^5 cells were seeded into the wells of a 12-well plate and grown to nearly 90% confluency. After transient transfection, artificial gaps were produced with a 200 μL sterile pipette tip. A microscope (Leica DM IL; Leica Microsystems Inc., Wetzlar, Hesse-Darmstadt, Germany) equipped with a digital camera (Leica DFC300FX; Leica Microsystems Inc., Wetzlar, Hesse-Darmstadt, Germany) was used to photograph the wounded areas.

Statistical Analysis

Statistical analyses were performed using SPSS 22.0 software (IBM Corp., Armonk, NY, USA). All data were expressed as the mean ± SD of at least three independent experiments. Chi-square tests and Student's *t*-tests were conducted to analyse the differences between groups. Survival curves were plotted using the Kaplan-Meier

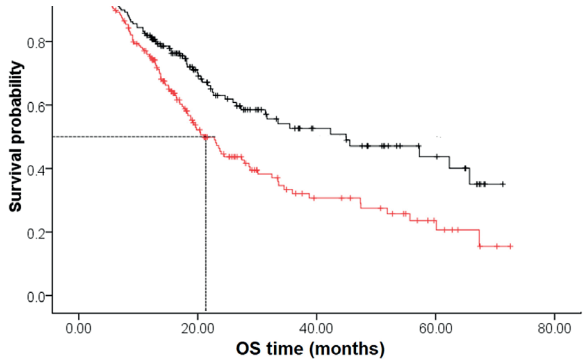

Figure 1. Survival curve of MIBC patients with different expression of MMP14 in six years according to TCGA. MMP14, matrix metalloproteinase 14. OS time, overall survival time. MIBC, muscle-invasive bladder cancer.

method with log-rank tests for statistical significance. The prognostic relevance of the clinicopathological and immunohistochemical data was analysed using univariate and multivariate Cox regression analyses. $p<0.05$ was considered statistically significant.

Results

Functional Prediction of MMP14 Based on the Online Dataset

To investigate MMP14 expression in MIBC, we analysed RNA-Seq data of 374 primary tumour and 19 solid normal tissue samples from TCGA. Compared to normal tissues, the MIBC tissues showed increased MMP14 expression ($p<0.001$) (Table I). To explore the potential predictive value of MMP14 in the short term, we evaluated 374 MIBC patients from TCGA possessing complete follow-up data using Kaplan-Meier analysis with a log-rank test. The group with high MMP14 expression exhibited a poorer prognosis than the group with low MMP14 expression at six years ($p<0.05$) (Figure 1).

To evaluate the mRNA expression levels of MMP14 in other tumours, we used the online tool GEPIA to analyse the differences in MMP14 expression between 33 tumours. The expression of MMP14 was significantly upregulated in 12 tumours, including lymphoid neoplasm diffuse large B-cell lymphoma, glioblastoma multiforme, head and neck squamous cell carcinoma, and renal clear cell carcinoma, as well as other cancers ($p<0.05$) (Figures 2A and 2B).

To evaluate the protein expression levels of MMP14 in other tumours, we used the online database The Human Protein ATLAS to obtain the relative protein expression levels in various tumours. The HPA051432 antibody was shown to have a moderate to a strong cytoplasmic immune reaction in papillary thyroid adenocarcinoma, melanoma, colorectal, pancreatic, ovarian, gastric, kidney and prostate cancer tissues (Figure 2C). Most kidney cancer tissues exhibited moderate staining with the CAB009918 antibody (Figure 2D).

High MMP14 Expression is Associated with Poor Prognostic Features in Clinical Samples

To further examine the relationship between expression and prognostic value of MMP14 in BC, we analysed the MMP14 immunostaining intensity in 113 BC patients from Peking University First Hospital. MMP14 expression was significantly higher in MIBC tissues than in adjacent normal bladder tissues ($p<0.05$) (Table II). Representative images of MMP14 expression in BC tissues and their adjacent normal tissues were presented in Figures 3A-3D. The results of Western blotting for 18 MIBC samples detected high MMP14 expression in cancer tissues (Figure 3E). Using the method described above for the public database, we assessed the potential predictive value of MMP14 for the clinical samples. The results showed that high expression of MMP14 in BC was closely associated with poorer overall survival and progression-free survival times compared with its low expression group in the short term ($p<0.05$) (Figures 3F and 3G).

