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Cytoplasmic-only Expression of Maspin Predicts Unfavorable Prognosis in Patients With Pancreatic Ductal Adenocarcinoma

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Abstract. *Background/Aim: Maspin is a tumor-suppressor protein expressed in >90% of pancreatic ductal adenocarcinoma (PDAC) cases. We aimed to assess the prognostic value of subcellular localization of maspin. Patients and Methods: Ninety-two resected PDAC specimens were immunohistochemically analyzed. Cytoplasmic-only expression observed in >10% of the tumor was defined as maspin-positive. Results: The maspin-positive status (21.7%) was inversely correlated with well-differentiated histological type and indicated a shorter recurrence-free survival (RFS) and overall survival (OS). Cox's multivariate analysis showed that maspin-positive status was an independent factor for shorter RFS and OS. Maspin was localized to cytoplasm in AsPC-1 cells, but to both nucleus and cytoplasm in BxPC-3 cells. In AsPC-1 cells, cell invasion was significantly reduced in response to maspin suppression via transfection with siRNA targeting maspin, whereas no reduction was observed in BxPC-3 cells. Conclusion: Cytoplasmic-only expression of maspin could be an independent unfavorable prognostic indicator for patients with PDAC.*

Pancreatic ductal adenocarcinoma (PDAC) has one of poorest prognoses of all malignant neoplasms worldwide, and it is the fourth leading cause of cancer-related deaths in many developed countries, with a 5-year survival rate of less than 9% (1). Despite of an understanding of PDAC-associated genetic mutations, including those in *KRAS, TP53, SMAD4* and *CDKN2A*, there has been limited clinical application of these findings as predictive biomarkers (2). Therefore, it is important to explore new prognostic factors for patients with PDAC to make prognostic decisions and treatment choices.

Maspin (a mammary serine protease inhibitor), a non-inhibitory member of the serpin (serine protease inhibitor) superfamily, was originally identified as a tumor-suppressor protein expressed in normal breast epithelial cells but not in breast carcinoma (3). Maspin inhibits both tumor growth, cell motility, invasiveness, angiogenesis and metastasis in multiple animal models and cancer cell lines whereas exerts pro-apoptotic effects (3-5). Although the exact biochemical mechanisms leading to these biological endpoints remain unknown, many studies have reported an association between maspin expression and clinicopathological factors in several types of cancers, including PDAC. However, there are conflicting results regarding whether maspin expression is a favorable or unfavorable indicator for cancer patients (6). The main factors contributing to this complexity may be the differences in "positive" criteria including subcellular localization, analytical methods and the study population. Maass *et al*. reported that more than 90% of PDAC cases as well as all high-grade precancerous lesions showed maspin expression, whereas no expression was observed in normal pancreatic epithelium and low-grade precancerous lesions (7, 8). Therefore, maspin may have important roles in the carcinogenesis and progression of PDAC. However, few reports have investigated the clinical significance of maspin expression in patients with PDAC (8, 9). In addition, only one study reported correlations between the subcellular localization of maspin and patient' prognosis (8). We previously reported that cytoplasmic-only expression of maspin was a poor prognostic indicator for patients with breast (10) and lung cancer (11-13). On the other hand, Goulet *et al*. reported that the nuclear localization of maspin is required for suppression of tumor growth and metastasis *in vivo* (14). Taken together, we hypothesized that cytoplasmic-only expression of maspin could correlate with unfavorable prognosis in patients with PDAC. The aim of the present study was to categorize patients according to the pattern of subcellular localization of maspin,

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and to clarify the prognostic impact of cytoplasmic-only expression of maspin in patients with PDAC. To substantiate the clinicopathological observations, we also investigated the subcellular localization of maspin protein expression, and the relationship between maspin expression and invasive abilities in several human PDAC cell lines.

Patients and Methods

Patients and tumor specimens. A total of 92 consecutive patients who underwent radical surgery between May 2006 and March 2017 at Tottori University Hospital (Yonago, Japan) for PDAC were included in the analysis. The protocol was approved by the Ethics Committee of Tottori University (approval number: 18A148), and all patients provided written informed consent for pathological analysis. All clinicopathological parameters and laboratory data of all patients were extracted from their electronic medical records. Patients' characteristics, including surgical and peri-operative parameters, were retrospectively analyzed. Pathological findings were classified according to the 7th edition of TNM classification.

Immunohistochemistry. All specimens were fixed in 10% neutral buffered formalin and embedded in paraffin, and a representative block was selected from each case. Sections (4 μm-thick) were deparaffinized, blocked to inhibit endogenous peroxidase activity, and then pre-treated in citrate buffer (0.01 M, pH 6.0) using a microwave oven for 20 min. We then performed immunohistochemical examination using a mouse monoclonal antibody for human maspin (clone G167-70, 1:500 dilution; BD Pharmingen, NJ, USA), as described elsewhere (15).

Evaluation of immunohistochemical findings. Cells were defined as positive when strong cytoplasmic-only staining was identified. Strong staining was defined as a staining intensity equal to that in myoepithelial cells in normal breast tissue. Cases with positive cells accounting for more than 10% of the total tumor were considered maspin-positive, as previously described (16). The subcellular localization of maspin was classified into four categories, cytoplasmic-only, pancellular (combined nuclear and cytoplasmic), nuclear-only, and no staining. All slides were evaluated independently by E.U. and Y.U., who were blinded to the patient' clinicopathological data.

