

# Identification of potential diagnostic biomarkers in MMPs for pancreatic carcinoma

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## Abstract

Pancreatic cancer (PC) is a malignant tumor which ranks fourth in cancer-related death. However, the specificity and sensitivity of traditional biomarkers such as carbohydrate antigen 19-9 no longer meet the clinical requirements.

Tools as ONCOMINE and Gene Expression Profiling Interactive Analysis (GEPIA) were used to analyze the differential expression of matrix metalloproteinases (MMPs) in PC and adjacent tissues. For further analysis, we adopted database for annotation, visualization and integrated discovery (DAVID 6.8), transcriptional regulatory relationships unraveled by sentence-based text (TRRUST) and other tools. We also identified drugs targeted the selected MMPs.

Eight MMPs (MMP1, MMP2, MMP7, MMP9, MMP11, MMP12, MMP14, and MMP28) were differentially expressed in PC and adjacent tissue. MMP1 ( $P=.0189$ ), MMP7 ( $P=.000216$ ), MMP11 ( $P=.0209$ ), MMP14 ( $P=.00611$ ) were correlated with the pathological stages of PC. Patients with higher expression of MMP1 ( $P=.0011$ ), MMP2 ( $P=.011$ ), MMP7 ( $P=.0081$ ), MMP9 ( $P=.046$ ), MMP11 ( $P=.0019$ ), MMP12 ( $P=.0011$ ), MMP14 ( $P=.0011$ ), and MMP28 ( $P=6.3e-06$ ) showed poor prognosis. Ten transcription factors were associated with the up-regulation of selected MMPs. Marimastat (DB00786) was found to target selected MMPs.

Our research revealed that selected MMPs played an important role in the early diagnosis and prognosis of PC.

**Abbreviations:** CA19-9 = carbohydrate antigen 19-9, cBioPortal = the cBio cancer genomics portal, DAVID = Database for Annotation, Visualization and Integrated Discovery, DB = DrugBank, DFS = disease free survival, ECM = extracellular matrix, ETS2 = ETS proto-oncogene 2, ETV4 = ETS variant transcription factor 4, FOS = Fos proto-oncogene, GEPIA = Gene Expression Profiling Interactive Analysis, JUN = Jun proto-oncogene, MAZ = MYC associated zinc finger protein, MF = Molecular function, MMPs = Matrix metalloproteinases, PC = pancreatic cancer, PPI = protein–protein interaction, RELA = RELA proto-oncogene, SP1 = Sp1 transcription factor, SRF = serum response factor, STAT3 = signal transducer and activator of transcription 3, TCGA = The Cancer Genome Atlas, TRRUST = transcriptional regulatory relationships unraveled by sentence-based text.

**Keywords:** biomarker, diagnosis, MMPs, pancreatic carcinoma

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## 1. Introduction

Pancreatic carcinoma (PC) is a malignant tumor which ranks fourth in cancer-related death.<sup>[1]</sup> It accounts for over 220,000 new cases and over 200,000 deaths worldwide each year and its incidence and mortality continue to increase.<sup>[2–55]</sup> There are several types of PC, 90% of which are pancreatic ductal adenocarcinoma.<sup>[6,7]</sup> Due to its extremely aggressive behavior and rapid progression, the 5-year survival rate of PC patients is less than 9%.<sup>[8]</sup> The only curable treatment for PC patients may be surgical resection.<sup>[9,10]</sup> However, most PC patients were diagnosed at unresectable stage and the effective screening methods are lacking.<sup>[11]</sup>

By searching useful biomarkers, early diagnosis is useful for improving the prognosis of PC patients.<sup>[12–14]</sup> Carbohydrate antigen 19-9 (CA19-9) is one biomarker mainly expressed in PC tissues with low specificity and sensitivity.<sup>[11,15]</sup> Previous studies had also identified several biomarkers which were useful in the diagnosis of PC.<sup>[16]</sup> Unfortunately, they have the same drawbacks with CA19-9.<sup>[17]</sup> Consequently, it is urgent to identify new biomarkers used for the early diagnosis of PC. The matrix metalloproteinases (MMPs) family consists 25 members, and most of them are expressed in human tissues.<sup>[18]</sup> Studies indicated that MMPs were essential in embryo formation, neovascularization, and the metastasis of malignant tumor cells including PC.<sup>[19]</sup> MMPs are involved in the development, progression and

invasion of tumor cells by modulating the cell-extracellular matrix (ECM) adhesion and promoting angiogenesis.<sup>[8,9]</sup> Hence, MMPs may be the potential biomarkers for PC.

Previous studies had already revealed the universal expression of MMPs in PC tissues. Their function and underlying mechanisms have also been clearly clarified.<sup>[10,12]</sup> However, further analysis should be performed to identify suitable subtypes of MMPs as diagnostic and prognosis biomarkers for PC. Recently, new high-throughput sequencing technology had developed rapidly and numerous databases had been established. It is possible to perform a comprehensive and integrated analysis for the expression of MMPs in PC.<sup>[20,21]</sup> We aimed to identify potential diagnostic and prognosis biomarkers in MMPs through comprehensive bioinformatics analysis for the diagnostic of PC based on public databases. The important role of selected MMPs in the progression of PC will also be explored.

## 2. Materials and methods

### 2.1. ONCOMINE

ONCOMINE ([www.oncomine.org](http://www.oncomine.org)) contains 65 microarray datasets, 4700 microarrays. More than 480 million gene expression data are presented.<sup>[22,23]</sup> Here, we evaluated the expression of MMPs in PC by using the data in ONCOMINE. A *P* value <.05, fold change of 2, and gene ranking in the top 10% were set as the significance thresholds in our study. Student *t* test was used to analyze the expression difference.

### 2.2. GEPIA (gene expression profiling interactive analysis)

Based on the Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression data, GEPIA is one useful tool to analyze the expression and interaction of cancer and normal genes.<sup>[24]</sup> Pathological stage, prognostic analysis, and the relative expression levels of MMPs in PC and normal tissues were performed with GEPIA. Student *t* test was used to analyze the expression difference, with a cutoff of 0.05. Besides, the cutoff value of fold change was set as 2. Prognostic analysis was presented using Kaplan–Meier curves and the Kaplan–Meier plotter online tool was also applied.

### 2.3. cBioPortal (cBio cancer genomics portal)

cBioPortal is one open platform and provide genomic data and clinical information from more than 215 studies for the study of multi-dimensional cancer genome. It enables researchers to access massive amounts of data from large-scale cancer genomics and convert them into visual charts quickly and efficiently.<sup>[25]</sup> The genetic changes of differentially expressed MMPs were also analyzed by cBioPortal. The co-expression and the network module of MMPs were also explored.

