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Original contribution

Clinicopathological and prognostic significance of MKK4 and MKK7 in resectable pancreatic ductal adenocarcinoma☆,☆☆

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Summary Mitogen-activated protein kinase kinase 4 (MKK4) and mitogen-activated protein kinase kinase 7 (MKK7) were shown to regulate biological behavior in many malignancies. In pancreatic ductal adenocarcinoma (PDAC), it remains controversial whether MKK4 and MKK7 have pro-oncogenic or tumor-suppressive activities. Furthermore, their clinicopathological and prognostic implications are unknown. In the present study, we detected MKK4 and MKK7 expressions in the nucleus and cytoplasm of resected PDAC tissues from 321 patients by tissue microarray–based immunohistochemistry. Cytoplasmic MKK4 and MKK7 expressions were significantly downregulated, whereas nuclear MKK4 expression was significantly upregulated in tumor tissues compared with nontumor tissues. Tumor cytoplasmic MKK4 and MKK7 expressions were significantly negatively associated with histologic grade. Cytoplasmic MKK4 expression was also negatively correlated with CA19-9 level. By univariate analysis, high cytoplasmic MKK4 expression was significantly associated with longer cancer-specific survival (hazard ratio [HR], 0.705; 95% confidence interval, 0.510-0.974), with a similar trend observed for MKK7 expression. High MKK4 and MKK7 messenger RNA expressions were significantly associated with longer overall survival in The Cancer Genome Atlas database. Although MKK4 expression was not significant in a multivariate Cox regression analysis, combination of MKK4/MKK7 and pN stage was identified as an independent prognostic indicator and had the lowest HR (HR, 0.308; 95% confidence interval, 0.126-0.752). Furthermore,

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combined analysis of MKK4 and MKK7 greatly increased the prognostic predictive power. In addition, downregulation of MKK4 or MKK7 increased proliferation of pancreatic cancer cells in vitro. In conclusion, high MKK4 expression and its combination with high MKK7 expression both predicted favorable prognosis in resectable PDAC.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC), comprising an impressive 85% of pancreatic cancer cases, remains a devastating cancer [1]. In the United States, the 5-year relative survival rate was pessimistically low, and pancreatic cancer ranked the fourth most common cause of death [2]. Because of its extremely poor prognosis, identification of new and specific factors related to PDAC prognosis is of great significance. Many clinicopathological parameters have been verified to be associated with its prognosis, including TNM staging [3] and CA19-9 level [4]. Recently, numerous molecular markers were proven to predict the prognosis of PDAC, such as GRK2 [5], HMGB1 [6], mTOR [7], and Smad4 [8]. Accordingly, more data for prognostic biomarkers with further validation and supplementation are urgently required.

Mitogen-activated protein kinase kinase 4 (MKK4) and mitogen-activated protein kinase kinase 7 (MKK7) are 2 significant regulators of c-Jun N-terminal kinase in the mitogen-activated protein kinase family. These kinases are stress activated and have key roles in inflammation and tissue homeostasis because they can regulate proliferation, differentiation, gene transcription, apoptosis, survival, and migration in specific cell types [9-14].

Loss-of-function mutations in MKK4 have been detected in pancreatic, biliary, and breast carcinomas [15,16]. There is substantial evidence for involvement of MKK4 and MKK7 in cancer cell proliferation, invasion, apoptosis, and metastasis [11,12,17-19]. However, in PDAC, it remains controversial whether MKK4 and MKK7 have pro-oncogenic or tumor-suppressive activities. In KrasG12D mice, absence of both MKK4 and MKK7 accelerated the development of malignant ductal lesions, suggesting their tumor-suppressive function [18]. In cell lines, ectopic expression of MKK4 promoted pancreatic cancer in vitro [19]. In tissues, MKK4 expression showed a prognostic trend within the limitation of relatively small sample sizes [20,21]. To date, the clinicopathological and prognostic implications of MKK7 in pancreatic cancer have not been explored.

Therefore, the objective of the present study was to clarify the clinical significance of MKK4 and MKK7 expressions in PDAC in a large cohort. We investigated their associations with clinicopathological characteristics and cancer-specific survival in PDAC patients after radical resection using tissue microarray (TMA)–based immunohistochemistry.

2. Materials and methods

2.1. Patients and follow-up

The criteria for patient eligibility included histologic confirmation of PDAC and radical resection without neoadjuvant therapy. Three hundred twenty-one patients were included. The patients comprised 172 men and 149 women, with a median age of 61 years (range, 29-82 years). Of the 321 patients, follow-up data were collected for 287 (89.41%), with follow-up periods ranging from 1 to 129 months (median, 18 months). Among these, 172 patients died and 115 remained alive. The clinicopathological characteristics of the patients are shown in Table 1. This study was approved by our institutional ethics committee.