Cell culture. PANC-1, MIA PaCa-2, and BxPC-3 cell lines were purchased from the American Type Culture Collection (Manassas, VA, USA). PANC-1 and MIA PaCa-2 cells were cultured in Dulbecco's modified Eagle medium (DMEM, Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% fetal bovine serum (FBS) at 37˚C with 5% CO_2, whereas AsPC-1 and BxPC-3 cells were cultured in RPMI 1640 (Nissui Pharmaceutical) supplemented with 10% FBS at 37˚C with 5% CO_2.

RNA extraction and quantitative polymerase chain reaction. Total RNA from PANC-1, MIA PaCa-2, AsPC-1 and BxPC-3 cells was extracted using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) and reverse-transcribed into complementary DNA (cDNA) using a High-Capacity RNA-to-cDNA Kit (Thermo Fisher Scientific) according to the manufacturer's protocol. Gene expression levels were measured using the TaqMan Gene Expression Assays (Thermo Fisher Scientific) with the following gene-specific primers: *GAPDH*, human *GAPDH* endogenous control (Hs99999905_m1, Thermo Fisher Scientific) and *maspin*, TaqMan Gene Expression Assays (Hs00985285_m1, Thermo Fisher Scientific).

Western blot analysis. Cells were lysed in protein extraction buffer (cat. no. 28941279; GE Healthcare, Buckinghamshire, UK) containing protease inhibitors (cat. no. 80650123; GE Healthcare). Protein concentrations were determined using the Bradford protein assay (Bio-Rad Laboratories, Hercules, CA, USA). Proteins were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to 0.2-μm polyvinylidene difluoride membranes (cat. no. 1704156; Bio-Rad Laboratories). Following incubation in 5% skim milk, the membranes were reacted with the primary antibodies. The primary antibodies used were mouse monoclonal anti-human maspin antibody (clone G167-70, 1:500 dilution; BD Pharmingen), rabbit monoclonal anti-GAPDH antibody (clone D16H11, 1:1,000 dilution; Cell Signaling Technology, Danvers, MA, USA), anti-HDAC1 antibody (clone 10E2, 1:1,000 dilution; Cell Signaling Technology) and anti-HSP90 antibody (clone C45G5, 1:1,000 dilution; Cell Signaling Technology). Then, horseradish peroxidase (HRP) conjugated anti-mouse IgG (cat. no. NA931; 1:3,000 dilution; GE Healthcare) or HRP-conjugated anti-rabbit IgG (cat. no. NA934, 1:4,000 dilution; GE Healthcare) were added to 5% skim milk. For AsPC-1 and BxPC-3 cells, subcellular protein fractionation was performed using a subcellular protein fractionation kit (Thermo Fisher Scientific) according to the manufacturer's instructions. The signals were visualized using the Immobilon Western chemiluminescent substrate (Millipore, Billerica, MA, USA) and quantified using the Image Quant LAS 4000 mini (GE Healthcare).

Immunofluorescence analysis. AsPC-1 and BxPC-3 cells were incubated for 24 h in 8-well chamber slides (Nalgen Nunc International, Rochester, NY, USA). After incubation, cells were fixed in 4% paraformaldehyde and permeabilized with methanol at 20˚C for 5 min. Samples were blocked by 5% blocking buffer (BLOCK ACE; Megmilk Snow Brand, Sapporo, Japan) for 60 min at room temperature. Immunofluorescence analysis was performed using an anti-maspin primary antibody (clone G167-70, 1:200 dilution; BD Pharmingen) as described elsewhere (15).

Plasmid and siRNA transfection. AsPC-1 and BxPC-3 cells were seeded into 6-well plates and allowed to adhere overnight. For each well, 10 nM siRNA targeting maspin (Silencer Select siRNA, s10466, Thermo Fisher Scientific), siRNA targeting maspin (Silencer Select siRNA, s10468, Thermo Fisher Scientific), or control siRNA (Silencer Negative Control #1; Thermo Fisher Scientific) was used to transiently transfect cells using 9 μl Lipofectamine RNAiMAX (cat. no. 13778030, Thermo Fisher Scientific) according to the manufacturer's instructions.

Cell invasion assay. Cell invasion assays were performed using a BioCoat Matrigel invasion chamber (BD Biosciences, Bedford, MA, USA) according to the manufacturer's protocol. Cells (AsPC-1: 2×10^5; BxPC-3: 1×10^5) transfected with siRNA were starved in serum-free medium for 24 h and seeded into the upper chambers of transwells on an 8.0 μm pore size membrane in 24-well plates. The lower chambers were filled with medium containing 10% FBS. After 24 h, non-invading cells were removed from the top of the filter with a cotton swab. The invading cells at the bottom of the filter were fixed with