### 2.4. STRING

Online database STRING provides information on protein-gene interaction pathways, co-expression, co-localization, and protein domain similarity.<sup>[26]</sup> To explore the interactions between MMPs, STRING was applied to differentially expressed MMPs for protein–protein interaction (PPI) network analysis.

### 2.5. GeneMANIA

GeneMANIA was applied to search related genes and protein interactions by searching the public biological datasets.<sup>[27]</sup>

### 2.6. DAVID 6.8 (database for annotation, visualization and integrated discovery)

To explore the biological significance of differently expressed MMPs in PC, gene ontology, and Kyoto Encyclopedia of Genes and Genome analysis were performed using DAVID 6.8 and top 10 pathways were selected.<sup>[28]</sup>

### 2.7. Drugbank (DB)

DB is a bioinformatics and chemical informatics database provided by University of Alberta. It was used to retrieve new drug targets, compare drug structures and study drug mechanisms.<sup>[29–31]</sup> Relevant matching drugs for differentially expressed MMPs were identified by DB.

### 2.8. TRRUST (transcriptional regulatory relationships unraveled by sentence-based text)

The transcriptional regulatory network analysis of differentially expressed MMPs was performed using the TRRUST.

### 2.9. Ethics

This study was approved by the Institutional Animal Care and Use Committee of Changhai Hospital, Second Military Medical University.

## 3. Results

### 3.1. Abnormal expression of MMPs in PC patients

The expression levels of 25 MMPs were explored in different cancer types and adjacent tissues using the ONCOMINE database. As shown in Figure 1 and Figure S1, Supplemental Digital Content, <http://links.lww.com/MD/G179>, the expression levels of MMP1, MMP2, MMP7, MMP9, MMP11, MMP12, MMP14, and MMP28 were significantly upregulated in PC tissues, while MMP24 and MMP25 were significantly down-regulated. Moreover, no changes were found among other MMPs. Detailed information on the abnormal expression of MMPs obtained by ONCOMINE was presented in Table 1. Consistently, Tito et al also found that the expression of MMP1 is associated with the prognosis of human pancreatic ductal adenocarcinoma.<sup>[32]</sup> Yueguang et al indicated that MMP2 was upregulated in PC tissues, which enhanced the invasion and metastasis of PC cells.<sup>[33]</sup> Compared with adjacent normal tissues, Lu-Lu Zhai et al also found a significant increase in the expression of MMP2 in PC tissues.<sup>[34–35]</sup> Besides, MMP7 and MMP11 were also overexpressed in PC tissues compared with chronic pancreatitis.<sup>[36]</sup> Jones et al also revealed the upregulated expression of MMP7, MMP8, MMP9, and MMP11 in PC tissues.<sup>[37]</sup>

Additionally, we also examined the relative expression of all MMPs in PC tissues using GEPIA. MMP7 was found to be the highest expressed MMP we evaluated (Fig. 2A). Moreover, the relative expression levels of MMP1, MMP2, MMP7, MMP9, MMP10, MMP11, MMP12, MMP14, MMP19, MMP23A, MMP23B, and MMP28 were found significantly increased in PC tissues compared with adjacent tissues (Fig. 2B). However, the relative expression levels of other MMPs in PC tissues were not significant (Figure S2, Supplemental Digital Content, <http://links.lww.com/MD/G180>). Combining the results from ONCOMINE and GEPIA, MMP1, MMP2, MMP7, MMP9, MMP11, MMP12, MMP14, and MMP28 were selected as the targets for our further study.

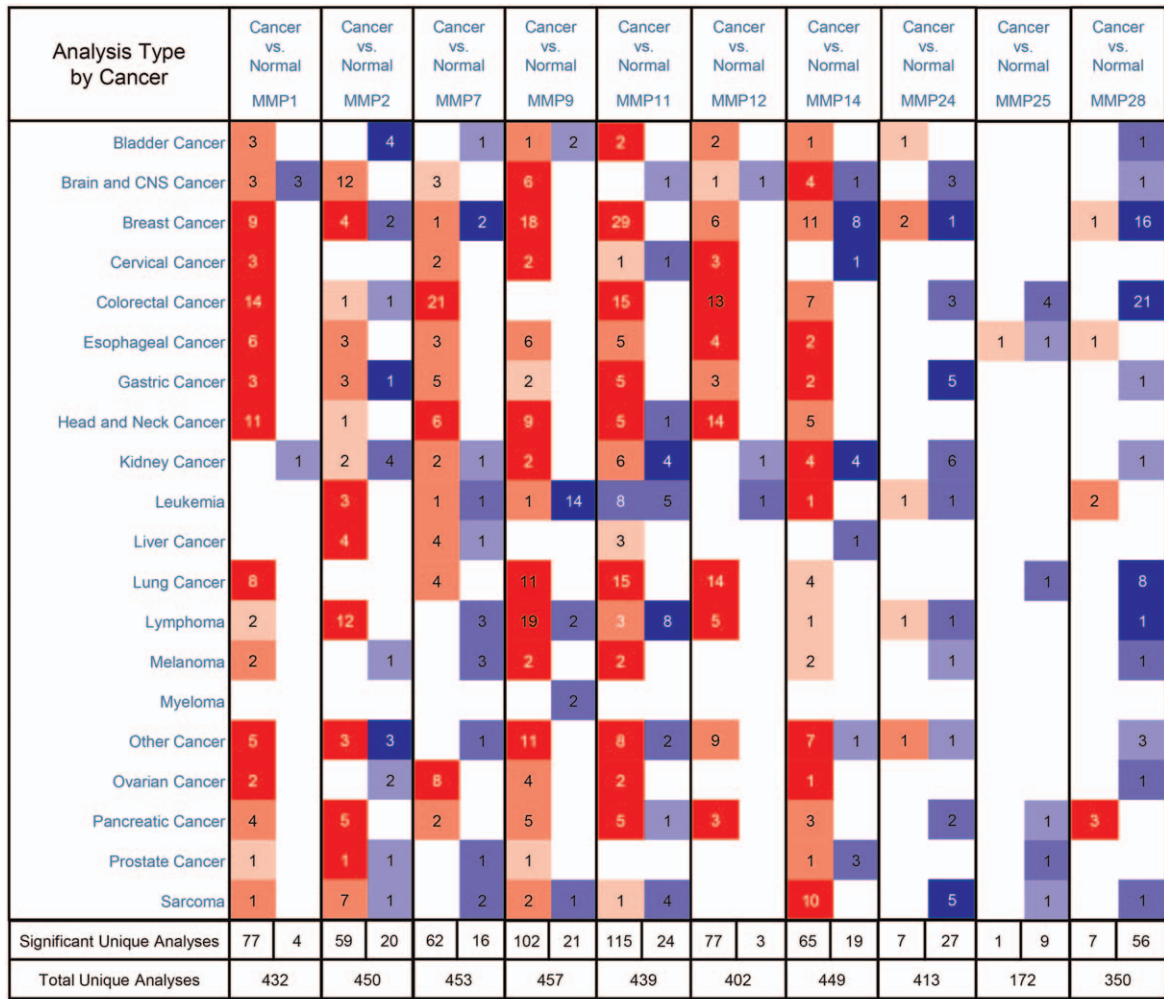


Figure 1. The expression levels of MMPs in PC and adjacent tissues from ONCOMINE database.