2.2. TMA construction and immunohistochemistry

TMA analyses were conducted on formalin-fixed, paraffin-embedded blocks with a manual tissue arrayer (Beecher Instruments, Sun Prairie, WI). Two cores (diameter, 1.5 mm) of representative tumor and nontumor tissues per PDAC patient were punched out after careful selection and review.

MKK4 and MKK7 expressions were detected by immunohistochemistry. Primary antibodies against MKK4 (ab90092; Abcam Biotech Company, Cambridge, United Kingdom) and MKK7 (ab52618; Abcam Biotech Company) and a 2-step staining kit (EnVision+ Kit; Dako, Glostrup, Denmark) were used for staining. Briefly, 4-μm-thick sections were mounted, deparaffinized in xylene, and rehydrated. Antigen retrieval was performed for 10 minutes in an autoclave using 0.01 mol/L citrate buffer (pH 6.0). Endogenous peroxidase activity was then blocked with 3% hydrogen peroxide. Subsequently, the sections were incubated with the primary antibodies (1:50 dilution) at 4°C overnight. After 4 washes with phosphate-buffered saline, horseradish peroxidase–conjugated secondary antibodies were added at room temperature for 30 minutes. After application of diaminobenzidine as a chromogen, the sections were counterstained with hematoxylin (Sigma-Aldrich, Munich, Germany). Nonimmune mouse and rabbit sera at the same dilution were adopted as negative controls.

Two experienced pathologists (Z. Y. L. and W. X. Z.) who were blinded to the clinicopathological and follow-up data evaluated the sections independently. The H-score [22], which

Table 1 Correlations between MKK4 and MKK7 expressions and clinicopathological features of PDAC

Variables	n	MKK4 expression [a]		*P*	n	MKK4 expression [b]		*P*	n	MKK7 expression		*P*
		Low	High			Low	High			Low	High	
Age (y)				.056				.888				.055
≤61	154	87	67		154	93	61		160	99	61	
>61	152	102	50		151	90	61		154	111	43	
Sex				.868				.281				.474
Male	164	102	62		164	103	61		169	116	53	
Female	142	87	55		141	80	61		145	94	51	
Tumor location				.961				.480				.100
Head	179	110	69		178	108	70		187	131	56	
Nonhead	115	71	44		115	65	50		115	70	45	
Tumor size (cm)				.942				.879				.850
<3.25	152	94	58		152	92	60		158	107	51	
≥3.25	127	78	49		127	78	49		129	86	43	
CA19-9				.003 *				.715				.576
<34	48	20	28		48	27	21		47	30	17	
≥34	209	136	73		208	123	85		216	147	69	
Histologic grade				.040 *				.622				.023 *
G1-2	168	93	75		167	96	71		172	106	66	
G3	89	61	28		89	54	35		90	68	22	
pT stage				.459				.345				.746
T1-2	17	12	5		17	12	5		17	12	5	
T3	284	175	109		283	167	116		292	195	97	
pN stage				.896				.283				.514
N0	131	81	50		130	83	47		137	94	43	
N1	163	102	61		163	94	69		166	108	58	

NOTE. Partial data were not available, and statistical analyses were based on available data. P-values were derived from the χ^2 test (2-sided).
Abbreviations: G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated; pT stage, pathologic T stage; pN stage, pathologic N stage.
[a] MKK4 expression the cytoplasm.
[b] MKK4 expression in the nucleus.
* $P < .05$.

considers both staining intensity and positive cell proportion [23], was used for staining evaluation. As previously reported [24], the H-score with the largest Youden index (YI) for survival status was identified as the cutoff value in the receiver operating characteristic (ROC) curve.

2.3. Cytology experiments in pancreatic cancer cell lines

For details, please see the Supplementary Data.

2.4. Computational survival analysis in The Cancer Genome Atlas database

For details, please see the Supplementary Data.

2.5. Statistical analyses

The MKK4/MKK7 H-scores in tumor and nontumor tissues were compared using the Mann-Whitney *U* test. The associations between MKK4/MKK7 expression and clinicopathological parameters were evaluated using the χ^2 test. Kaplan-Meier survival curves were compared using the log-rank test. Cox regression (proportional hazards model) was applied for univariate and multivariate prognostic factor identifications. The data were expressed as mean ± SEM in the cell experiments. Differences between 2 groups were analyzed using an unpaired Student *t* test. *P* values of less than .05 were considered statistically significant. Statistical software package IBM SPSS Statistics 22.0 (IBM, Chicago, IL) was applied for all analyses.