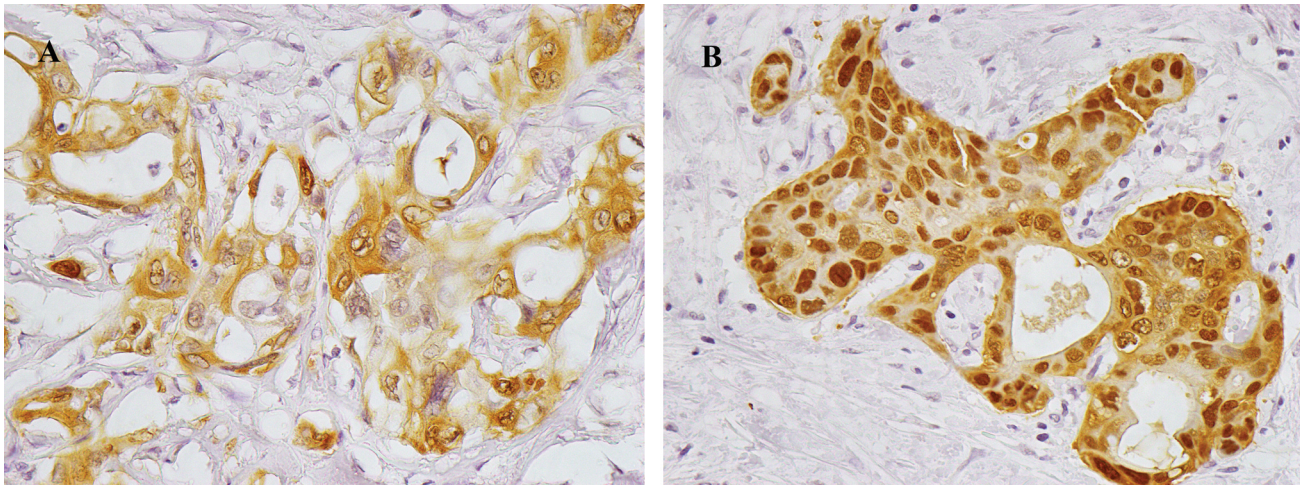

Figure 1. *Immunohistochemical staining pattern of maspin in pancreatic ductal adenocarcinoma. A) Cytoplasmic-only expression of maspin. B) Pancellular expression of maspin.*

methanol for 10 min, stained with 0.2% crystal violet, and counted in five different fields of view using a microscope (ECLIPSE Ts2; Nikon, Tokyo, Japan) (magnification, 200×). The mean values of three independent experiments were used for statistical analysis.

Statistical analysis. All statistical analyses were performed using SPSS version 25 (IBM SPSS Statistics; IBM Corporation, Armonk, NY, USA). Overall survival (OS) and recurrence-free survival (RFS) rates were calculated using the Kaplan–Meier method and compared using log-rank tests. OS was calculated from the date of surgery to the date of death or last visit. RFS was calculated from the date of surgery to the date of disease recurrence or last visit. Relative maspin mRNA expression was compared using one-way analysis of variance and the Tukey honest significant difference test. Differences in proliferation and invasion were evaluated using Student's *t*-tests. All tests were considered significantly different when the *p*-value was less than 0.05. All continuous values are presented as means±standard deviations.

Results

Immunohistochemistry. Representative immunohistochemical staining patterns for maspin are shown in Figure 1. Normal pancreatic tissue (ductal epithelium, acinar epithelium, and islet cells) stained negatively. The subcellular localization of maspin expression consisted of cytoplasmic-only staining (20 cases: 21.7%), pancellular (combined nuclear and cytoplasmic) staining (54 cases: 58.7%), and no staining (18 cases: 19.6%). No nuclear-only staining was observed.

Clinicopathological characteristics and association with maspin expression status. The median age of the 92 patients at the time of surgery was 73.5 years (range=49-87 years); 57 patients were men, and 35 were women. The correlations between maspin status and clinicopathological factors are summarized in Table I. The status of maspin positivity was inversely correlated with the well-differentiated histological type (p=0.007). The status of pancellular expression was significantly correlated with a well-differentiated histological type compared with the status of cytoplasmic-only expression (p=0.01; Table II).

Survival analysis. The median follow-up period was 28.5 months (range=1.7-101.3 months). Seventy patients experienced locoregional and/or distant recurrences. Fifty-four patients died because of pancreatic cancer progression. The survival curves for the patients are shown in Figures 2 and 3. Three-year RFS rates of the maspin-positive and maspin-negative groups were 25% [95% confidence interval (CI)=6.0-44.0%] and 49.7% (95%CI=37.9-61.5%), respectively. Three-year RFS rates were 25.0% (95%CI=6.0-44.0%) in the cytoplasmic-only staining group, 48.1% (95%CI=34.5-61.7%) in the pancellular staining group, and 54.3% (95%CI=30.7-77.9%) in the no staining group.

Three-year OS rates of the maspin-positive and maspin-negative groups were 25.0% (95%CI=6.0-44.0%) and 53.8% (95%CI=42.0-65.6%), respectively. Three-year OS rates were 25.0% (95%CI=6.0-44.0%) in the cytoplasmic-only staining group, 52.4% (95%CI=38.9-65.9%) in the pancellular staining group, and 57.9% (95%CI=33.8-82.1%) in the no staining group. Log-rank tests showed that maspin-positive status was associated with significantly shorter RFS (p=0.037; Figure 2A) and OS (p=0.014; Figure 3A). Cytoplasmic-only staining was associated with a significantly shorter OS than pancellular staining (p=0.025; Figure 3B) and no staining (p=0.044; Figure 3B). Univariate analysis showed a significant association between shorter RFS and high stage (p<0.001), high Dupan-2 (p=0.007), and maspin-positive status (p=0.039). Multivariate