Table I. Expression of MMP14 in MIBC compared with normal tissues in 393 cases from TCGA.

Sample	Number of patients	MMP14		p
		Low (%)	High (%)	
Normal tissues	19	17 (89.5)	2 (10.5)	<0.001*
MIBC	374	179 (48.2)	195 (52.1)	

Note: MIBC, muscle-invasive bladder cancer. MMP14, matrix metalloproteinase 14. Paired Chi-square test. *p <0.05.

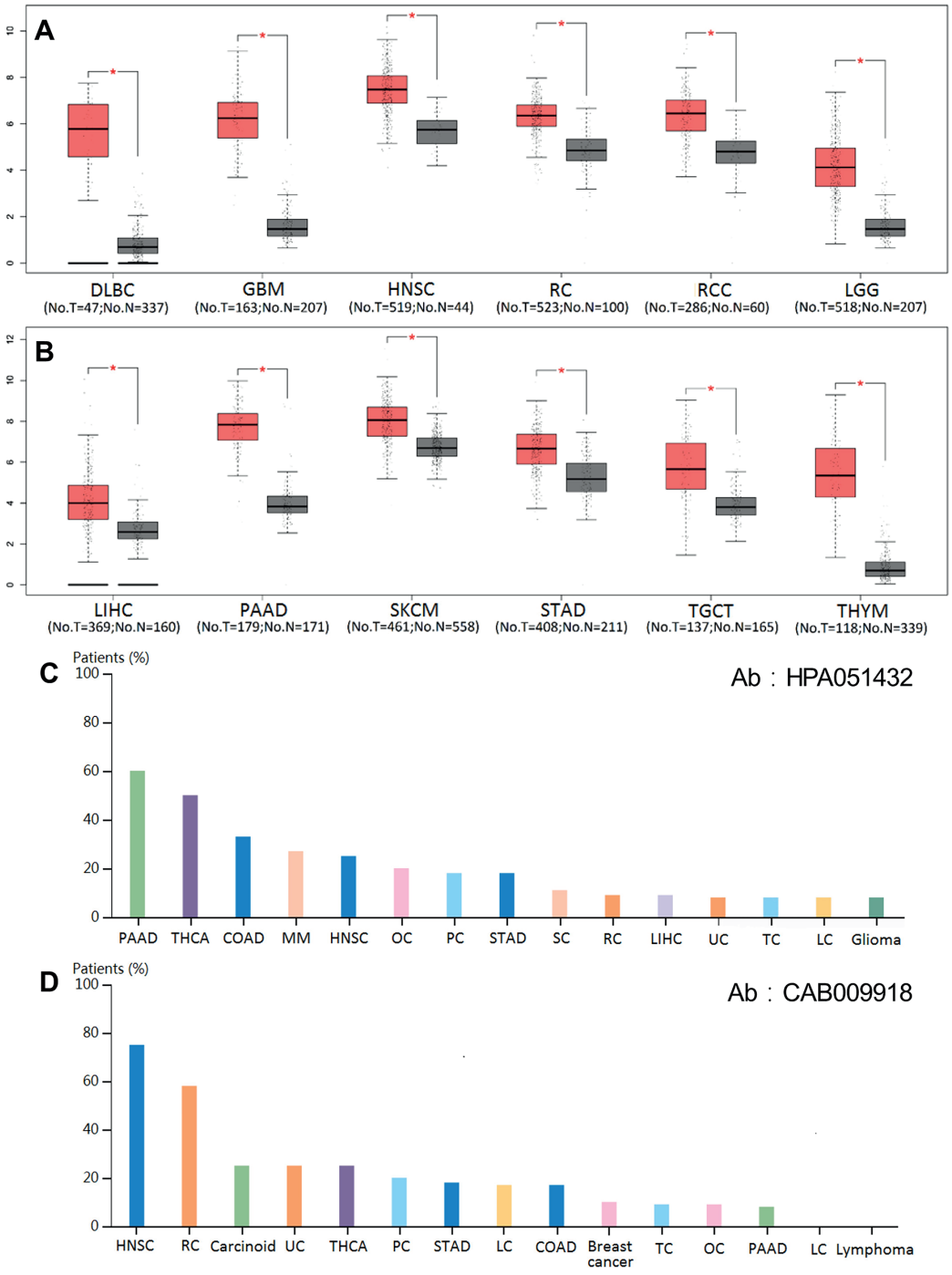

Figure 2. mRNA and protein expression levels of MMP14 in other tumours. **A,** Expression of MMP14 mRNA across six tumour types (DLBC, GBM, HNSC, KIRC, KIRP, and LGG) and paired normal tissues. **B,** Expression of MMP14 mRNA across six other tumour types (LIHC, PAAD, SKCM, STAD, TGCT, and THYM) and paired normal tissues. **C,** Expression of MMP14 protein across fifteen tumour types with antibody HPA051432. **D,** Expression of MMP14 protein across fifteen tumour types with antibody CAB009918. The data in (**C**) and (**D**) are derived from The Human Protein Atlas (https://www.proteinatlas.org/). MMP14, matrix metalloproteinase 14. COAD, colon adenocarcinoma. DLBC, lymphoid neoplasm diffuse large B-cell lymphoma. GBM, glioblastoma multiforme. HNSC, head and neck squamous cell carcinoma. LC, lung cancer. LGG, brain lower-grade glioma. LIHC, liver hepatocellular carcinoma. MM, malignant melanoma. OC, ovarian cancer. PAAD, pancreatic adenocarcinoma. PC, prostate cancer. RC, renal cancer. RCC, renal papillary cell carcinoma. SC, skin cancer. SKCM, skin cutaneous melanoma. STAD, stomach adenocarcinoma. TC, testis cancer. TGCT, testicular germ cell tumours. THCA, thyroid carcinoma. THYM, thymoma. UC, urothelial cancer. $^{*}p<0.05$.

