Received: 17 July 2022 | Revised: 19 September 2022 | Accepted: 20 September 2022

DOI: 10.1002/jcla.24719

RESEARCH ARTICLE

WILEY

Downregulation of MMP1 functions in preventing perineural invasion of pancreatic cancer through blocking the NT-3/TrkC signaling pathway

Xiaoqing Xu[1,2] | Xiaomin Lu[3] | Liping Chen[1] | Ke Peng[1] | Fuhai Ji[1]

[1]Department of Anesthesiology, The First Affiliated Hospital of Soochow University, Suzhou, China

[2]Department of Pain Medicine, The Haian Hospital Affiliated to Nantong University, Nantong, China

[3]Department of Oncology, The Haian Hospital Affiliated to Nantong University, Nantong, China

Correspondence
Fuhai Ji, Department of Anesthesiology, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu 215006, China.
Email: jifuhai@suda.edu.cn

Funding information
National Natural Science Foundation of China, Grant/Award Number: 82072130 and 81873925; Natural Science Foundation of Jiangsu Province, Grant/Award Number: BK20191171; Six talent peaks project in Jiangsu Province, Grant/Award Number: WSN-022

Abstract

Background: Pancreatic cancer (PC) is a fatal malignancy that frequently involves perineural invasion (PNI). This study aims to investigate the function and underlying mechanisms of matrix metalloproteinase-1 (MMP1) in PNI of PC.

Methods: Human pancreatic cancer PANC-1 cells were co-cultured with dorsal root ganglion in vitro. The expression of MMP1, epithelial–mesenchymal transition (EMT) markers, Schwann cell markers, neurotrophic factors, NT-3, and TrkC was measured by qRT-PCR or Western blot. Transwell assay was performed to evaluate cell migration and invasion. In vivo model of PNI was established via inoculating PANC-1 cells into mice. PANC-1 cells and mice were also treated with LM22B-10 (an activator of TrkC) to confirm the mechanisms involving NT-3/TrkC in PNI of PC both in vivo and in vitro.

Results: The expression of MMP1 was significantly higher in PDAC tissues than non-cancerous tissues, which was positively associated with PNI. MMP1 knockdown repressed the migration and invasion of PANC-1 cells. Except for E-cadherin, the expression of EMT markers, Schwann cell markers, neurotrophic factors, NT-3, and TrkC was inhibited by MMP1 silencing. The same effects of MMP1 knockdown on the above factors were also observed in the PNI model. Moreover, MMP1 knockdown elevated the sciatic nerve function and reduced PNI in the model mice. LM22B-10 partially abolished the effects of MMP1 knockdown both in vivo and in vitro.

Conclusions: Silencing of MMP1 prevents PC cells from EMT and Schwann-like cell differentiation via inhibiting the activation of the NT-3/TrkC signaling pathway, thus alleviating the PNI of PC.

KEYWORDS
epithelial–mesenchymal transition, matrix metalloproteinase-1, NT-3/TrkC signaling pathway, perineural invasion, Schwann-like cell differentiation

Xiaoqing Xu and Xiaomin Lu contributed equally.

1 | INTRODUCTION

Since 2016, pancreatic cancer (PC) has become the third leading cause of cancer death.[1] Pancreatic ductal adenocarcinoma (PDAC), accounting for more than 90% of PC cases, is the most frequent type of PC.[2,3] From 1990 to 2017, the number of patients with pancreatic cancer has increased from 196,000 to 441,000 worldwide.[4] At an early stage, PC is difficult to be diagnosed. Thus, a majority of PC patients present at an advanced stage along with surgically inoperable.[5] Most patients experience relapse even after a potential radical treatment, presenting a low 5-year survival rate of only 2–9%.[6]

Perineural invasion (PNI) refers to the invasion of malignant cells into or around nerves, which is a major pathologic hallmark of a variety of malignancies, including PDAC. PNI occurs in approximately 100% patients with PDAC.[7–9] The invasion of cancer cells into peripheral nerves provides a route for the metastasis through cross-talking with neuron cells.[7,10,11] Additionally, PNI is also related to an increased risk of local recurrence and decreased survival rate.[12,13] It is critical to understand how cancer invades nerves and to develop relevant therapeutic strategies.

Matrix metalloproteinase-1 (MMP1) is a MMP family member that functions in degrading the extracellular matrix (ECM) and basement membrane components.[14,15] MMP1 enhances the migration and invasion of tumor cells and is strongly associated with adverse outcomes of tumors.[16–18] A gene expression profile of MMPs revealed that MMP1 expression is significantly upregulated in 11 different cancer types, such as bladder, breast, lung squamous, and other cancers.[19] The upregulation of MMP1 has also been detected in human PDAC tissues, and silencing of MMP1 mediated by miRNA can inhibit PC metastasis.[20] Besides, the expression of MMP1 is associated with PNI in prostate carcinoma.[21] Recently, a study reported that MMP1 modulates early PNI of PC cells,[22] which indicates a crucial role of MMP1 in the PNI of PC.

Neurotrophin-3 (NT-3) is a neurotrophic factor that works by interacting with a specific downstream receptor, tropomyosin receptor kinase C (TrkC). NT-3 and TrkC can mediate osteosarcoma cell growth and metastasis.[23] The expression of NT-3 and TrkC is both up-regulated in PC and prostate cancer with PNI.[24,25] Also, a previous study indicated that the NT-3/TrkC axis facilitates the PNI progression in human SACC.[26] These findings suggest a vital role of the NT-3/TrkC signaling pathway in PNI.

Based on the previous findings, we hypothesize that MMP1 may regulate the PNI of PC via regulating the NT-3/TrkC signaling pathway. Thus, this study sought to elucidate the function of MMP1 in the PNI of PC, and the underlying mechanism involving the NT-3/TrkC signaling. Our study may reveal a novel action mechanism of MMP1 in PNI and also provide a potential therapeutic target for PC.

2 | MATERIALS AND METHODS

2.1 | Tumor specimens

Between April 2020 and October 2020, a total of 170 PDAC tissues and matched adjacent non-tumor tissues were excised from patients at Soochow University's First Affiliated Hospital. This study was performed in accordance with the principles of the Declaration of Helsinki. Each patient signed an informed consent form. This research was approved by the Ethics Committee of Soochow University's First Affiliated Hospital. The study is also reported in accordance with ARRIVE guidelines.

2.2 | Cell culturing and transfection

Normal pancreatic epithelial cell line HPC-Y5 was purchased from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, and human pancreatic cancer cell lines, SW1990 and PANC-1, were obtained from CTCC. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS; Gibco). Nerve-derived cell microglia BV2 was obtained from Procell Life Science&Technology Co., Ltd. and cultured in DMEM containing 10% FBS and 1% penicillin–streptomycin (Thermo Fisher Scientific). All cells were cultured at 37°C with 5% CO_2. In addition, a siRNA targeting MMP1 (si-MMP1) and a negative siRNA (si-NC) that synthesized in GenePharma were transfected into PANC-1 cells (2×10^5 cells/well) using Lipofectamine 3000 (Invitrogen). After the transfection for 48 hours, cells were harvested for the following assays.

2.3 | Dorsal root ganglion (DRG) co-culturing

The newborn Balb/c mice were euthanatized, and DRGs were isolated as previously described.[11] The harvested DRGs were immediately placed in PBS and implanted in a drop of growth factor-depleted Matrigel.[27] DRGs in Matrigel drops were incubated in DMEM containing 10% FBS. Subsequently, 5×10^4 cancer cells were added around the DRG at 7 days after DRG implantation. PANC-1 cells were co-cultured with DRG for 24 h and then used for the following analysis.