### 3.2. The prognostic value of selected MMPs in PC patients

We then evaluated the correlation between selected MMPs and the pathological stage of PC patients. We found a significant association between MMP1 ( $P=.0189$ ), MMP7 ( $P=.000216$ ), MMP11 ( $P=.0209$ ), MMP14 ( $P=.00611$ ), and the pathological

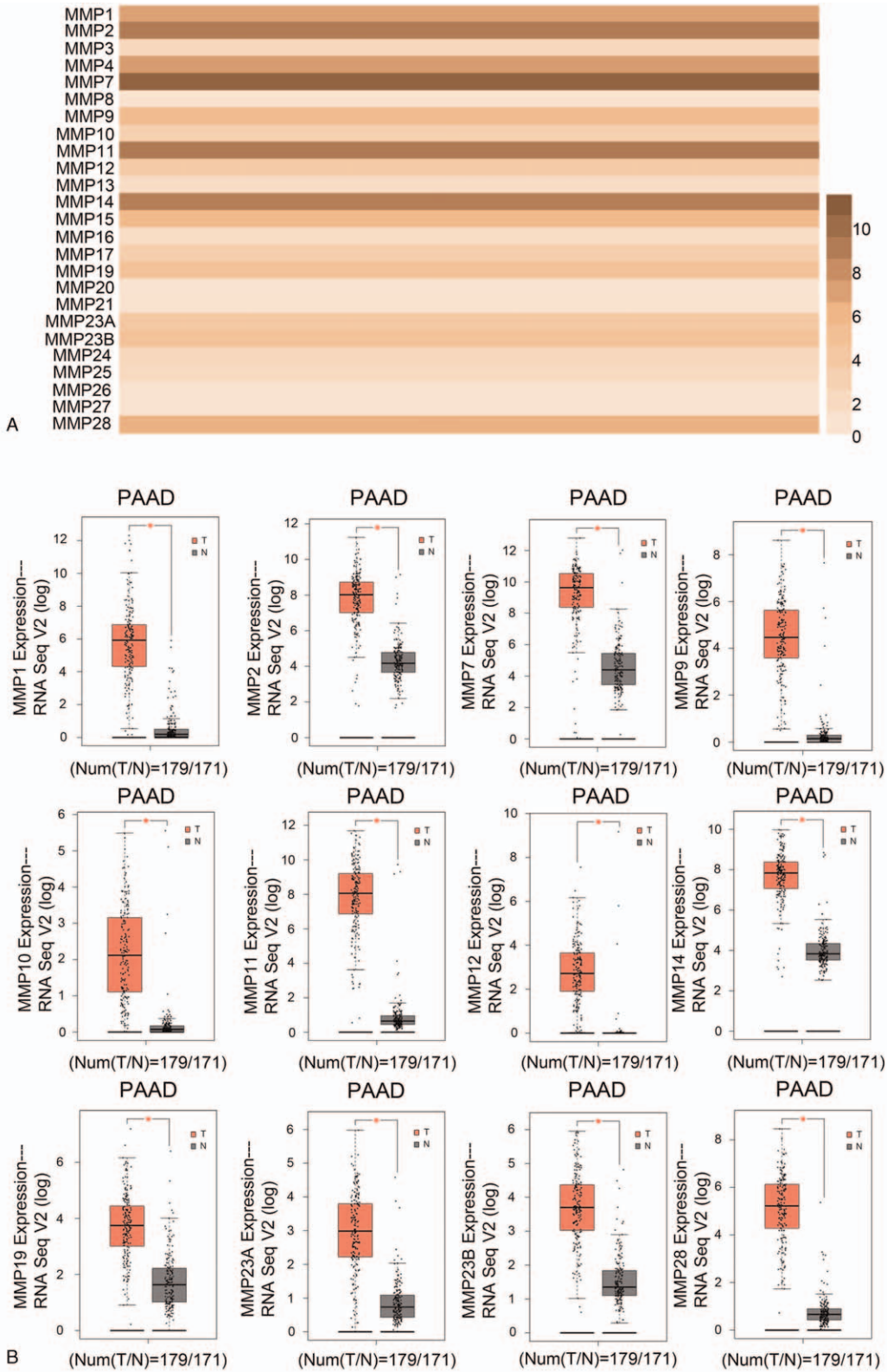
stages of PC patients (Fig. 3A). Thus, MMP1, MMP7, MMP11, and MMP14 may be the useful prognostic markers for PC patients.

Furthermore, survival analysis indicated that PC patients with highly expressed MMP1 (HR=2.28 (1.37–3.79),  $P=.0011$ ), MMP2 (HR=1.96 (1.15–3.34),  $P=.011$ ), MMP7 (HR=2.02