Table I. *Association between clinicopathologic characteristics and maspin status.*

Variables	Maspin-positive (n=20)	Maspin-negative (n=72)	*p*-Value
Age (years)			
<70	6	23	0.868
≥70	14	49	
Gender			
Male	14	43	0.402
Female	6	29	
Pathological tumor status			
pT1	0	3	0.644
pT2	1	4	
pT3	19	65	
Stage (UICC 7th)			
I+IIA	5	31	0.143
IIB+III+IV	15	41	
Adjuvant chemotherapy			
Present	12	41	0.807
Absent	8	31	
Histological type			
Well	4	39	0.007
Mod+por+others	16	33	
Neural invasion			
Negative	1	2	0.621
Positive	19	70	
Span-1			
<30	5	30	0.191
≥30	14	40	
Dupan-2			
<150	11	46	0.529
≥150	8	24	

Mod: Moderate; por: poor.

Table II. *Comparison of clinicopathologic factors between cytoplasmic-only and pancellular (combined nuclear and cytoplasmic) expression of maspin.*

Variables	Cytoplasmic-only (n=20)	Pancelluar (n=54)	*p*-Value
Age (years)			
<70	6	17	0.903
≥70	14	37	
Gender			
Male	14	31	0.324
Female	6	23	
Pathological tumor status			
pT1	0	3	0.549
pT2	1	2	
pT3	19	49	
Stage (UICC 7th)			
I+IIA	5	23	0.166
IIB+III+IV	15	31	
Adjuvant chemotherapy			
Present	12	31	0.841
Absent	8	23	
Histological type			
Well	4	29	0.010
Mod+por+others	16	25	
Neural invasion			
Negative	1	2	0.802
Positive	19	52	
Span-1			
<30	5	23	0.190
≥30	14	30	
Dupan-2			
<150	11	33	0.737
≥150	8	20	

Mod: Moderate; por: poor.

analysis using the Cox regression hazard model showed that maspin-positive status was an independent factor for shorter RFS (p=0.004) in addition to high stage (p<0.001) and high Dupan-2 (p<0.001; Table III). Univariate analysis showed a significant association between shorter OS and gender (p=0.049), high stage (p<0.001), not-well differentiated histological type (p=0.033), high Span-1 (p=0.031), high Dupan-2 (p=0.002), and maspin-positive status (p=0.016). According to multivariate analysis using the Cox regression hazard model, maspin-positive status was an independent factor for shorter OS (p=0.039) in addition to gender (p=0.005), high stage (p<0.001), and high Dupan-2 (p<0.001; Table IV).

Maspin expression in PANC-1, MIA PaCa-2, AsPC-1, and BxPC-3 cells. The mRNA and protein expression of maspin in PANC-1, MIA PaCa-2, AsPC-1 and BxPC-3 cell lines were assessed by reverse transcription polymerase chain reaction and western blot analysis, respectively. AsPC-1 and BxPC-3 cells showed increased mRNA and protein expression (Figure 4A and 4B). Immunofluorescence analysis in AsPC-1 and BxPC-3 cells showed that maspin protein was localized to exclusively in the cytoplasm of AsPC-1 cells but to both the nucleus and cytoplasm of BxPC-3 cells (Figure 5A). Western blot analysis confirmed the results of immunofluorescence analysis (Figure 5B).

Cell invasion assays. The efficacy of siRNA targeting maspin in AsPC-1 and BxPC-3 cells was determined. In both cell types, siRNA (S10466) was found to efficiently reduce maspin mRNA expression (Figure 6A). In AsPC-1 cells, cell invasion was significantly reduced in response to maspin suppression (p<0.01), whereas no reduction was observed in BxPC-3 cells (Figure 6B and C).

Discussion

Many reports have described the association between maspin expression and clinicopathological factors in various cancers (6). However, the prognostic value of maspin expression is still unclear. The factors contributing to the potential complexity of