2.4 | Transwell assay

For detecting cell migration, the upper chambers were loaded with approximately 1×10^5 PANC-1 cells suspended in serum-free medium. The medium containing 10% FBS was added to the lower chamber. For detecting cell invasion, the steps were the same as

those of cell migration. The only difference was that the transwell membrane was pre-coated with fresh Matrigel (Corning) before cell seeding in the upper chamber. Cells not migrated or invaded were scraped off at 48 h later. Cells on the lower chamber were fixed with methanol and then stained with crystal violet. Finally, the images were captured and cells were counted under a microscope.

2.5 | qRT-PCR

Total RNAs in samples were extracted using TRIzol reagent (Takara). Equal quantity of RNAs was synthesized into first cDNAs using M-MLV Reverse Transcriptase (Vazyme). Interest genes were amplified using a SYBR green pro kit (Accyrate Biology). Gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method and normalized to GAPDH. Table 1 presented the primers of different genes.

2.6 | Western blot

Pancreatic cancer tissues or PANC-1 cells were lysed in RIPA buffer (Solarbio) containing 1% PMSF. A BCA kit (Solarbio) was used to quantify the protein concentration. Subsequently, the proteins were separated by SDS-PAGE and transferred onto PDVF membranes (Roche). The membranes were blocked with 5% nonfat milk, incubated with primary antibodies against NT-3 (ab16640, Abcam), TrkC (ab227289, Abcam), Vimentin (ab8978, Abcam), N-Cadherin (ab76011, Abcam), E-Cadherin (ab1416, Abcam), and GAPDH (ab8245, Abcam) at 4°C overnight, and then incubated with a horseradish peroxidase-conjugated secondary antibody. The bands of proteins were visualized using an ECL Plus detection system, and the gray value was normalized to GAPDH.

2.7 | In vivo PNI model

Animal experiments were approved by the Ethics Committee of The First Affiliated Hospital of Soochow University in accordance with the National Institutes of Health's guidelines for the care and use of laboratory animals. Twenty nude mice (5 weeks old; Antaik Biotechnology) were used for the establishment of in vivo model of PNI following one week of acclimation. Mice were randomly divided into three groups, including the si-NC group (si-NC-transfected cells; $n = 6$), si-MMP1 group (si-MMP1-transfected cells; $n = 7$), and si-MMP1 + LM22B-10 group (si-MMP1-transfected cells and LM22B-10; $n = 7$). Mice were anesthetized and inguinally injected with 2.5×10^6 PANC-1 cells of different groups. The mice in the si-MMP1 + LM22B-10 group were treated with 50 mg/kg LM22B-10 (a TrkC activator) daily for 2 weeks. The following indexes were recorded every week to assess the function of the hind limbs in mice: (a) Sciatic nerve function index represents the extension length between the first and fifth toes of the hind limbs; (b) Hind limb function is classified from 4 (normal) to 1 (total paw paralysis) based on the response of hind limb to manual extension. After the injection for 7 weeks, all mice were euthanized by intraperitoneal injection of pentobarbital sodium (100 mg/kg body weight). The tumor xenografts were excised and used for subsequent experiments.

2.8 | Histological analyses

The histological changes in tumor xenografts were detected by hematoxylin and eosin (H&E) staining. The paraffin-embedded tissue sections (5 μm) were deparaffinized with xylene and then stained with hematoxylin and eosin. The morphologic changes of tumor xenografts were observed under a light microscope. In addition, the sections of PC tissues and non-tumor tissues (normal control) from patients were used to detect MMP1 expression by immunohistochemistry. The sections were incubated at high temperature for 30 mines for antigen retrieval, blocked with blocking solution, and incubated with anti-MMP1 antibody (ab52631, Abcam) at 4°C overnight. The sections were then incubated with the secondary IgG antibody (ab6728, Abcam) for 30 min at 37°C. After exposed to diaminobenzidine (DAB) and counterstained with hematoxylin, positive MMP1 staining was observed in tissues.

2.9 | Statistical analysis

Data were presented as mean ± SD. The statistical significance was analyzed using SPSS software. The differences between two groups

TABLE 1 Primers used for qRT-PCR

Genes	Forward (5′-3′)	Reverse (5′-3′)
MMP1	AACCGGATCAGCCATGAGGGT	CCAGCCTAGTCGGTACTCCCA
BDNF	TAACGGCGGCAGACAAAAAGA	TGCACTTGGTCTCGTAGAAGTAT
GDNF	AGAGGAAAAGGTCGGAGAGG	CCTTGGTTTCATAGCCCAGA
NGF	TGTGGGTTGGGGATAAGACCA	GCTGTCAACGGGATTTGGGT
NRTN	GCTGTCCATCTGGATGTGTCG	CAGGAGTGCACGGTACTGG
S100A4	CTCAGCGCTTCTTCTTTC	GGGTCAGCAGCTCCTTTA
GFAP	CCTCTCCCTGGCTCGAATG	GGAAGCGAACCTTCTCGATGTA
P75NTR	GGAAGCGAACCTTCTCGATGTA	TGAAGGCTATGTAGGCCACAA
GAPDH	GCATTGCCCTCAACGACCAC	CCACCACCCTGTTGCTGTAG

were assessed by Student's *t* test, and one-way or two-way ANOVA followed by Tukey's test was used to analyze the differences among multiple groups. The associations between MMP1 expression and other factor expression were evaluated using two-tailed Pearson correlation analysis. The significant level was defined as a p-value < 0.05.

3 | RESULTS

3.1 | MMP1 is overexpressed in PC and correlated with PNI

The expression of MMP1 in PC tissues and non-tumor tissues was determined by immunohistochemistry. As shown in Figure 1A, MMP1 expression in non-cancerous tissues was negative, but most PDAC tissues showed positive MMP1 staining. Afterward, we examined the mRNA expression of MMP1 in normal pancreatic epithelial cell line HPC-Y5, human pancreatic cancer cell lines SW1990 and PANC-1, and mouse microglial line BV2. As a result, SW1990, PANC-1, and BV2 cells exhibited a higher MMP1 expression compared with HPC-Y5 cells, and BV2 cells displayed the highest MMP1 expression among these four cell lines (Figure 1B). Besides, the increased mRNA expression of MMP1 was also observed in PDAC tissues and isolated DRGs (Figure 1C). The data in Table 2 showed that MMP1 expression in PDAC patients is positively associated with PNI. These results indicate that MMP1 may play a positive role in PNI and PC progression. A loss-of-function analysis was then performed to evaluate the function of MMP1 in PC. We detected the efficiency of siRNAs targeting MMP1 in PANC-1 cells. The results in Figure 1D showed that si-MMP1 effectively inhibited MMP1 expression in PANC-1 cells.