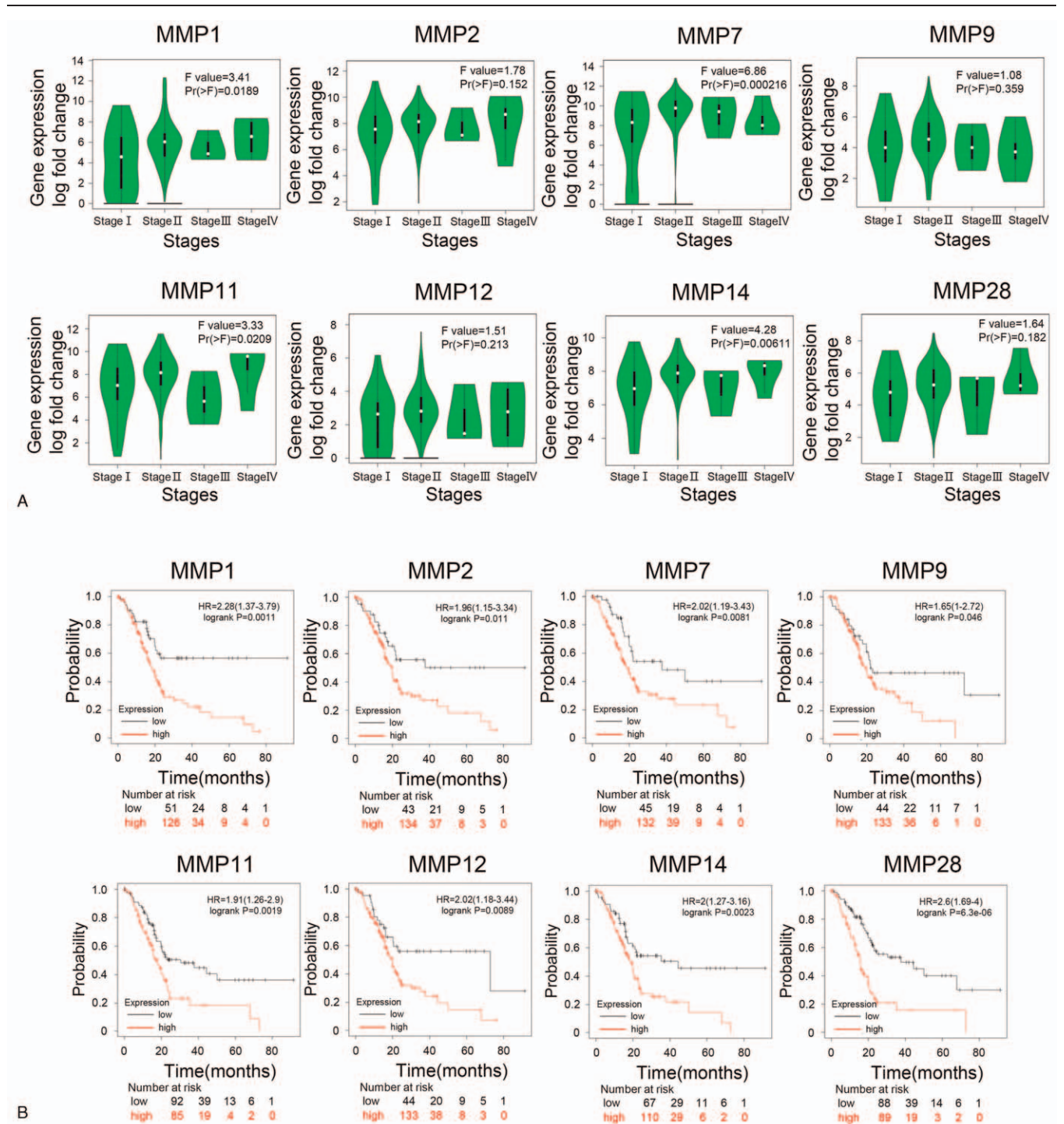
Table 1

Differential expression of MMP family in pancreatic cancer and adjacent tissue.

Gene name	Type	Fold change	P value	T test	References (PMID)
MMP1	Pancreatic Ductal Adenocarcinoma	11.671	1.23E-09	6.824	19260470
MMP2	Pancreatic Ductal Adenocarcinoma	5.996	3.48E-14	9.92	19260470
MMP7	Pancreatic Ductal Adenocarcinoma	10.743	1.21E-12	8.69	19260470
MMP9	Pancreatic Adenocarcinoma	11.597	6.28E-05	6.908	12750293
MMP11	Pancreatic Adenocarcinoma	15.423	3.45E-07	10.534	12750293
MMP12	Pancreatic Adenocarcinoma	317.536	8.19E-08	14.368	12750293
MMP14	Pancreatic Carcinoma	2.018	5.91E-06	5.018	19732725
MMP24	Pancreatic Ductal Adenocarcinoma	-1.79	0.000485	-4.801	16103885
MMP25	Pancreatic Ductal Adenocarcinoma	-1.646	0.008	-2.835	16103885
MMP28	Pancreatic Carcinoma	4.456	3.6E-09	7.155	19732725



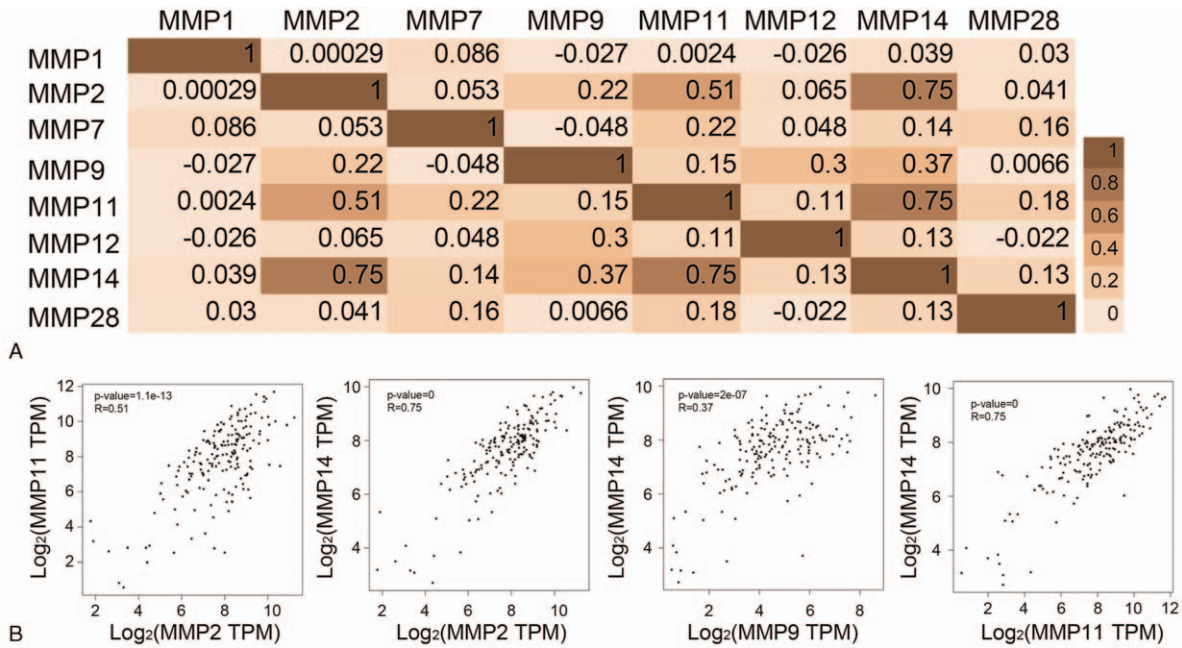
**Figure 2.** (A) Relative expression levels of all MMPs in PC tissues with GEPIA. (B) MMPs expression profile in PC and normal pancreatic tissues from GEPIA database.