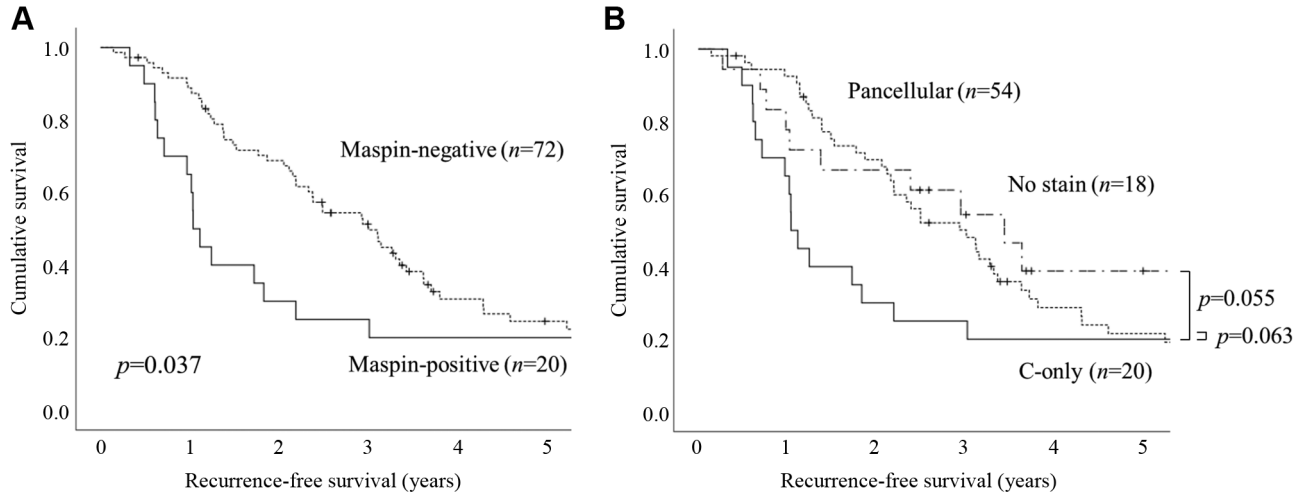

Figure 2. *Kaplan–Meier survival curves for recurrence-free survival in 92 patients according to maspin expression status (cytoplasmic-only staining versus all other categories) (A) and subcellular localization of maspin expression (B). C-only: cytoplasmic-only.*

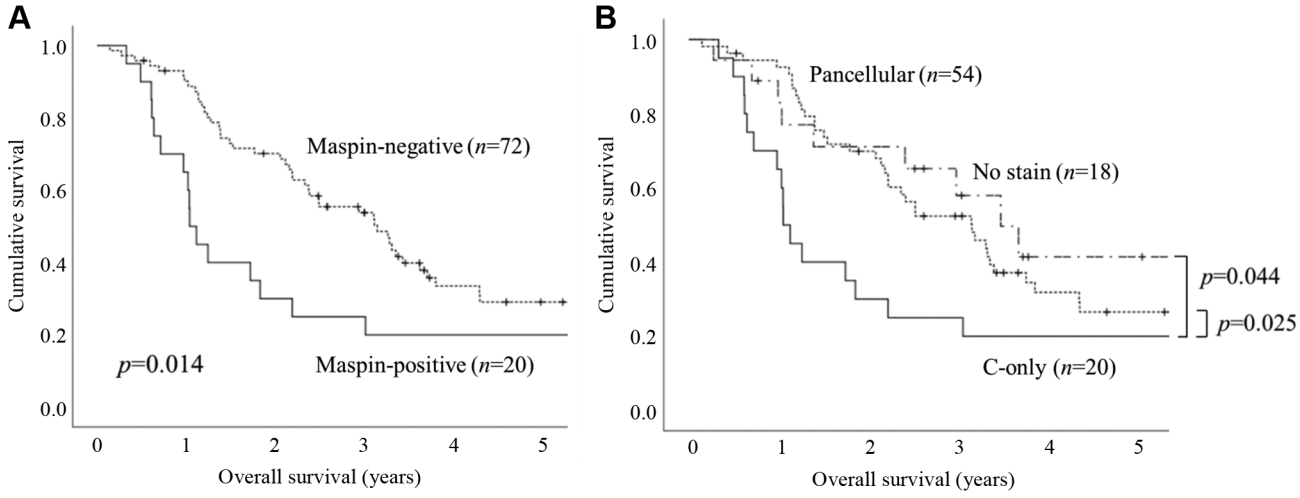

Figure 3. *Kaplan–Meier survival curves for overall survival in 92 patients according to maspin expression status (cytoplasmic-only staining versus all other categories) (A) and subcellular localization of maspin expression (B). C-only: cytoplasmic-only.*

the prognostic value include differences in the positive criteria, histological type, antibodies used and study population. In particular, the lack of standardization in evaluating the positivity of maspin at the subcellular level may yield profound discrepancies in evaluating prognostic significance. We have reported that cytoplasmic-only expression of maspin is an independent unfavorable prognostic factor for patients with breast cancer (10), lung adenocarcinoma (11, 13), and lung squamous cell carcinoma (12). Moreover, maspin is frequently expressed in PDAC, PanIN3 and intraductal carcinoma extension, but not the normal pancreatic epithelium and low-grade precancerous lesions (8). Thus, maspin may have important roles in the carcinogenesis and progression of PDAC. Additionally, maspin is upregulated during the progression of mammary ductal carcinoma (15), although a separate report demonstrated conflicting results (17). Therefore, we hypothesized that cytoplasmic-only expression of maspin may be a poor prognostic factor in patients with PDAC.

Few reports have investigated the clinical value of maspin expression in patients with PDAC (8, 9). To the best of our knowledge, only one report demonstrated the correlation between subcellular localization of maspin and patient'

Table III. *Univariate and multivariate analysis of various factors of recurrence-free survival in patients with pancreatic ductal adenocarcinoma.*

Prognostic factors	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value
Age (year)						
≥70 *vs.* <70	1.501	0.888-2.538	0.130			
Gender						
Male *vs.* Female	1.381	0.846-2.255	0.197			
Pathological tumor status						
pT3 *vs.* pT1+2	1.531	0.614-3.818	0.360			
Stage (UICC 7th)						
≥IIB *vs.* ≤IIA	3.482	2.015-6.019	<0.001	3.602	2.071-6.264	<0.001
Adjuvant chemotherapy						
Present *vs.* Absent	0.997	0.616-1.615	0.991			
Histological type						
Mod+por+others *vs.* Well	1.465	0.913-2.351	0.113			
Neural invasion						
Positive *vs.* Negative	1.361	0.188-9.837	0.760			
Span-1						
≥30 *vs.* <30	1.612	0.983-2.643	0.058			
Dupan-2						
≥150 *vs.* <150	1.968	1.208-3.207	0.007	2.616	1.572-4.351	<0.001
Maspin status						
Positive *vs.* Negative	1.787	1.029-3.106	0.039	2.336	1.311-4.163	0.004

HR: Hazard ratio; CI: confidence interval. Mod: moderate; por: poor.