3.2 | MMP1 knockdown suppresses Epithelial–mesenchymal transition (EMT) and Schwann-like cell differentiation of PC cells

PANC-1 cells were co-cultured with excised murine DRG for 24 hours. The effect of MMP1 silencing on PC metastasis was assessed in vitro. Transwell assay revealed that MMP1 downregulation significantly inhibited the migration and invasion of PANC-1 cells (Figure 2A,B). EMT is a key process in the migration and invasion of cancer cells.[28] Also, we performed Western blot to detect the levels of EMT-related proteins, including Vimentin, N-cadherin, and E-cadherin in PANC-1 cells. As expected, knockdown of MMP1 significantly increased E-cadherin expression but reduced N-cadherin and Vimentin expression in PANC-1 cells (Figure 2C). These results indicate that MMP1 knockdown represses the migration and invasion of PC cells via inhibiting EMT. Moreover, the mRNA expression of pancreatic neurotrophic factors, including nerve growth factor (NGF), brain-derived neurotrophic

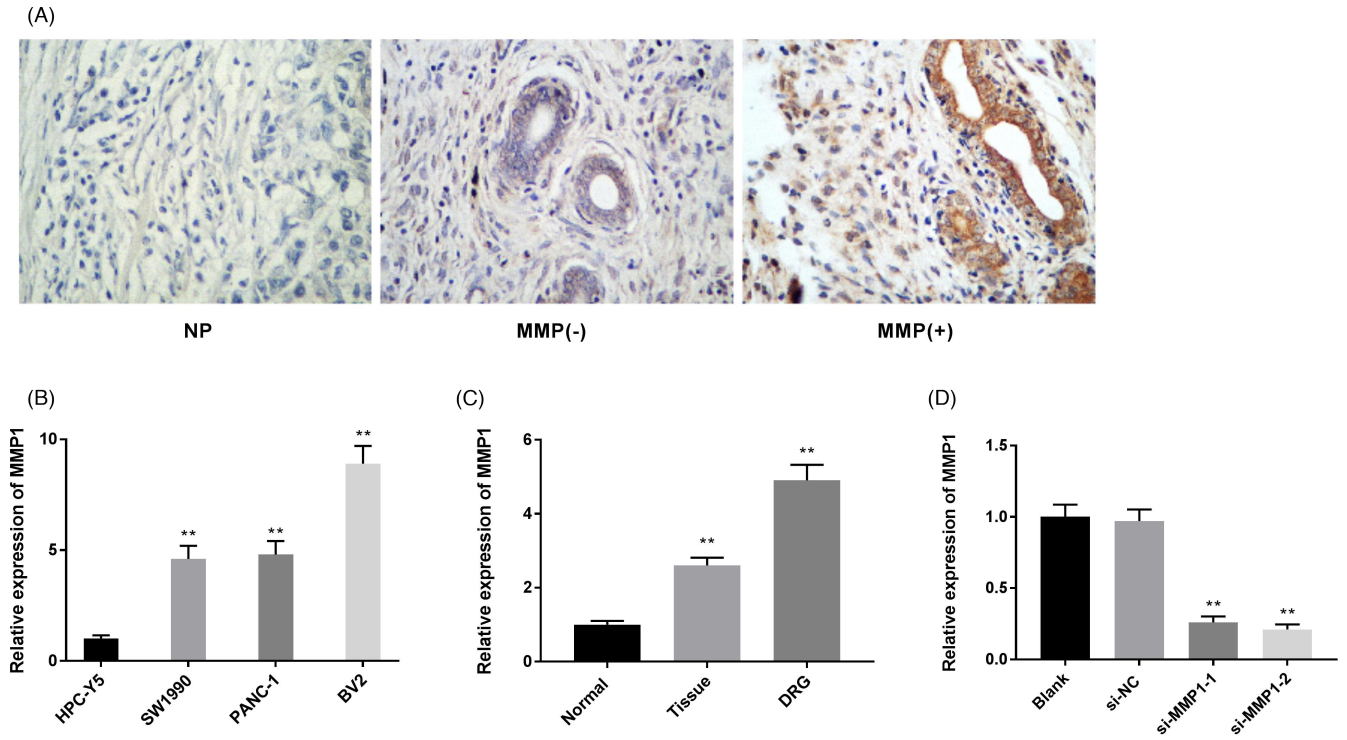

FIGURE 1 MMP1 is highly expressed in PC tissues and cell lines. (A) Representative images of immunohistochemistry of MMP1 expression in PDAC tissues and corresponding non-cancerous pancreatic tissues (NP). (B) Relative mRNA expression of MMP1 in HPC-Y5, SW1990, PANC-1, and BV2 cells was measured by qRT-PCR. (C) Relative mRNA expression of MMP1 in normal pancreatic tissues and PDAC tissues from patients, and DRG isolated from mice was measured by qRT-PCR. (D) The efficiency of MMP1 knockdown was examined by qRT-PCR in PANC-1 cells transfected with si-MMP1-1/2.

TABLE 2 Correlations between MMP1 expression and clinicopathological parameters in PDAC patients

Clinicopathological parameters	cases 170	MMP1 high expression 85	MMP1 low expression 85	*p*-Value
Age(years)				
<50	93	42	51	0.218
≥50	77	43	34	
Sex				
Male	87	45	42	0.759
Female	83	40	43	
Tumor size				
<5 cm	95	42	53	0.122
≥5 cm	75	43	32	
Histological grade				
G1	48	15	33	0.005**
G2	63	39	24	
G3	59	31	28	
Lymph node metastasis				
N0	95	40	55	0.005**
N1	75	45	30	
pTNM stage				
I	21	3	18	<0.001***
II	84	59	25	
III	65	49	16	
Perineural invasion (PNI)				
−	99	37	62	<0.001***
+	71	48	23	

Note: $^{**}p < 0.01$ and $^{***}p < 0.001$ represent significantly different between MMP1 high expression and MMP1 low expression.

factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), and neurturin (NRTN) was all down-regulated by the knockdown of MMP1 in PANC-1 cells (Figure 2D), indicating that MMP1 may contribute to PNI by promoting the expression of neurotrophic factors in PC. Since these neurotrophic factors were secreted by Schwann cells, the expression of Schwann cell markers, including S100 calcium-binding protein A4 (S100A4), p75 neurotrophin receptor (p75NTR), and glial fibrillary acidic protein (GFAP), was detected. The results showed that MMP1 knockdown significantly down-regulated the expression of these markers in PC cells (Figure 2E), indicating a critical role of MMP1 in the differentiation of PC cells into Schwann-like cells.

3.3 | MMP1 knockdown inactivates the NT-3/TrkC signaling pathway

The NT-3/TrkC signaling pathway is involved in tumor growth and metastasis. We further measured the protein levels of NT-3 and TrkC in PANC-1 cells. As shown in Figure 3, silencing of MMP1 reduced the expression of NT-3 and TrkC in PANC-1 cells. The downregulation of MMP1 may block the activation of the NT-3/TrkC signaling pathway in PC.

3.4 | The effects of MMP1 on EMT and Schwann-like cell differentiation are mediated by the NT-3/TrkC signaling pathway

To confirm the action mechanisms of MMP1 involving the NT-3/TrkC signaling pathway in the PNI of PC, LM22B-10, an activator of TrkC was used to activate TrkC in PANC-1 cells. Cell migration and invasion suppressed by MMP1 knockdown were partially restored by LM22B-10 treatment, indicating that TrkC facilitates the PC metastasis in vitro (Figure 4A,B). Besides, LM22B-10 decreased the protein expression of E-cadherin while increasing that of N-cadherin and Vimentin (Figure 4C). Furthermore, the activation of TrkC also enhanced the expression of neurotrophic factors BDNF, GDNF, NRTN, and NGF, as well as Schwann markers S100A4, P75, and GFAP (Figure 4D,E). To confirm these findings in vivo, PANC-1 cells were injected into mice to establish the PNI model. The mice in the si-MMP1 group exhibited higher sciatic nerve function index and sciatic nerve score compared with mice in the si-NC group, and these changes were weakened by LM22B-10 treatment (Figure 5A,B). Tumor xenografts were excised for HE staining at 7 weeks after injection. MMP1 knockdown decreased the incidence of PNI and this inhibitory effect was abolished by LM22B-10 treatment (Figure 5C).