**Figure 3.** (A) The relationship between the transcriptional expression of each MMP family member and pathological stage of PC patients. (B) Survival plots of selected MMPs in pancreatic cancer using the Kaplan–Meier plotter online tool.

(1.19–3.43),  $P = .0081$ ), MMP9 (HR = 1.65 (1–2.72),  $P = .046$ ), MMP11 (HR = 1.91 (1.26–2.9),  $P = .0019$ ), MMP12 (HR = 2.02 (1.18–3.44),  $P = .0011$ ), MMP14 (HR = 2 (1.27–3.16),  $P = .0011$ ), and MMP28 (HR = 2.6 (1.69–4),  $P = 6.3e-06$ ) showed poor prognosis (Fig. 3B). We also assessed the correlation between selected MMPs and their clinical outcome by GEPIA.

Disease free survival (DFS) curves of all selected MMPs were shown in Supplementary Figure 3A, <http://links.lww.com/MD/G181>. We found that low expression of both MMP14 ( $P = .004$ ) and MMP28 ( $P = 5.2e-05$ ) was significantly associated with longer DFS. The value of selected MMPs in the OS were also evaluated with GEPIA. Results indicated that patients with high expression of MMP1 ( $P = .03$ ), MMP11 ( $P = .042$ ), MMP14 ( $P = .033$ ) and MMP28 ( $P = 7e-06$ ) showed shorter overall



**Figure 4.** (A) Pearson correlation of selected MMPs in PC via the GEPIA online tool. (B) Correlations among MMP2, MMP9, MMP11, and MMP14 by scatter plots.

survival (Figure S3, Supplemental Digital Content, <http://links.lww.com/MD/G181>). These results suggested that the selected MMPs were involved in the progression of PC.

### 3.3. Co-expression, genetic alteration, neighbor gene interaction network analysis of selected MMPs in PC patients

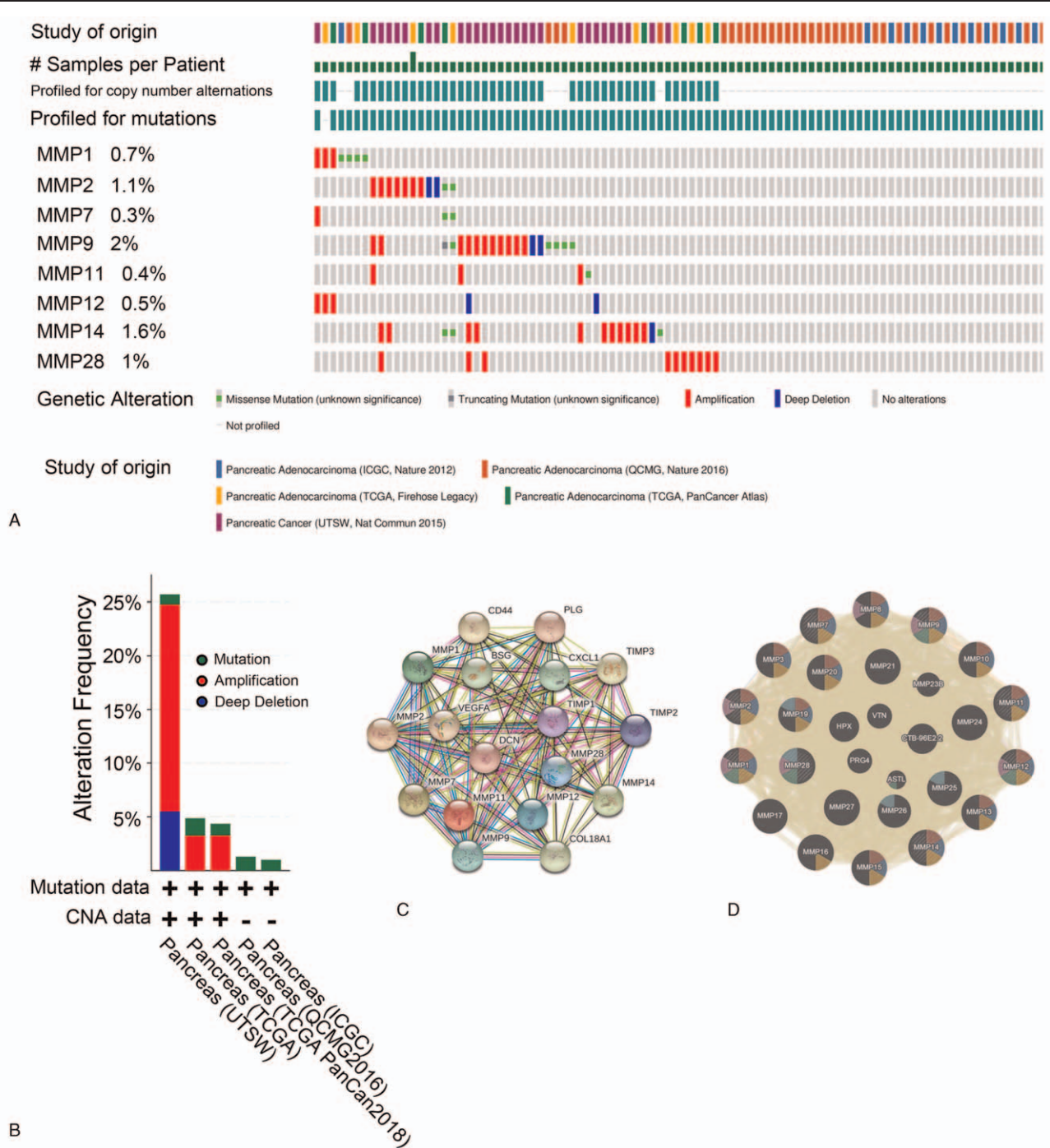
We then performed a comprehensive analysis of the molecular characteristics of selected MMPs. Firstly, we evaluated the correlations between selected MMPs by using the GEPIA online tool. The expression of MMP2 was moderately correlated with MMP11 (0.51) and MMP9 with also moderately correlated MMP14 (0.37). The correlation between the expression of MMP2 and MMP14 (0.75), MMP11 and MMP14 (0.75) were highly correlated (Fig. 4A). Furthermore, the correlation among MMP2, MMP9, MMP11, and MMP14 were also presented by scatter plots (Fig. 4B).