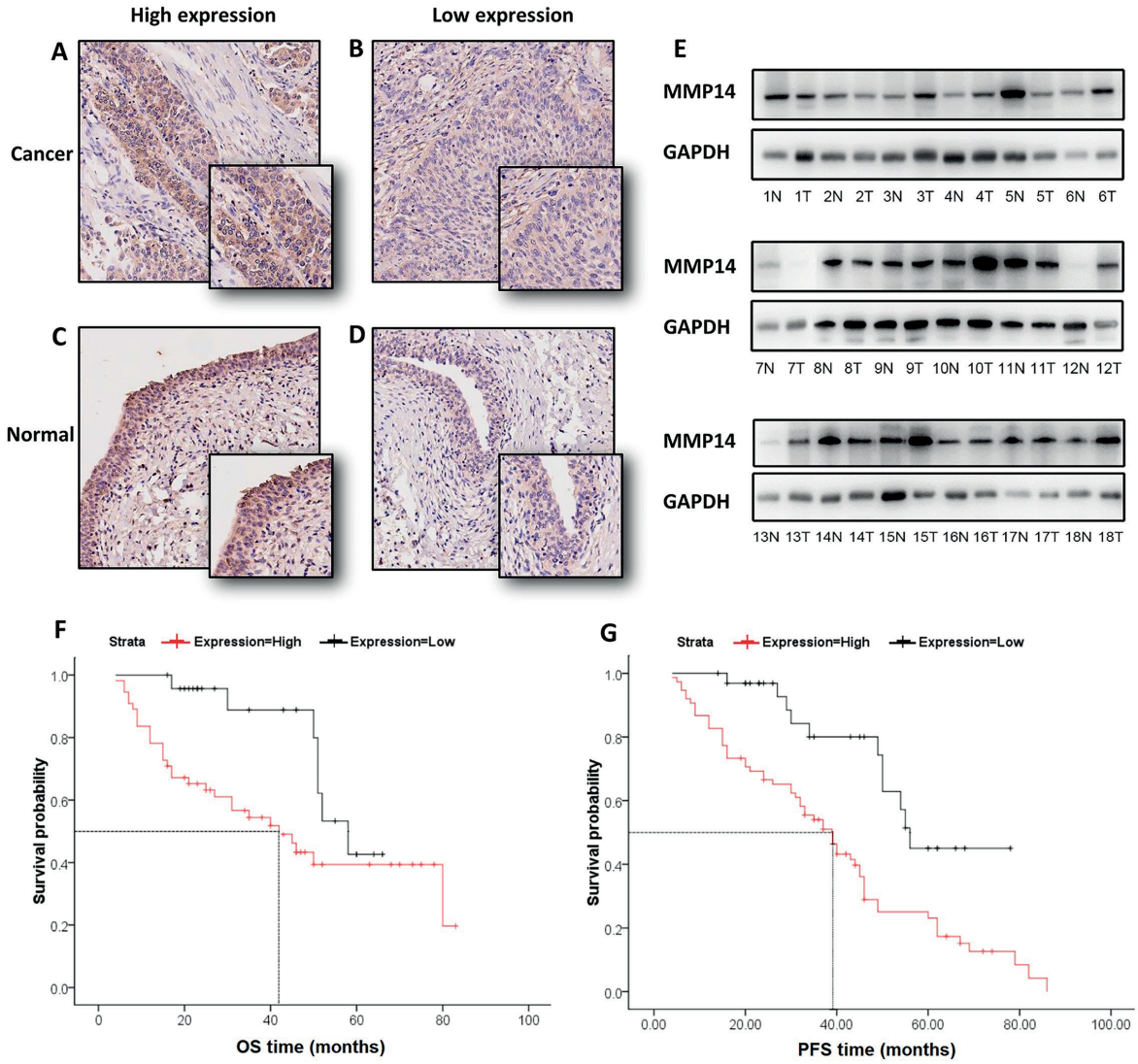

Figure 3. MMP14 is highly expressed in MIBC cancer tissues and is associated with poor prognostic features of clinical patients. **A-D,** Representative microarray images of MMP14 expression in BC tissues and their adjacent normal tissues. Original magnifications ×100 and ×260 (lower panels). **A,** and **B,** MMP14 was highly or weakly expressed in bladder cancer tissues. **C,** and **D,** MMP14 was highly or weakly expressed in adjacent normal tissues. **E,** The Western blot results for eighteen MIBC samples confirmed the observed expression of MMP14 in cancer tissues. **F,** and **G,** OS time and PFS time of BC patients with different expression levels of MMP14 among 113 patients from Peking University First Hospital. MMP14, matrix metalloproteinase 14. BC, bladder cancer. OS time, overall survival time. PFS time, progression-free survival time.

Table II. Expression of MMP14 in BC tissues compared with adjacent normal tissues in 113 patients from Peking University First Hospital..

Sample	Number of patients	MMP14		*p*
		Low (%)	High (%)	
NMIBC	31	31 (100.0)	0 (0)	<0.001*
Adjacent normal tissues	31	9 (29.0)	22 (71.0)	
MIBC	82	76 (92.7)	6 (7.3)	<0.001*
Adjacent normal tissues	82	25 (30.5)	57 (69.5)	

Note: NMIBC, non-muscle-invasive bladder cancer. MIBC, muscle-invasive bladder cancer. MMP14, matrix metalloproteinase 14. Paired Chi-square test. *$p<0.05$.

Table III. Correlation of MMP14 expression to BC clinicopathologic characteristics in 113 patients from Peking University First Hospital.

Sample	Number of patients	MMP14		p
		Low (%)	High (%)	
Gender				
Female	19	7 (36.8)	12 (63.2)	0.482
Male	94	27 (28.7)	67 (71.3)	
Age (years)				
<66	52	16 (30.8)	36 (69.2)	0.884
≥66	61	18 (29.5)	43 (70.5)	
Metastasis				
No	80	24 (30.0)	56 (70.0)	0.032*
Yes	32	10 (31.2)	22 (68.8)	
Grade				
G2	27	9 (33.3)	18 (66.7)	0.673
G3	86	25 (29.1)	61 (70.9)	
T staging				
Tis+T1	31	9 (29.0)	22 (71.0)	0.880
T2+T3+T4	82	25 (30.5)	57 (69.5)	
N staging				
N0	73	28 (31.1)	62 (68.9)	0.639
N1+N2+N3	23	6 (26.1)	17 (73.9)	
Smoking				
No	78	22 (28.2)	56 (71.8)	0.515
Yes	35	12 (34.3)	23 (65.7)	

Note: MMP14, matrix metalloproteinase 14. Chi-square test. *$p<0.05$.