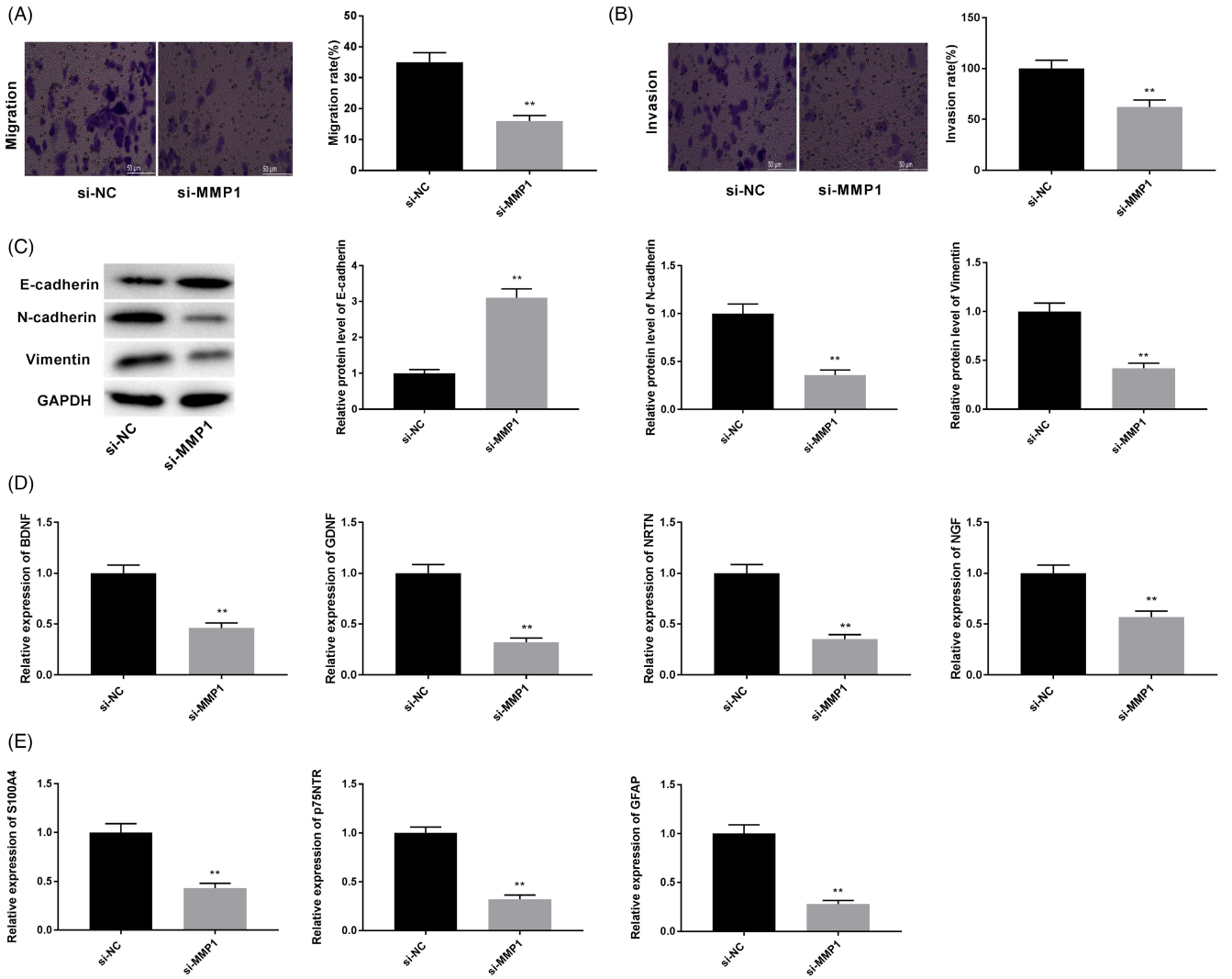

FIGURE 2 Knockdown of MMP1 represses the migration, invasion, EMT, and Schwann-like cell differentiation in PC cells. (A and B) Transwell assay was performed to detect the migration (A) and invasion (B) of PANC-1 cells. (C) Relative protein expression of E-cadherin, N-cadherin, and Vimentin in PANC-1 cells was detected by Western blot. (D and E) Relative mRNA expression of neurotrophic factors (BDNF, GDNF, NRTN, and NGF) (D) and Schwann cell markers (S100A4, P75NTR, and GFAP) (E) in PANC-1 cells.

These results indicate that the activation of the NT-3/TrkC signaling pathway aggravates PNI. In consistent with the results in vitro, LM22B-10 treatment exhibited similar effects on EMT, neurotrophic factors, and Schwann-like cell differentiation in vivo (Figure 5D–F). The above results demonstrate that MMP1 plays a key role in PNI through regulating the NT-3/TrkC signaling pathway.

3.5 | MMP1 expression is closely correlated with the expression of EMT markers, neurotrophic factors, and Schwann cell markers

In human PC specimens, the associations of MMP1 expression with the expression of EMT markers, neurotrophic factors, and Schwann cell markers were analyzed. As shown in Figure 6A, MMP1 was negatively correlated with E-cadherin ($R = -0.5027$), while positively correlated with N-cadherin ($R = 0.632$) and Vimentin ($R = 0.594$). Besides, MMP1 was also positively correlated with Schwann cell markers, including S100A4 ($R = 0.662$), P75NTR ($R = 0.499$), and GFAP ($R = 0.121$) (Figure 6B), as well as neurotrophic factors, including BDNF ($R = 0.572$), GDNF ($R = 0.439$), and NRTN ($R = 0.405$) and NGF ($R = 0.650$) (Figure 6C). Thus, MMP1 expression was positively correlated with EMT, neurotrophic factor production, and Schwann-like cell differentiation in the PNI of PC.

4 | DISCUSSION

Perineural invasion is often the first route of metastasis and a prominent characteristic of PC.[13,29] In this study, we assessed the function of MMP1 in PNI of PC and identified a novel action mechanism

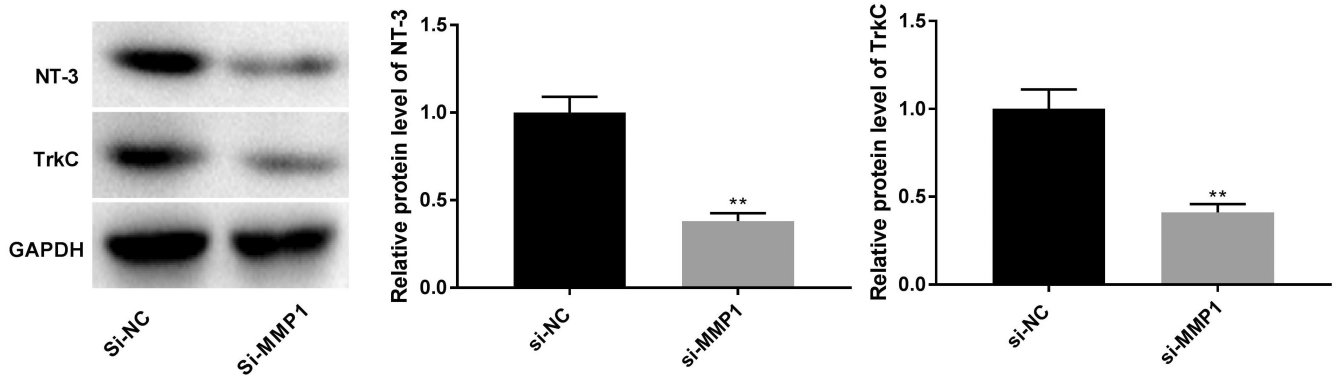

FIGURE 3 Knockdown of MMP1 inactivates the NT-3/TrkC signaling pathway. The protein expression of NT-3 and TrkC in PANC-1 cells was detected by Western blot.

involving the NT-3/TrkC signaling pathway. We found that MMP1 expression was up-regulated in PC tissues and cells. Knockdown of MMP1 inhibited cell migration and invasion, EMT, Schwann-like cell differentiation, and neurotrophic factor expression to retrain the PNI of PC, and these effects were associated with the deactivation of the NT-3/TrkC signaling pathway.