3. Results

3.1. Expression of MKK4 and MKK7 in PDAC tissue

MKK4-positive staining was observed in both the cytoplasm and the nucleus. The cytoplasmic H-score for MKK4 staining was significantly higher in normal pancreatic duct cells in the adjacent nontumor tissues than in PDAC cells ($P < .001$, Fig. 1A-C). The nuclear expression of MKK4 showed the opposite findings; namely, its expression in tumor tissues was

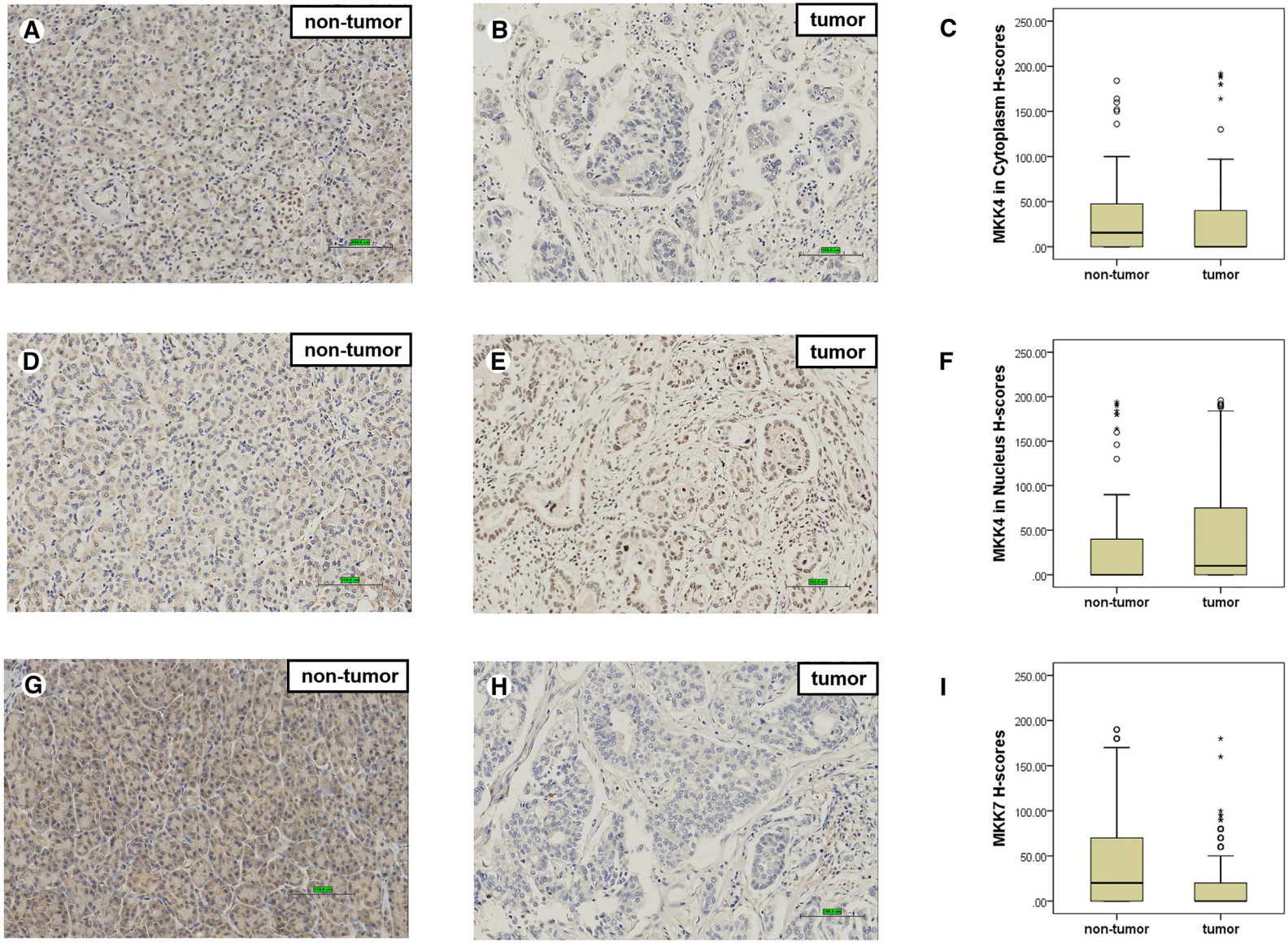

Fig. 1 Expression of MKK4/MKK7 in PDAC. A, MKK4 expression in the cytoplasm of nontumor tissues. B, MKK4 expression in the cytoplasm of tumor tissues. C, Comparison of H-scores for cytoplasmic MKK4 between tumor and nontumor tissues ($P < .001$, Mann-Whitney U test). D, MKK4 expression in the nucleus of nontumor tissues. E, MKK4 expression in the nucleus of tumor tissues. F, Comparison of H-scores for nuclear MKK4 between tumor and nontumor tissues ($P < .001$, Mann-Whitney U test). G, MKK7 expression in the cytoplasm of nontumor tissues. H, MKK7 expression in the cytoplasm of tumor tissues. I, Comparison of H-scores for cytoplasmic MKK7 between tumor and nontumor tissues ($P < .001$, Mann-Whitney U test). A, B, D, E, G, and H, Original magnification ×200; bar, 100 μm.