Besides, clinicopathological information for 113 patients was analysed to evaluate associations between MMP14 expression and clinicopathological features. According to the results, MMP14 expression was significantly associated with metastasis ($p<0.05$) but not with other clinicopathological features ($p>0.05$) (Table III).

Furthermore, univariate and multivariate Cox regression analyses indicated that pathologic T stage [hazard ratio (HR)=1.412, 95% confidence interval (CI)=1.121-1.779, $p=0.003$] and metastasis (HR=2.256, 95% CI=1.242-4.100, $p=0.008$) to be unfavourable prognostic factors in BC patients (Table IV). However, there were no significant associations between MMP14 expression and other factors.

MMP14 is Highly Expressed in BC Cell Lines

To assess baseline MMP14 expression *in vitro*, we performed qRT-PCR and Western blot analysis in the immortalized human bladder epithelium cell line (SV-HUC-1) and five BC cell lines (5637, T24, J82, BIU-87, and UMUC-3). qRT-PCR revealed that MMP14 expression was elevated in five bladder cell lines. MMP14 expression in the BC cell lines was 1.4 to 24.7 times higher than that in the SV-HUC-1 cell line (Figure 4A). The Western blot results were consistent with the qRT-PCR results (Figure 4B).

MMP14 Downregulation Reduces Cell Invasion and Migration

To assess the influence of MMP14 downregulation on invasion and migration in BC cell lines, transwell cell invasion and wound-healing assays were performed. The effectiveness of MMP14 knockdown in these cells was verified by qRT-PCR. Post transfection with MMP14 siRNA, the mRNA level of MMP14 was reduced by 1 and 1.1 times respectively in T24 and UMUC-3 cells (Figures 4C and 4D).

The results of transwell cell invasion assay showed that downregulation of MMP14 in T24 and UMUC-3 cells significantly suppressed cell invasion (Figures 4E and 4F). Wound-healing assays demonstrated that cell migration was strongly attenuated in T24 and UMUC-3 cells with downregulated MMP14 compared with control cells (Figures 4G and 4H).