Mechanisms of matrix metalloproteinase-1 are keys enzymes function in regulating ECM turnover in tumor microenvironment, and their expression is closely associated with the tumorigenesis and progression of multiple tumors.[30] MMP1 is a collagenase in the MMP family, which involves the progression of a variety of tumors. For instances, the upregulation of MMP1 contributes to the growth and metastasis of non-small cell lung cancer and breast cancer.[31,32] Downregulation of MMP-1 represses the invasion and migration of gastric cancer.[33] These findings indicate a positive role of MMP1 in the progression of cancers. Similarly, we discovered that MMP1 was overexpressed in PC tissues and cell lines, and siRNA-mediated knockdown of MMP1 weakened the migratory and invasive abilities of PC cells. Additionally, MMP1 expression was positively related to PNI in PC patients. Based on these findings, we suspect that MMP1 is involved in the development of PNI in PC.

The loss of epithelial marker E-cadherin and the acquisition of mesenchymal markers Vimentin and N-cadherin occur in EMT, and this is a biological process in which cells lose their epithelial traits and acquire mesenchymal ones.[34] EMT is associated with a variety of tumor behaviors, including tumorigenesis, progression, metastasis, and other phenotypes.[35] It has been reported that PNI is related to EMT in human cutaneous squamous cell carcinoma.[36] Here, we assessed the function of MMP1 on EMT in PC both in vivo and in vitro. MMP1 knockdown up-regulated E-cadherin and down-regulated N-cadherin and Vimentin in PC cells, indicating an inhibitory effect of MMP1 silencing on EMT. In consistent with our findings, a previous study showed that MMP1 knockdown enhances E-cadherin expression and inhibits Vimentin expression in cervical cancer.[37]

Schwann cells are the primary glia of the vertebrate peripheral nervous system, presenting a range of activities in peripheral nerves and ganglia.[38] Researchers also point out that the differentiation of cancer cells into Schwann-like cells may be an inducer in the occurrence of PNI in tumors.[39,40] Meanwhile, increasing evidence has demonstrated that Schwann cell markers S100A4, P75NTR, and GFAP are up-regulated in multiple tumors with PNI, including breast cancer, prostate cancer, and colorectal cancer.[41–43] BDNF, GDNF, NRTN, and NGF are neurotrophic factors secreted by Schwann cells that can promote neuronal growth and PNI progression.[12] NGF, GDNF, and NRTN are significantly increased during the progression of pancreatic intraepithelial neoplasm (PanIN).[44] In our study, the downregulation of MMP1 restricted the expression of Schwann cell markers and neurotrophic factors in PC, which indicates that MMP1 downregulation prevents the differentiation of PC cells into Schwann-like cells. These findings also suggest that MMP1 may contribute to PNI progression through enhancing the differentiation of PC cells toward Schwann-like cells. Similarly, a previous study also indicated that macrophage migration inhibitory factor mediates the EMT and Schwann-like cell differentiation to promote the PNI of SACC.[45] Based on these findings, we conclude that MMP1 regulates PNI of PC via facilitating EMT and the differentiation of PC cells into Schwann-like cells.

However, the underlying down-steam signaling pathway for MMP1 in regulating PNI remains unclear. TrkC is a receptor tyrosine kinase that binds NT-3. After binding with NT-3, TrkC manifests autophosphorylation and results in the activation of distinct intracellular signaling pathways.[46] Previous researches have demonstrated that NT-3 and TrkC expression are increased in PC and prostate cancer with PNI.[24,25] In this study, the protein expression of NT-3 and TrkC was inhibited by MMP1 knockdown, while promoted by TrkC activator. The activation of the NT-3/TrkC signaling pathway promoted PNI of PC via facilitating EMT and Schwann-like cell differentiation. These findings are similar to a previous research that miR-429-mediated inhibition of NT-3 mitigates PNI of PC.[47] Moreover, abnormal activation of TrkC and TrkC fusion proteins