The online tool cBioPortal was used for evaluating the genetic alterations of the selected MMPs in queried PC samples. As shown in Figure 5A, MMP1, MMP2, MMP7, MMP9, MMP11, MMP12, MMP14, and MMP28 altered 0.7%, 1.1%, 0.3%, 2%, 0.4%, 0.5%, 1.6% and 1%, respectively. Genetic alterations of selected MMPs in cBioPortal included amplification, deep deletion and mutation (Fig. 5B). These data were obtained from 5 studies including pancreatic adenocarcinoma (ICGC, Nature 2012), pancreatic adenocarcinoma (TCGA, Firehose Legacy), pancreatic cancer (ICGC, Nat Commun 2015), pancreatic adenocarcinoma (QCMG, Nature 2016) and pancreatic adenocarcinoma (TCGA, PanCancer Atlas). Meanwhile, we also conducted a protein-protein interaction (PPI) network analysis of selected MMPs with STRING to explore their potential interaction. As illustrated in Figure 5C, we obtained 18 nodes and 166 edges in the PPI network. There were also 10 other interactors which interacted with selected MMPs. The function of

these interactors were associated with cell migration, inflammation, oncogenesis, metastasis, tumorigenesis and progression<sup>[38]</sup> pro-inflammatory, angiogenesis<sup>[38–40]</sup> tissue inhibitors of matrix metalloproteinases, aggressive phenotype of pancreatic carcinoma,<sup>[35,41]</sup> the distant metastasis, and aggressive malignant behaviors of pancreatic cancer.<sup>[42]</sup> Moreover, results of GeneMANIA also indicated that selected MMPs were strongly associated with each other, mainly related to oxidative stress, proteoglycan, lysosomal glycosidase and promotion of cell adhesion and spreading (Fig. 5D).

### 3.4. Potential transcription factors of selected MMPs

MMP1, MMP2, MMP7, MMP9, MMP11, MMP12, MMP14, and MMP28 were significantly up-regulated in PC tissues compared with adjacent tissues. Thus, we explored the potential transcription factors regulating the differentially expressed MMPs in PC patients by TRRUST. Totally, we found 10 transcription factors, including ETS variant transcription factor 4 (ETV4), signal transducer and activator of transcription 3 (STAT3), Jun proto-oncogene (JUN), MYC associated zinc finger protein (MAZ), RELA proto-oncogene (RELA), serum response factor (SRF), ETS proto-oncogene 2 (ETS2), Sp1 transcription factor (SP1), Fos proto-oncogene (FOS) and Nuclear factor- $\kappa$ B (NF- $\kappa$ B), were associated with the up-regulated of selected MMPs (Table 2). ETV4 was the key transcription factor of MMP1, MMP2, MMP7, and MMP14 ( $p=9.66E-11$ ,  $FDR=1.84E-09$ ). STAT3 was the key transcription factor of MMP1, MMP2, MMP7, MMP9, and MMP14 ( $P=1.23E-09$ ,  $FDR=9.97E-09$ ). JUN was the key transcription factor of MMP1, MMP2, MMP7, and MMP12 ( $P=1.57E-09$ ,  $FDR=9.97E-09$ ). MAZ was the key transcription factor of MMP1, MMP9, and MMP14 ( $P=2.80E-09$ ,  $FDR=1.33E-08$ ). RELA was the key transcription factor of MMP1, MMP2, MMP9, MMP12, and MMP14 ( $P=5.37E-08$ ,  $FDR=2.04E-07$ ). SRF was the key transcription factor of



**Figure 5.** (A) Genetic alterations of the selected MMPs in the queried PC samples by the online tool cBioPortal. (B) Analyses of genetic variations of MMP family member. (C) Protein-protein interaction (PPI) network analysis of selected MMPs. (D) PPI network and functional analysis from GENEMANIA.

MMP2, MMP9, and MMP14 ( $P=1.15E-07$ ,  $FDR=3.63E-07$ ). ETS2 was the key transcription factor of MMP1, MMP2, and MMP9 ( $P=2.47E-07$ ,  $FDR=6.69E-07$ ). SP1 was the key transcription factor of MMP2, MMP9, MMP11, MMP14, and MMP28 ( $P=5.04E-07$ ,  $FDR=1.20E-06$ ). FOS was the key transcription factor of MMP1, MMP7, and MMP9 ( $P=1.45E-06$ ,  $FDR=3.06E-06$ ). NF- $\kappa$ B was the key transcription factor of MMP1, MMP2, and MMP14 ( $P=4.33E-06$ ,  $FDR=7.60E-06$ ). ETV4 was up-regulated in CIC-deficient hepatocellular carcinoma

cells, which induces the expression of MMP1<sup>[43]</sup> and STAT3 was associated with proliferation, growth, and invasion of pancreatic cancer.<sup>[44,45]</sup> MAZ was significantly upregulated in PC tissues ( $P<.0001$ ) and significantly correlated with certain clinical characteristics of PC patients, such as age, tumor diameter, tumor number, serum CA19-9 level ( $P<.05$ ) and survival time ( $P=.0365$ ).<sup>[46]</sup> RelA/p65 is found to be activated in most of PC cell lines.<sup>[47]</sup> Elk-1/SRF pathway is a cancer-associated pathway.<sup>[48]</sup> ETS2 is associated with carcinoma

**Table 2**  
**Key regulated transcription factors of MMPs.**

Key TF	Description	Regulated gene	P value	FDR
ETV4	Ets variant 4	MMP1,MMP2,MMP7,MMP14	9.66E-11	1.84E-09
STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)	MMP1,MMP2,MMP7,MMP9,MMP14	1.23E-09	9.97E-09
JUN	Jun proto-oncogene	MMP1,MMP2,MMP7,MMP9,MMP12	1.57E-09	9.97E-09
MAZ	MYC-associated zinc finger protein (purine-binding transcription factor)	MMP1,MMP9,MMP14	2.80E-09	1.33E-08
RELA	V-rel reticuloendotheliosis viral oncogene homolog A (avian)	MMP1,MMP2,MMP9,MMP12,MMP14	5.37E-08	2.04E-07
SRF	Serum response factor (c-fos serum response element-binding transcription factor)	MMP2,MMP9,MMP14	1.15E-07	3.63E-07
ETS2	V-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	MMP1,MMP2,MMP9	2.47E-07	6.69E-07
SP1	Sp1 transcription factor	MMP2,MMP9,MMP11,MMP14,MMP28	5.04E-07	1.20E-06
FOS	FBJ murine osteosarcoma viral oncogene homolog	MMP1,MMP7,MMP9	1.45E-06	3.06E-06
NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	MMP1,MMP2,MMP14	4.33E-06	7.60E-06

progression and also plays a role in the progression of pancreatic adenocarcinoma, mainly in advanced stages.<sup>[49]</sup> SP1 is known as a key regulators in the carcinogenesis of pancreatic carcinoma.<sup>[50]</sup> FOS is a proto-oncogene in some types of tumors.<sup>[51]</sup> NF- $\kappa$ B, which helps to suppress anti-tumor immune responses, might increase anti-tumor immunity.<sup>[52]</sup>