Table IV. *Univariate and multivariate analysis of various factors of overall survival in patients with pancreatic ductal adenocarcinoma.*

Prognostic factors	Univariate analysis			Multivariate analysis		
	HR	95%CI	*p*-Value	HR	95%CI	*p*-Value
Age (year)						
≥70 *vs.* <70	1.481	0.844-2.599	0.171			
Gender						
Male *vs.* Female	1.719	1.001-2.952	0.049	2.254	1.274-3.987	0.005
Pathological tumor status						
pT3 *vs.* pT1+2	1.723	0.623-4.767	0.295			
Stage (UICC 7th)						
≥IIB *vs.* ≤IIA	2.906	1.651-5.113	<0.001	3.149	1.721-5.760	<0.001
Adjuvant chemotherapy						
Present *vs.* Absent	0.939	0.565-1.560	0.808			
Histological type						
Mod+por+others *vs.* Well	1.736	1.046-2.881	0.033	1.378	0.767-2.477	0.284
Neural invasion						
Positive *vs.* Negative	0.648	0.158-2.664	0.548			
Span-1						
≥30 *vs.* <30	1.816	1.058-3.118	0.031	1.245	0.682-2.273	0.475
Dupan-2						
≥150 *vs.* <150	2.302	1.371-3.865	0.002	2.957	1.678-5.210	<0.001
Maspin status						
Positive *vs.* Negative	2.023	1.139-3.595	0.016	2.010	1.036-3.899	0.039

HR: Hazard ratio; CI: confidence interval. Mod: moderate; por: poor.

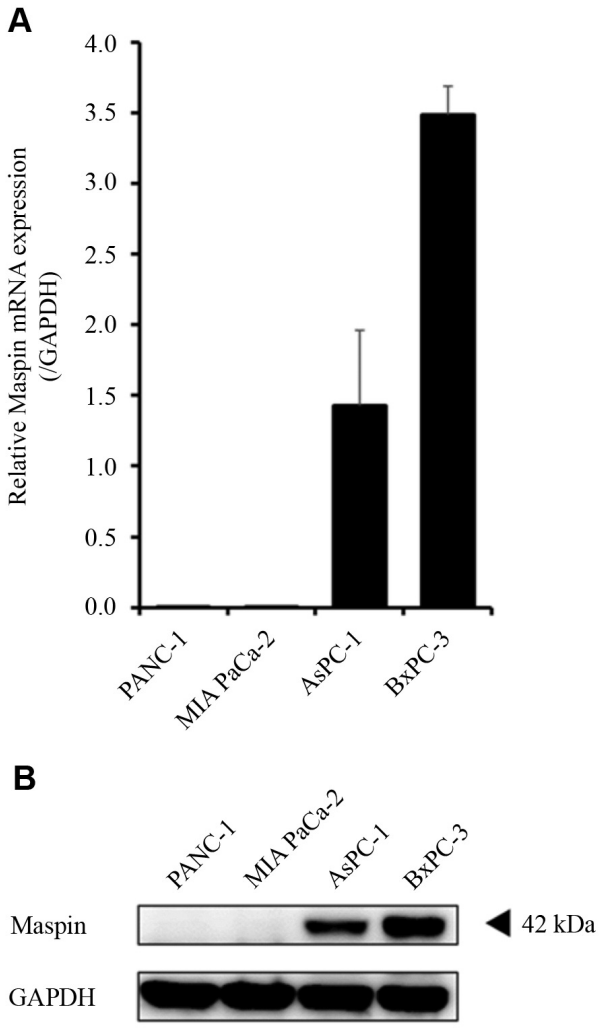

Figure 4. *Maspin expression was assessed by RT-PCR (A) and western blot analysis (B) in PANC-1, MIA PaCa-2, AsPC-1, and BxPC3 cell lines. Maspin mRNA expression was normalized to the expression of GAPDH.*

prognosis (8). Therefore, in this study, we explored the correlations between the subcellular localization of maspin and clinical significance in patients with PDAC. In the largest series of 223 surgically resected PDAC by Cao *et al*., maspin over-expression, defined as cytoplasmic-only or pancellular, was an independent poor prognostic factor (8). However, the researchers failed to find a significant difference in OS between cytoplasmic-only and pancellular staining groups, in contrast to our results. The difference in cohort size may be the main reason for this discrepancy, although differences in the ratios of cytoplasmic-only maspin expression to maspin expression could also contribute (182/209, 87.1% *versus* 20/75, 26.7%). Regarding histological grade, Ohike *et al*. (18) reported that maspin-high tumors predominantly showed a low histological grade, whereas Cao *et al*. (8) showed that the cytoplasmic-only expression of maspin was inversely correlated with tumor differentiation compared with the pancellular status, consistent with our results.