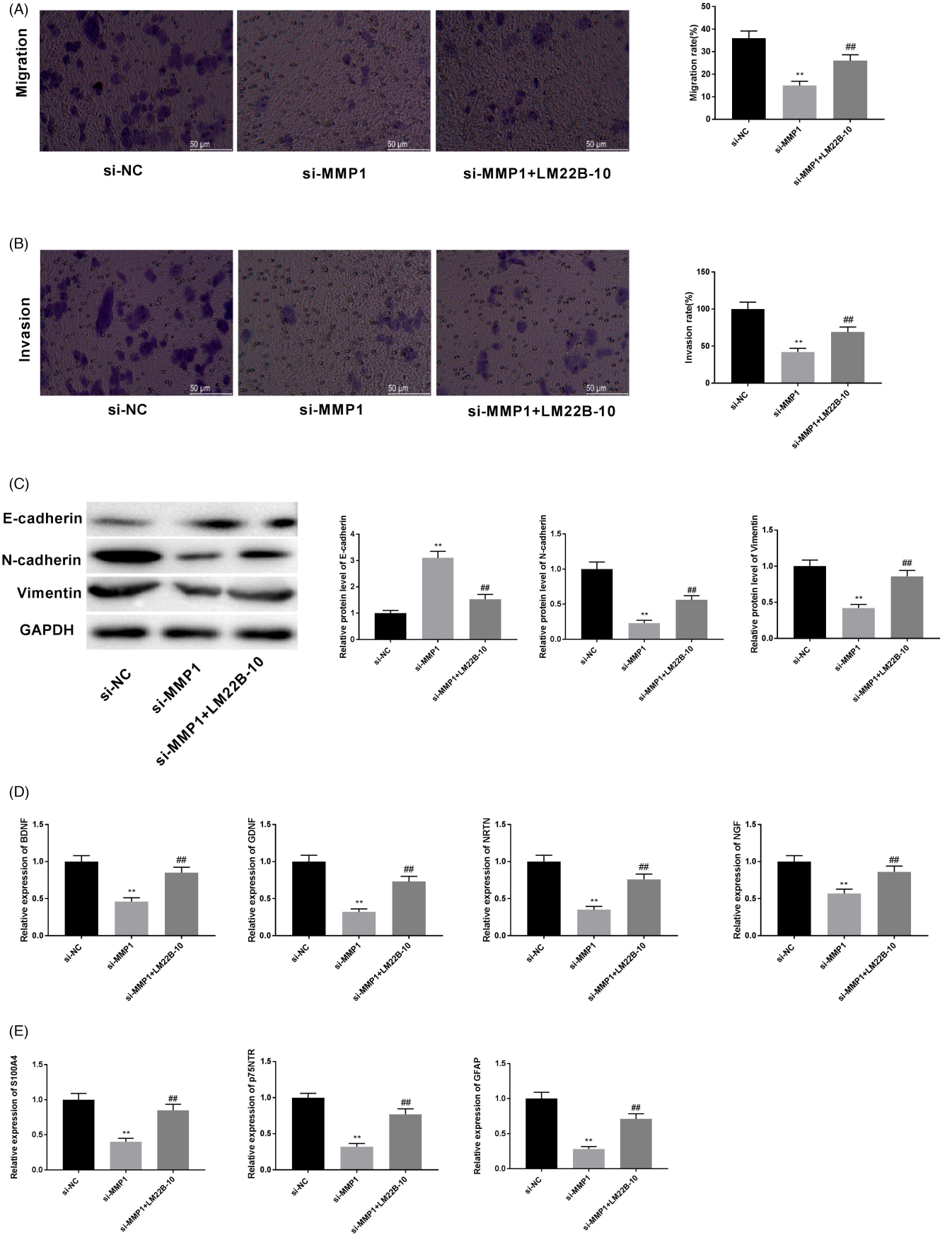

FIGURE 4 Action mechanism of MMP1 on EMT and PNI is associated with the NT-3/TrkC signaling pathway. (A-B) The migration (A) and invasion (B) of PANC-1 cells treated with LM22B-10 were determined by Transwell assay. (C) Relative protein expression of E-cadherin, N-cadherin, and Vimentin in LM22B-10-treated PANC-1 cells was detected by Western blot. (D–E) Relative mRNA expression of neurotrophic factors (BDNF, GDNF, NRTN, and NGF) (D) and Schwann cell markers (S100A4, P75NTR, and GFAP in PANC-1 cells) (E).

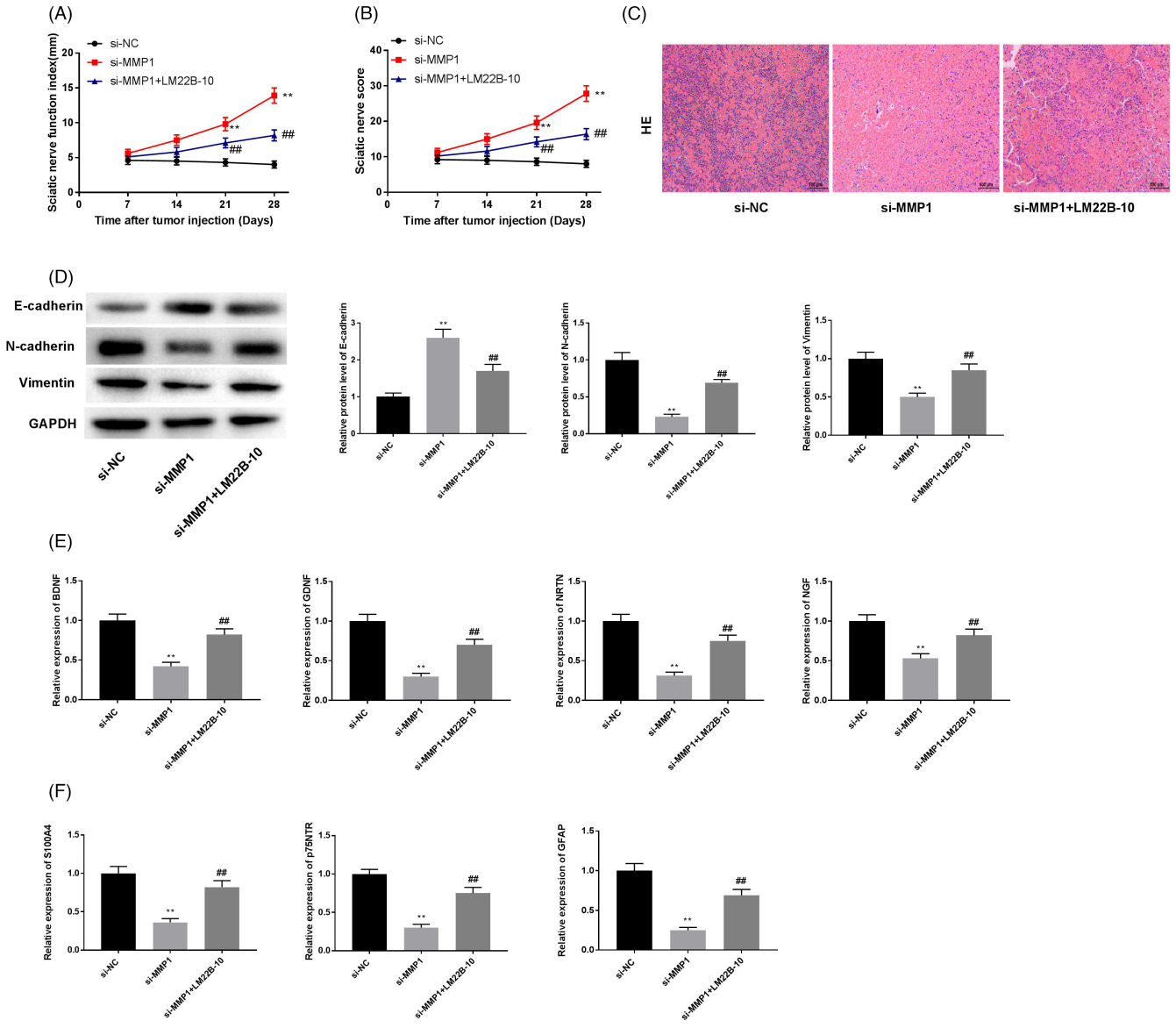

FIGURE 5 Knockdown of MMP1 inhibits PNI of PC in vivo. (A) The sciatic nerve function index. (B) The sciatic nerve score. (C) Representative images of HE staining of PNI in tumor xenografts. Scale bar = 100 μm. (D) Relative protein expression of E-cadherin, N-cadherin, and Vimentin in tumor xenografts. (E, F) Relative mRNA expression of neurotrophic factors (BDNF, GDNF, NRTN, and NGF) (E) and Schwann cell markers (S100A4, P75NTR, and GFAP) (F) in tumor xenografts.

significantly promotes EMT.[48] The function of the NT-3/TrkC signaling pathway in EMT and Schwann-like cell differentiation has also been confirmed in SACC.[26] Therefore, MMP1 may contribute to the PNI of PC via activating the NT-3/TrkC signaling pathway.

According to the findings of this study, we consider the NT-3/TrkC signaling pathway is involved in the action mechanisms of MMP1 in PNI of PC. Overall, MMP1 silencing decreases the expression of NT-3 and TrkC, thereby leading to the inactivation of this pathway. The blocking of the NT-3/TrkC signaling pathway then inhibits the EMT and Schwann-like cell differentiation, thus suppressing the PNI of PC. In addition, the effects of MMP1 knockdown were abrogated by a TrkC activator in this study, which further illustrate that MMP1 functions in the PNI of PC through regulating the NT-3/TrkC signaling pathway. Indeed, the activation of the NT-3/TrkC signaling pathway can also activate a variety of other signaling pathways, including PI3K/Akt, MAP kinase, and Bcl2 signaling pathways.[23] However, the downstream effectors of the NT-3/TrkC signaling pathway in PNI of PC are not explored here, which need to be studied in future experiments.

5 | CONCLUSIONS

In conclusion, MMP1 knockdown inhibits the EMT and Schwann-like cell differentiation of PC cells via blocking the NT-3/TrkC signaling pathway, thus suppressing the PNI of PC.