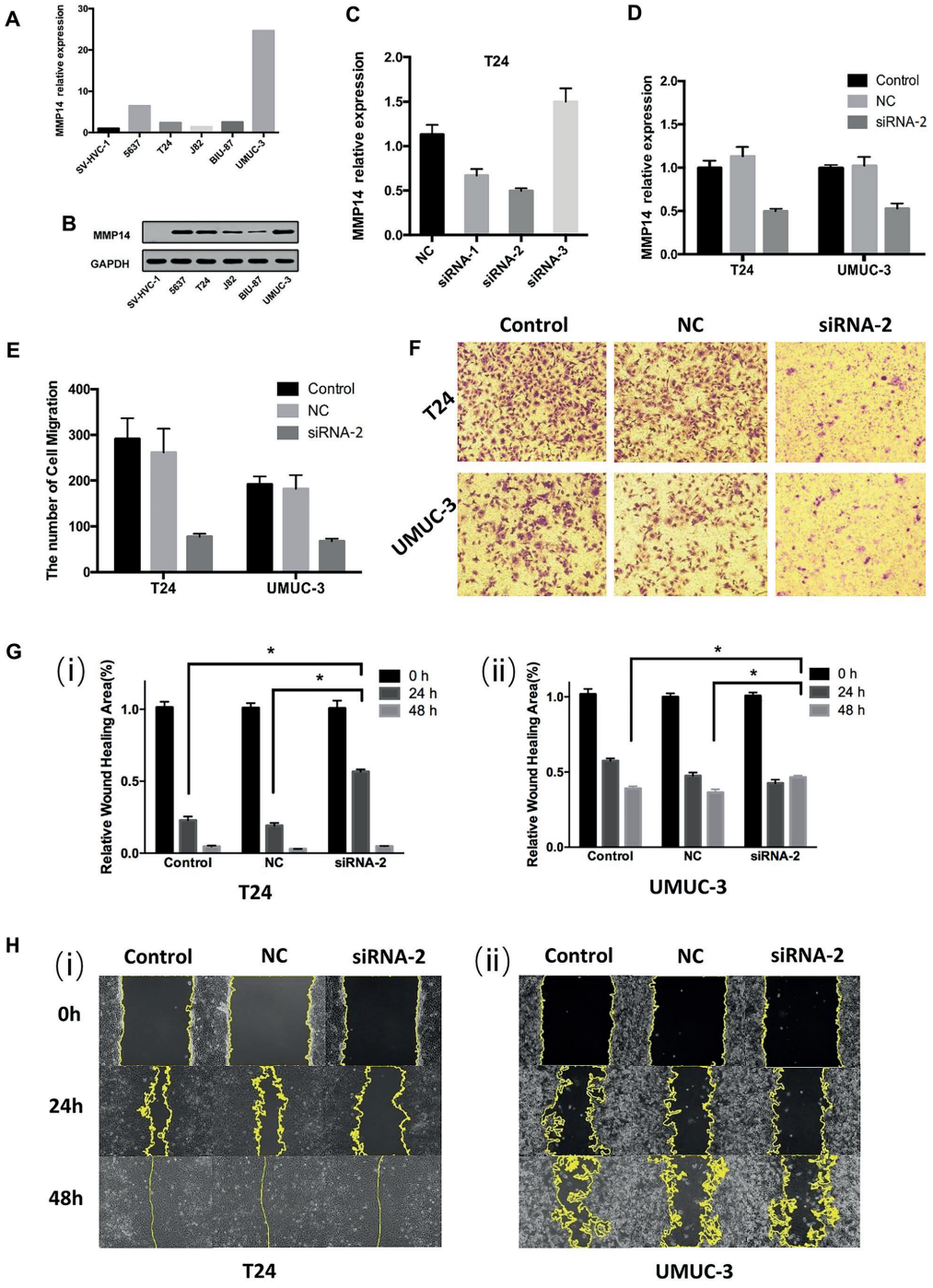

Figure 4. Downregulation of MMP14 reduced cell invasion and migration ability *in vitro*. **A,** MMP14 mRNA was more highly expressed by 1.4 to 24.7 times in BC cell lines than in SV-HUC-1 cells, as shown by qRT-PCR. **B,** High expression of MMP14 in BC cell lines compared with SV-HUC-1, as shown by Western blotting. **C,** MMP14 downregulation was achieved by transfecting three different predesigned siRNAs directed against human MMP14, which was functionally verified by qRT-PCR. **D,** The knockdown efficiency of MMP14 in T24 and UMUC-3 cells was verified by qRT-PCR after transfection. **E,** Statistical results from the cell invasion assay. **F,** Representative images from the cell invasion assay after 72 h for T24 and UMUC-3. Original magnifications ×200. **G,** Statistical results from wound-healing assays for T24 (i) and UMUC-3 (ii). **H,** Representative microarray images from wound-healing assays after 0 h, 24 h, and 48 h for T24 (i) and UMUC-3 (ii). Original magnification ×200. MMP14, matrix metalloproteinase 14. qRT-PCR, quantitative Rea Time-Polymerase Chain Reaction. NC, negative control. $^{*}p<0.05$.

Discussion

The MMP family plays an important role in tumour metastasis and invasion. MMP7 and MMP9 can be used as markers of poor prognosis in BC[22,23], and the possibility of using MMP14 as a marker for colon cancer and gastric cancer was also reported recently[24,25]. Nonetheless, the prognostic value of MMP14 in BC has not been fully studied.