### 3.5. Functional enrichment analysis of selected MMPs in PC patients

We used DAVID 6.8 and metascap to perform functional enrichment analysis. Ten most enriched gene ontology items in the biological process, molecular function (MF), and cellular component (CC) categories were shown in Tables S1–S3, Supplemental Digital Content, <http://links.lww.com/MD/G182>, <http://links.lww.com/MD/G183>, <http://links.lww.com/MD/G184>, respectively. In the biological process category, extracellular matrix organization (FDR = 2.44E-44), extracellular matrix disassembly (FDR = 1.03E-31), collagen metabolic process (FDR = 3.84E-18), regulation of cell motility (FDR = 6.16E-17), and regulation of cell migration (FDR = 1.76E-16) were considered to be associated with the progression of PC. And cell adhesion molecule binding (FDR = 7.81E-10) may be directly related to the metastasis of PC in the MF category. In contrast, most of the items in the CC category were related to extracellular or secretory components such as extracellular matrix (FDR = 6.05E-25) and secretory granule (FDR = 9.26E-19). These results suggests that selected MMPs were involved in the tumorigenesis of PC.

Moreover, Kyoto Encyclopedia of Genes and Genome pathway analysis was also performed. As illustrated in Table S4, Supplemental Digital Content, <http://links.lww.com/MD/G185>, proteoglycans in cancer (FDR = 3.35E-18), bladder cancer (FDR = 9.33E-11), Rap1 signaling pathway (FDR = 1.25E-09), ECM-receptor interaction (FDR = 6.67E-09), HIF-1 signaling pathway (FDR = 3.97E-07), PI3K-Akt signaling pathway (FDR = 1.24E-06), and focal adhesion (FDR = 1.95E-06) were the most enriched pathways, which are also commonly reported to be significantly associated with tumorigenesis of PC. Lorenz et al found Epac1, a Rap1-specific exchange factor, is highly expressed in pancreatic tumor cells and tissue, and that activation of the Epac1/Rap1 pathway is related to the growth control in pancreatic cancer cell lines.<sup>[53]</sup> Zhang et al reported that Rap1 activity is a key factor in determining the aggressiveness of tumor cells.<sup>[54]</sup> Wang et al revealed the overrepresented ECM-receptor interaction in pancreatic ductal adenocarcinoma.<sup>[55]</sup> Konstantinos Parperis et al revealed that

pancreatic carcinoma may be accompanied by paraneoplastic arthritides such as rheumatoid arthritis.<sup>[56]</sup> PI3K-Akt signaling pathway is related to the proliferation, invasion, metastasis and apoptosis of PC.<sup>[57,58]</sup> Focal adhesion kinase expression was significantly correlated with tumor size in PC ( $P = .004$ ).<sup>[59]</sup>

### 3.6. Seeking potential drugs for selected MMPs Using DB

In the development of many diseases, drugs interacted directly or indirectly with pathogenic biomolecules to achieved therapeutic goals.<sup>[60]</sup> Pathogenic biomolecules belonging to the same family could be inhibited by the same drug.<sup>[60]</sup> Thus, we first queried DB with the selected MMPs and found 5 drugs (DB00786, DB07556, DB07926, DB08403, DB 08482, and DB08491) directly targeting MMP1 (Table 3). For MMP2, there were 6 drugs (DB00786, DB01630, DB04866, DB05387, DB06423, and DB01197) targeting; MMP7 had 4 drugs (DB00786, DB08170, DB08489, and DB08493) targeting; MMP11 had 2 drugs (DB04318 and DB00786) targeting; MMP9 had 10 drugs (DB00786, DB01017, DB01296, DB01949, DB03683, DB05387, DB05495, DB06423, DB01197, and DB07117) targeting; and MMP12 had 10 drugs (DB02118, DB00551, DB03367, DB03880, DB04405, DB05387, DB00786, DB07026, DB07446, and DB07556) targeting (Table 3). However, there was only 1 drug for MMP14 (DB00786) and 1 for MMP28 (DB00786), respectively.

Among drugs we found to target the selected MMPs, DB00786 (Marimastat) was found to target all the MMPs differentially expressed in PC. We also queried DB with marimastat and found marimastat was categorized as amines, hydroxy Acids, metalloendopeptidases, antagonists, inhibitors, and hydroxylamines. Moreover, using the group status of marimastat as an investigational drug, query of DB indicated that it is a drug widely used for the treatment of various cancers. Such as orthotopic oral squamous cell carcinoma,<sup>[61]</sup> gastric carcinoma,<sup>[62,63]</sup> small-cell lung cancer,<sup>[64]</sup> glioma.<sup>[65]</sup> As one matrix-metalloproteinase inhibitor, marimastat is an anti-angiogenic anti-antitumor chemotherapeutic drug.<sup>[66]</sup> Long-term oral administration of marimastat is feasible and safe and is expected to be a first-line therapy for patients with unresectable PC.<sup>[67,68]</sup>

## 4. Discussion

MMPs are a family of zinc-dependent endopeptidases which play crucial role in many physiological processes including tissue remodeling, degradation of various proteins, cell proliferation,