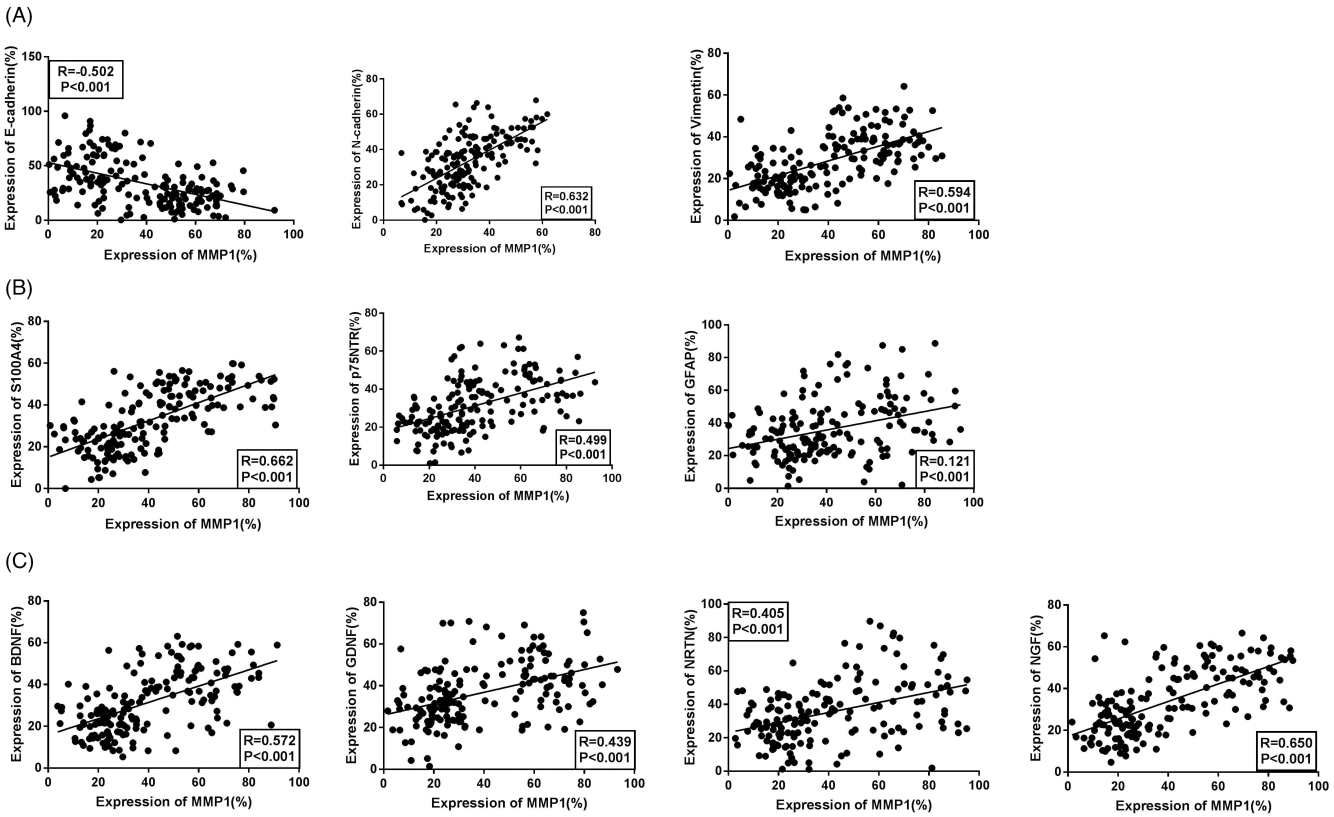

FIGURE 6 MMP1 is closely correlated with EMT markers, neurotrophic factors, and Schwann cell markers in tumor tissues from PC patients. The correlations of the expression of MMP1 with the expression of EMT markers (E-cadherin, N-cadherin, and Vimentin) (A), Schwann cell markers (S100A4, P75NTR, and GFAP) (B), and neurotrophic factors (BDNF, GDNF, NRTN, and NGF) (C) were analyzed by Pearson correlation analysis.

AUTHOR CONTRIBUTIONS

Xiaoqing Xu contributed to the writing—original draft, funding acquisition, conceptualization, formal analysis, and project administration. Xiaomin Lu and Liping Chenk contributed to the resources, writing—review and editing, data curation, and investigation. Ke Peng and Fuhai Ji contributed to the methodology, supervision, validation, and writing—review and editing. All authors read and approved the final study.

ACKNOWLEDGEMENT

Not applicable.

FUNDING INFORMATION

This work was supported by the National Natural Science Foundation of China (82072130 and 81873925 to FHJ), Natural Science Foundation of Jiangsu Province (BK20191171 to FHJ), and Six talent peaks project in Jiangsu Province (WSN-022 to FHJ).

CONFLICT OF INTEREST

The authors state that there are no conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

The datasets generated or analyzed during the current study are available from the corresponding author on reasonable request.

INFORMED CONSENT

Each patient signed an informed consent form.

ORCID

Fuhai Ji https://orcid.org/0000-0003-0426-9624

REFERENCES

1. Tempero MA. NCCN guidelines updates: pancreatic cancer. *J Natl Compr Canc Netw*. 2019;17:603-605.
2. Goral V. Pancreatic cancer: pathogenesis and diagnosis. *Asian Pac J Cancer Prev*. 2015;16:5619-5624.
3. Zhang L, Sanagapalli S, Stoita A. Challenges in diagnosis of pancreatic cancer. *World J Gastroenterol*. 2018;24:2047-2060.
4. The global, regional, and national burden of pancreatic cancer and its attributable risk factors in 195 countries and territories, 1990-2017: a systematic analysis for the global burden of disease study 2017. Lancet. *Gastroenterol Hepatol*. 2019;4:934-947.
5. Saluja A, Maitra A. Pancreatitis and pancreatic cancer. *Gastroenterology*. 2019;156:1937-1940.
6. McGuigan A, Kelly P, Turkington RC, Jones C, Coleman HG, McCain RS. Pancreatic cancer: a review of clinical diagnosis, epidemiology, treatment and outcomes. *World J Gastroenterol*. 2018;24:4846-4861.
7. Amit M, Na'ara S, Gil Z. Mechanisms of cancer dissemination along nerves. *Nat Rev Cancer*. 2016;16:399-408.
8. Liu B, Lu KY. Neural invasion in pancreatic carcinoma. *Hepatobiliary Pancreat Dis Int*. 2002;1:469-476.