In general, PDAC shows rapid local invasion to adjacent tissues and early metastasis to lymph nodes. Although few studies have evaluated the effect of maspin on the invasive abilities of PDAC cells, conflicting results have been reported. Hong *et al*. (19) reported that maspin-transfected PDAC cells (Panc-1 cells) show reduced invasive ability compared with Panc-1-mock transfected cells, whereas Mardin *et al*. (20) revealed that increased maspin mRNA expression is correlated with increased metastasis in 16 PDAC cell lines. We demonstrated that cell invasion was significantly reduced in AsPC-1 cells (showing cytoplasmic-only localization of maspin) in response to maspin suppression, whereas no reduction was observed in BxPC-3 cells (showing pancellular localization of maspin). The different results between the two cell lines may be related to differences in invasive ability, efficiencies of siRNA transfection, and subcellular localization of maspin.

The nuclear localization of maspin in cancer cells is necessary for its tumor-suppressor activity, and when maspin is excluded from the nucleus, it does not exhibit tumor-suppressor activity (14). Thus, the localization of maspin in the nucleus, irrespective of the cytoplasmic localization, may be correlated with favorable prognosis, whereas cytoplasmic-only expression could indicate an unfavorable prognosis. Although our results are not definitive owing to the limitations of the study, including the small number of patients and retrospective nature of the analysis, our findings demonstrating that cytoplasmic-only expression of maspin was an independent poor prognostic indicator in patients with PDAC may support these experimental findings. Further studies of the molecular mechanisms regulating the activities and cellular localization of maspin could facilitate the development of targeted therapies to control PDAC progression or metastasis.

In conclusion, we demonstrated that cytoplasmic-only expression of maspin was an independent poor prognostic indicator in patients with PDAC. Although further studies with larger patient cohorts are needed, our results suggested that the immunohistochemical detection of maspin could help to predict aggressive tumor behavior in patients with PDAC.

Conflicts of Interest

The Authors have no conflicts of interest to disclose regarding this study.

Authors' Contributions

Conception and design: E.U. and Y.U.; acquisition of data: T.H., N.T., T.S., and S.H.; experiments: E.U.; analysis and interpretation of data: E.U., T.S., and Y.U.; writing, review, and/or revision of manuscript: E.U., T.S., Y.U., and Y.F. All Authors read and approved the final version.