**Table 3**

**Targeted drugs for MMPs family.**

Query_MMPs (8)		Matched_drugs			
Symbol	UniProtKB_AC	Entrez_ID	DB_ID	Name	Group
MMP1	P03956	4312	DB00786	Marimastat	investigational
			DB07556	CGS-27023	experimental
			DB07926	N-[3-(N'-HYDROXYCARBOXAMIDO)-2-(2-METHYLPROPYL)-PROPANOYL]-O-TYROSINE-N-METHYLAMIDE	experimental
			DB08403	METHYLAMINO-PHENYLALANYL-LEUCYL-HYDROXAMIC ACID	experimental
			DB08482	[[1-[N-HYDROXY-ACETAMIDYL]-3-METHYL-BUTYL]-CARBONYL-LEUCINYL]-ALANINE ETHYL ESTER	experimental
MMP2	P08253	4313	DB00786	Marimastat	investigational
			DB01630	SC-74020	experimental
			DB04866	Halofuginone	investigational, vet_approved
			DB05387	AE-941	investigational
			DB06423	Endostatin	investigational
MMP7	P50280	4316	DB01197	Captopril	approved
			DB00786	Marimastat	investigational
			DB08170	(1R)-N,6-DIHYDROXY-7-METHOXY-2-[(4-METHOXYPHENYL)SULFONYL]-1,2,3,4-TETRAHYDROISOQUINOLINE-1-CARBOXAMIDE	experimental
			DB08489	N4-HYDROXY-2-ISOBUTYL-N1-(9-OXO-1,8-DIAZA-TRICYCLO[10.6.1.013,18]NONADEC-12(19),13,15,17-TETRAEN-10-YL)-SUCCINAMIDE	experimental
			DB08493	5-METHYL-3-(9-OXO-1,8-DIAZA-TRICYCLO[10.6.1.013,18]NONADEC-12(19),13,15,17-TETRAEN-10-YLCARBAMOYL)-HEXANOIC ACID	experimental
MMP9	P14780	4318	DB00786	Marimastat	investigational
			DB01017	Minocycline	approved, investigational
			DB01296	Glucosamine	approved, investigational
			DB01949	2-Amino-N,3,3-Trimethylbutanamide	experimental
			DB03683	2-[[Formyl (Hydroxy)Amino]Methyl]-4-Methylpentanoic Acid	experimental
			DB05387	AE-941	investigational
			DB05495	PG-530742	investigational
			DB06423	Endostatin	investigational
			DB01197	Captopril	approved
			DB07117	5-(4-PHENOXYPHENYL)-5-(4-PYRIMIDIN-2-YLPIPERAZIN-1-YL)PYRIMIDINE-2,4,6 (2H,3H)-TRIONE	experimental
MMP11	P24347	4320	DB04318	N $\alpha$ -[[2S]-2-[[[S]-[(1S)-1-[[[Benzyloxy]carbonyl]amino]-2-phenylethyl]](hydroxy)phosphoryl]methyl]-5-phenylpentanoyl]-L-tryptophanamide	experimental
MMP12	P39900	4321	DB00786	Marimastat	investigational
			DB02118	CP-271485	experimental
			DB00551	Acetohydroxamic acid	approved
			DB03367	PF-00356231	experimental
			DB03880	Batimastat	experimental
			DB04405	2-[2-(1,3-Dioxo-1,3-Dihydro-2h-Isoindol-2-Yl)Ethyl]-4-(4'-Ethoxy-1,1'-Biphenyl-4-Yl)-4-Oxobutanoic Acid	experimental
			DB05387	AE-941	investigational
			DB00786	Marimastat	investigational
DB07026	(1S,5S,7R)-N~7~-((BIPHENYL-4-YLMETHYL)-N~3~-HYDROXY-6,8-DIOXA-3-AZABICYCLO[3.2.1]OCTANE-3,7-DICARBOXAMIDE	experimental			
DB07446	N-(biphenyl-4-ylsulfonyl)-D-leucine	experimental			
DB07556	CGS-27023	experimental			
MMP14	P50281	4323	DB00786	Marimastat	investigational
MMP28	Q9H239	79148	DB00786	Marimastat	investigational

migration, differentiation, apoptosis, angiogenesis, tissue repair, and immune response.<sup>[19]</sup> During the progression of tumor, extracellular proteinases mediate changes in microenvironment. Moreover, degradation of ECM is an essential step in tumor metastasis and MMPs are playing vital roles in this process. MMPs have also been proved to be correlated with the invasiveness of PC cells.<sup>[33]</sup> However, it is unclear whether MMPs can be diagnostic biomarkers for PC. The expression of MMPs and their correlation with the pathological stages of PC were explored for the first time. We found that several MMPs were significantly upregulated while 2 MMPs were down-regulated in PC tissues compared with adjacent pancreatic tissues. MMP1, MMP2, MMP7, MMP9, MMP11, MMP12, MMP14, and MMP28 were selected as the targets of our further study. The expression of MMP1, MMP7, MMP11, and MMP14 increased as PC progressed. All target MMPs with high expression showed poor prognosis. Moreover, low expression of both MMP14 and MMP28 was significantly associated with longer DFS, and high expression levels of MMP1, MMP11, MMP14, and MMP28 were significantly associated with shorter OS in PC patients. The above data suggested that these differentially expressed MMPs may play vital roles in the progression of PC. Previous studies have also showed the abnormal expression of MMP1, MMP2, MMP7, MMP8, MMP9, and MMP11 in PC tissues.<sup>[34,35,37]</sup>

We then explored the co-expression, genetic alteration, neighbor gene interaction network analysis of selected MMPs in PC patients to provide an integrated and comprehensive analysis of the molecular characteristics of MMPs. The genetic alterations of the selected MMPs included amplification, deep deletion and mutation. The potential interaction analysis revealed a correlation between selected MMPs and cell adhesion, spreading promotion, cell migration, inflammation, oncogenesis, metastasis, and tumorigenesis and progression. These data suggested that the selected MMPs play a synergistic role in the progression of PC. We then identified the potential transcription factors of the selected differentially expressed MMPs, and we found that ETV4, STAT3, JUN, MAZ, RELA, SRF, ETS2, SP1, FOS, and NF- $\kappa$ B may be key transcription factors for selected MMPs. Overexpression of ETV4 promotes progression, proliferation and invasion of many carcinomas.<sup>[43,69,70]</sup> STAT3 regulates glycolysis in hepatocellular carcinoma cells,<sup>[71]</sup> is associated with inhibition of epithelial-mesenchymal transition in hepatocellular carcinoma,<sup>[72]</sup> and is related to proliferation promotion and apoptosis inhibition in esophageal squamous cell carcinoma.<sup>[73,74]</sup> Previous studies have also identified a role for STAT3 in the regulation of PC.<sup>[44]</sup> JUN, MAZ, RELA, SRF, ETS2, SP1, FOS, and NF- $\kappa$ B were activated in many cancers including PC. Our study provided additional data on the complicated relationship among MMPs, PC, and transcription factor-related signaling pathways. Furthermore, extracellular matrix disassembly, regulation of cell motility, regulation of cell migration, cell adhesion molecule binding and extracellular or secretory components all played vital roles as expectation. Our study suggested that the selected MMPs were involved in the tumorigenesis of PC. We also focused on drugs targeting selected MMPs, which helped us to get a clear understanding of the biological function and mechanism of these drugs. DB00786 (marimastat) was found to target all differentially expressed MMPs in PC, which provides novel insights and additional support for the use of marimastat in PC patients

Our study has some limitations and further exploration is needed. Information from blood samples has an irreplaceable role in exploring diagnostic tumor biomarkers. Given that we currently do not have access to high-quality blood sample information, the results of this paper need to be further validated by *in vivo* and *in vitro* experiments. In general, our study provided potential diagnostic biomarkers and therapeutic target for PC patients.

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