As an important member of the matrix metalloproteinase family, MMP14 is a housekeeping protein that regulates reproduction, tissue remodelling and other processes under physiological conditions. MMP14 regulates cell migration and invasion through the epithelial-mesenchymal transition and can also play a role as a regulator of tumour microenvironment communication in connective tissues[26,27]. MMP14 exhibits a certain amount of background expression in the normal bladder mucosa and plays an important role in maintaining the fluidity and deformability of the normal bladder mucosal epithelium, which partly accounts for higher recurrence and invasion rate in BC than in other tumours. At present, research on MMP14 is predominantly focused on the mechanism and regulation of MMP14. For example, the downregulation of lncRNA MFI2 Antisense RNA 1 restrains the aggressive phenotypes of glioma cells by reducing the expression of MMP14[28]. MiR-337-3p directly binds to the MMP14 promoter to repress myeloid zinc finger 1 facilitated MMP14 expression, thus suppressing the progression of gastric cancer[29]. Other studies[25,30] have concentrated on MMP14 as a predictive marker for tumour prognosis, and MMP14 has been used as a potential marker in breast cancer and colon cancer. However, there is no reliable evidence regarding the prognostic value of MMP14 in BC. Also, a certain amount of background MMP14 expression in the bladder mucosa makes it difficult to study the differential expression of MMP14 between normal and BC tissue.