9. Marchesi F, Piemonti L, Mantovani A, Allavena P. Molecular mechanisms of perineural invasion, a forgotten pathway of dissemination and metastasis. *Cytokine Growth Factor Rev*. 2010;21:77-82.
10. Ayala GE, Dai H, Powell M, et al. Cancer-related axonogenesis and neurogenesis in prostate cancer. *Clin Cancer Res*. 2008;14:7593-7603.
11. Gil Z, Cavel O, Kelly K, et al. Paracrine regulation of pancreatic cancer cell invasion by peripheral nerves. *J Natl Cancer Inst*. 2010;102:107-118.
12. Liebig C, Ayala G, Wilks JA, Berger DH, Albo D. Perineural invasion in cancer: a review of the literature. *Cancer*. 2009;115:3379-3391.
13. Bapat AA, Hostetter G, Von Hoff DD, Han H. Perineural invasion and associated pain in pancreatic cancer. *Nat Rev Cancer*. 2011;11:695-707.
14. Wang Y, McNiven MA. Invasive matrix degradation at focal adhesions occurs via protease recruitment by a FAK-p130Cas complex. *J Cell Biol*. 2012;196:375-385.
15. Pittayapruek P, Meephansan J, Prapapan O, Komine M, Ohtsuki M. Role of matrix metalloproteinases in photoaging and Photocarcinogenesis. *Int J Mol Sci*. 2016;17:868.
16. Said AH, Hu S, Abutaleb A, et al. Interacting post-muscarinic receptor signaling pathways potentiate matrix metalloproteinase-1 expression and invasion of human colon cancer cells. *Biochem J*. 2017;474:647-665.
17. Kumar JD, Steele I, Moore AR, et al. Gastrin stimulates MMP-1 expression in gastric epithelial cells: putative role in gastric epithelial cell migration. *Am J Physiol Gastrointest Liver Physiol*. 2015;309:G78-G86.
18. Boire A, Covic L, Agarwal A, Jacques S, Sherifi S, Kuliopulos A. PAR1 is a matrix metalloprotease-1 receptor that promotes invasion and tumorigenesis of breast cancer cells. *Cell*. 2005;120:303-313.
19. Gobin E, Bagwell K, Wagner J, et al. A pan-cancer perspective of matrix metalloproteases (MMP) gene expression profile and their diagnostic/prognostic potential. *BMC Cancer*. 2019;19:581.
20. Chen Y, Peng S, Cen H, et al. MicroRNA hsa-miR-623 directly suppresses MMP1 and attenuates IL-8-induced metastasis in pancreatic cancer. *Int J Oncol*. 2019;55:142-156.
21. Ozden F, Saygin C, Uzunaslan D, Onal B, Durak H, Aki H. Expression of MMP-1, MMP-9 and TIMP-2 in prostate carcinoma and their influence on prognosis and survival. *J Cancer Res Clin Oncol*. 2013;139:1373-1382.
22. Huang C, Li Y, Guo Y, et al. MMP1/PAR1/SP/NK1R paracrine loop modulates early perineural invasion of pancreatic cancer cells. *Theranostics*. 2018;8:3074-3086.
23. Ivanov SV, Panaccione A, Brown B, et al. TrkC signaling is activated in adenoid cystic carcinoma and requires NT-3 to stimulate invasive behavior. *Oncogene*. 2013;32:3698-3710.
24. Ohta T, Numata M, Tsukioka Y, et al. Neurotrophin-3 expression in human pancreatic cancers. *J Pathol*. 1997;181:405-412.
25. Weeraratna AT, Arnold JT, George DJ, DeMarzo A, Isaacs JT. Rational basis for Trk inhibition therapy for prostate cancer. *Prostate*. 2000;45:140-148.
26. Li H, Yang Z, Wang W, et al. NT-3/TrkC Axis contributes to the perineural invasion and the poor prognosis in human salivary adenoid cystic carcinoma. *J Cancer*. 2019;10:6065-6073.
27. Tonge DA, Golding JP, Edbladh M, Kroon M, Ekström PE, Edström A. Effects of extracellular matrix components on axonal outgrowth from peripheral nerves of adult animals in vitro. *Exp Neurol*. 1997;146:81-90.
28. Mittal V. Epithelial mesenchymal transition in tumor metastasis. *Annu Rev Pathol*. 2018;13:395-412.
29. Pour PM, Bell RH, Batra SK. Neural invasion in the staging of pancreatic cancer. *Pancreas*. 2003;26:322-325.
30. Maller O, Martinson H, Schedin P. Extracellular matrix composition reveals complex and dynamic stromal-epithelial interactions in the mammary gland. *J Mammary Gland Biol Neoplasia*. 2010;15:301-318.
31. Wang Y, Ding X, Liu B, et al. ETV4 overexpression promotes progression of non-small cell lung cancer by upregulating PXN and MMP1 transcriptionally. *Mol Carcinog*. 2020;59:73-86.
32. Liu H, Kato Y, Erzinger SA, et al. The role of MMP-1 in breast cancer growth and metastasis to the brain in a xenograft model. *BMC Cancer*. 2012;12:583.
33. Chen YJ, Liang L, Li J, et al. IRF-2 inhibits gastric cancer invasion and migration by Down-regulating MMP-1. *Dig Dis Sci*. 2020;65:168-177.
34. Pastushenko I, Blanpain C. EMT transition states during tumor progression and metastasis. *Trends Cell Biol*. 2019;29:212-226.
35. De Craene B, Berx G. Regulatory networks defining EMT during cancer initiation and progression. *Nat Rev Cancer*. 2013;13:97-110.
36. Brugière C, El Bouchtaoui M, Leboeuf C, et al. Perineural invasion in human cutaneous squamous cell carcinoma is linked to Neurotrophins, epithelial-mesenchymal transition, and NCAM1. *J Invest Dermatol*. 2018;138:2063-2066.
37. Tian R, Li X, Gao Y, Li Y, Yang P, Wang K. Identification and validation of the role of matrix metalloproteinase-1 in cervical cancer. *Int J Oncol*. 2018;52:1198-1208.
38. Zorick TS, Lemke G. Schwann cell differentiation. *Curr Opin Cell Biol*. 1996;8:870-876.
39. Demir IE, Boldis A, Pfitzinger PL, et al. Investigation of Schwann cells at neoplastic cell sites before the onset of cancer invasion. *J Natl Cancer Inst*. 2014;106:dju184.
40. Deborde S, Wong RJ. How Schwann cells facilitate cancer progression in nerves. *Cell Mol Life Sci*. 2017;74:4405-4420.
41. Kwon SY, Bae YK, Gu MJ, et al. Neuroendocrine differentiation correlates with hormone receptor expression and decreased survival in patients with invasive breast carcinoma. *Histopathology*. 2014;64:647-659.
42. Park K, Chen Z, MacDonald TY, et al. Prostate cancer with Paneth cell-like neuroendocrine differentiation has recognizable histomorphology and harbors AURKA gene amplification. *Hum Pathol*. 2014;45:2136-2143.
43. Lee SJ, Choi SY, Kim WJ, et al. Combined aberrant expression of E-cadherin and S100A4, but not β-catenin is associated with disease-free survival and overall survival in colorectal cancer patients. *Diagn Pathol*. 2013;8:99.
44. Stopczynski RE, Normolle DP, Hartman DJ, et al. Neuroplastic changes occur early in the development of pancreatic ductal adenocarcinoma. *Cancer Res*. 2014;74:1718-1727.
45. Zhang M, Li ZF, Wang HF, et al. MIF promotes perineural invasion through EMT in salivary adenoid cystic carcinoma. *Mol Carcinog*. 2019;58:898-912.
46. Huang EJ, Reichardt LF. Trk receptors: roles in neuronal signal transduction. *Annu Rev Biochem*. 2003;72:609-642.
47. Liu D, Song L, Dai Z, et al. MiR-429 suppresses neurotrophin-3 to alleviate perineural invasion of pancreatic cancer. *Biochem Biophys Res Commun*. 2018;505:1077-1083.
48. Jin W. Roles of TrkC signaling in the regulation of tumorigenicity and metastasis of cancer. *Cancers (Basel)*. 2020;12:147.

How to cite this article: Xu X, Lu X, Chen L, Peng K, Ji F. Downregulation of MMP1 functions in preventing perineural invasion of pancreatic cancer through blocking the NT-3/TrkC signaling pathway. *J Clin Lab Anal*. 2022;36:e24719. doi: 10.1002/jcla.24719