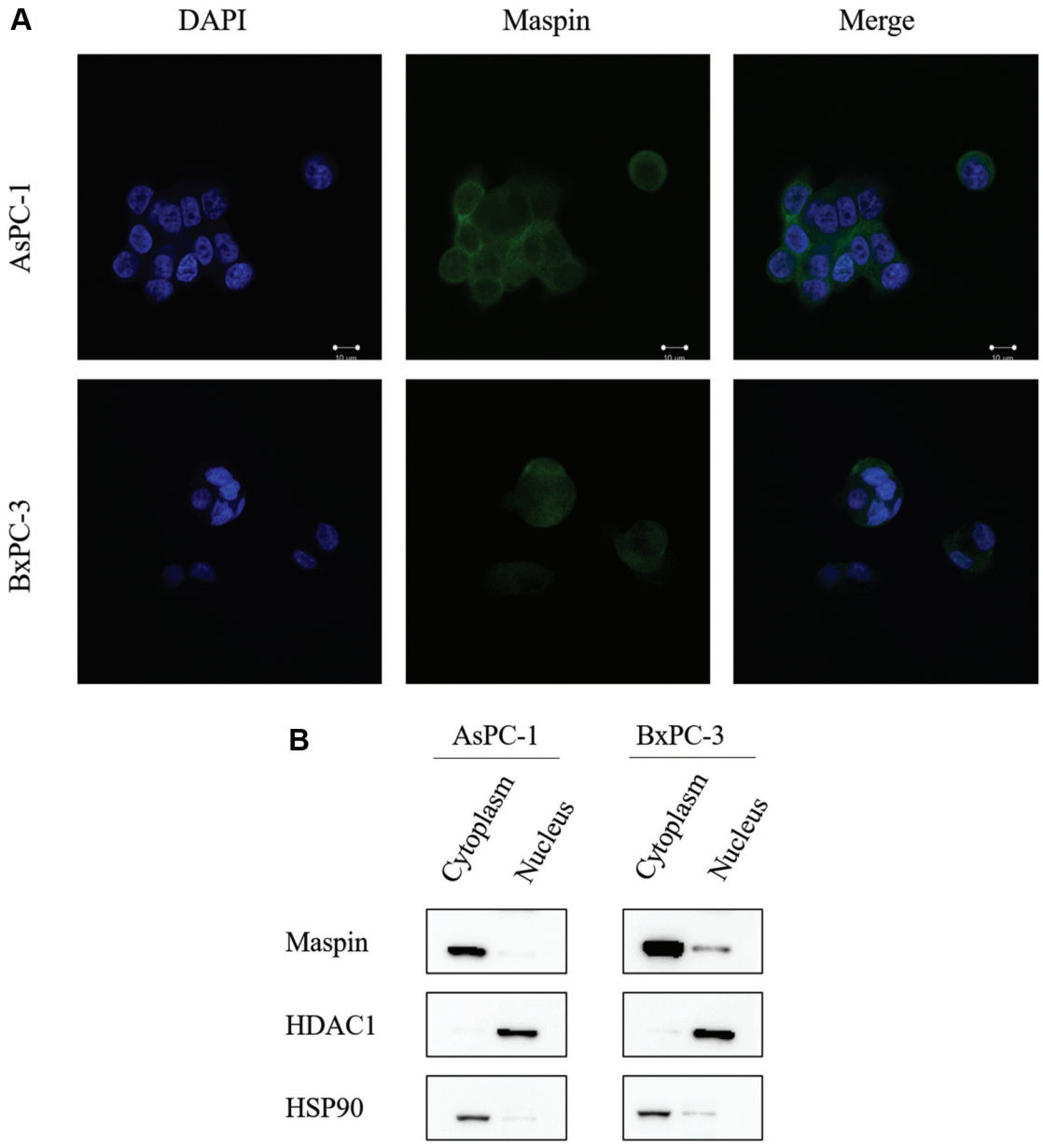

Figure 5. *Subcellular localization of maspin in AsPC-1 and BxPC-3 cells. A) Immunofluorescence analysis. B) Western blot analysis.*

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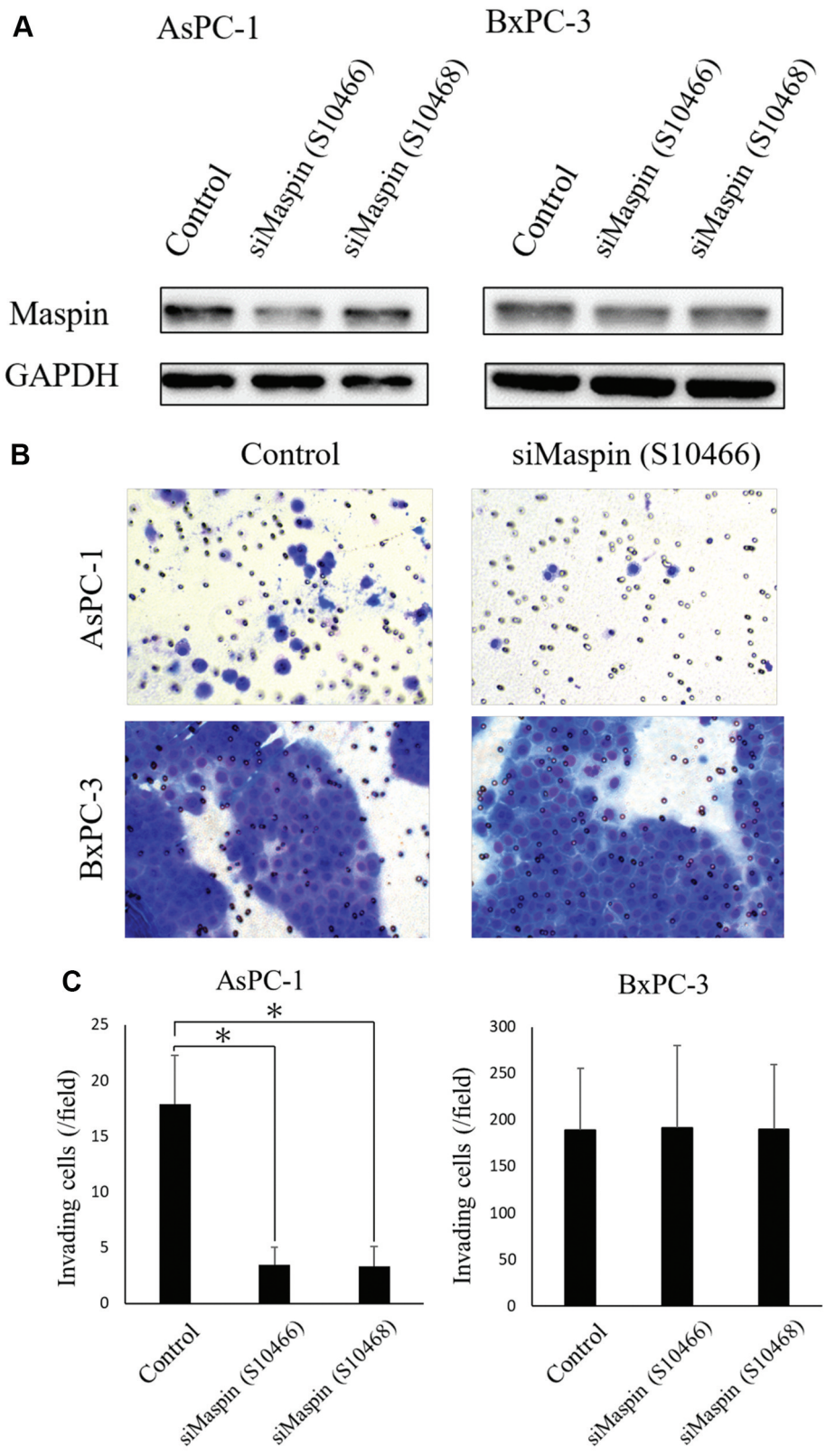

Figure 6. *The association between maspin expression and cell invasion. Knockdown experiment by siRNA targeting maspin in AsPC-1 and BxPC-3 cells (A). Cell invasion analysis of AsPC-1 and BxPC-3 cells using transwell assays (B, C). *p<0.01.*

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