Our study found that MMP14 exhibits differential expressions between cancerous and normal tissue, and MMP14 expression is positively correlated with the pathological stage, which is consistent with the immunostaining results of our clinical samples. Using GEPIA and The Human Protein ATLAS, we found increased MMP14 mRNA and protein expression in various tumours, including head and neck, renal, pancreatic, and other cancers, which is consistent with literatures[31-34]. Therefore, MMP14 is closely related to the occurrence and development of BC.

Survival analysis was also performed in this study. We plotted a six-year survival curve of MIBC with TCGA data and found that high MMP14 expression was associated with a poor prognosis. The results were detected in our clinical samples, indicating that MMP14 is a meaningful marker, at least for short-term

Table IV. Univariate and multivariate Cox regression of MMP14 expression for overall survival in 113 BC patients from Peking University First Hospital.

	Univariate analysis		Multivariate analysis	
Factors	**HR (95% CI)**	***p***	**HR (95% CI)**	***p***
Gender Female *vs.* Male	1.329 (0.717-2.464)	0.367		
Age (year) ≥66 *vs.* <66	0.922 (0.502-1.692)	0.792		
Grade G2 *vs.* G3	1.542 (0.898-2.648)	0.116		
Pathologic T staging T1 *vs.* T2 *vs.* T3 *vs.* T4	1.442 (1.175-1.777)	0.000*	1.412 (1.121-1.779)	0.003*
Pathologic N staging N0 *vs.* N1 *vs.* N2	1.595 (1.142-2.227)	0.060		
Complication No *vs.* Yes	1.797 (0.854-3.781)	0.123		
Metastasis No *vs.* Yes	3.014 (1.824-5.283)	0.000*	2.256 (1.242-4.100)	0.008*
Smoking No *vs.* Yes	1.461 (0.882-2.420)	0.141		

Note: HR, hazard ratio. 95% CI, 95% confidence interval. vs, verse. Univariate and multivariate Cox regression. *$p<0.05$.

MIBC prognostic predictions. Nevertheless, the relationship between MMP14 expression and long-term prognosis requires further large-scale and long-term follow-up validation. Additionally, univariate and multivariate Cox regression analyses showed that pathological T stage and metastasis were unfavourable prognostic factors in BC patients, which is consistent with clinical experience.

To further validate the functional significance of MMP14, we performed cell function experiments *in vitro*. MMP14 was highly expressed in a series of BC cell lines and may be involved in BC pathogenesis. Cell migration and invasion abilities were reduced in BC cells with MMP14 expression downregulation. Similarly, downregulation of MMP14 in breast cancer and gastric cancer cells reduces the growth rate of the primary tumour[17,18]. Therefore, we are convinced that MMP14 plays an important biological role in the metastasis and invasion of BC.

Along with the data-mining of the target gene from public databases, we performed multi-angle studies on MMP14 using cell lines and clinical samples. However, there are several limitations to this research. Cancer and normal tissue data in the public database were derived from different patients, rather than tissues from the same individual, which may generate bias. Furthermore, there is no uniform standard for immunohistochemical scoring. Currently, image processing software and human eye-based recognition have certain limitations, which may also cause some bias. Moreover, the long-term prognostic value of MMP14 in BC still requires further confirmation by studies with larger sample sizes and longer follow-up times.

Conclusions

In summary, our study demonstrated that MMP14 expression is upregulated in human MIBC and is significantly related to tumour invasion and migration. MMP14 is associated with the progression and poor short-term prognosis in MIBC.

Funding Acknoledgments

This study was funded by the National Natural Science Foundation of China (No. 81772703) and Natural Science Foundation of Beijing (No. 7172219).

Conflict of Interest

The Authors declare that they have no conflict of interests.

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