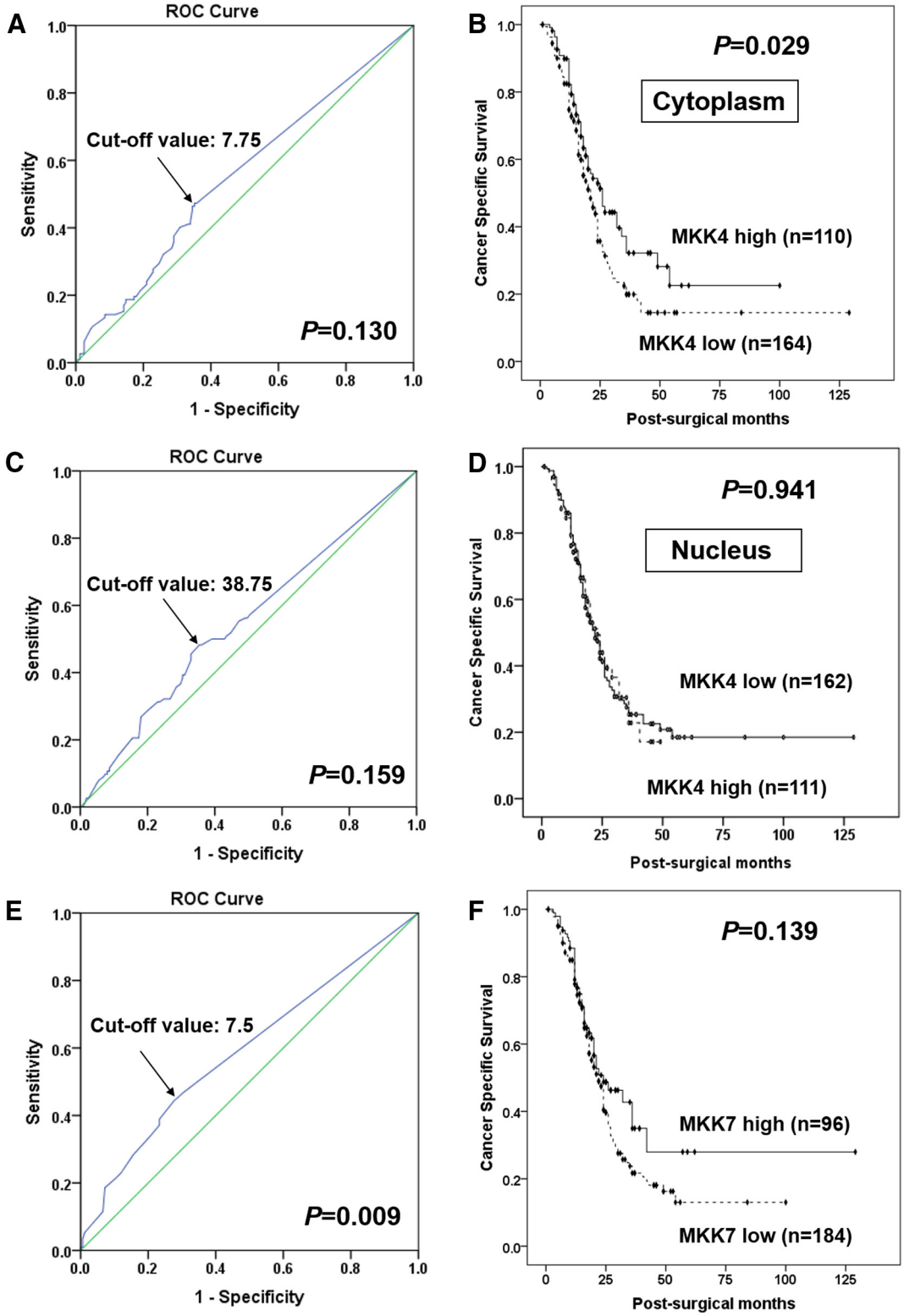

Fig. 2 Prognostic impact of MKK4/MKK7 in PDAC. A, ROC curves for tumor cytoplasmic MKK4 H-scores for cancer-specific survival (AUC, 0.554). B, Cancer-specific survival curves for patients with high or low tumor cytoplasmic MKK4 expression ($P = .029$, log-rank test). C, ROC curves for tumor nuclear MKK4 H-scores for cancer-specific survival (AUC, 0.550). D, Cancer-specific survival curves for patients with high or low tumor nuclear MKK4 expression ($P = .941$, log-rank test). E, ROC curves for tumor cytoplasmic MKK7 H-scores for cancer-specific survival (AUC, 0.592). F, Cancer-specific survival curves for patients with high or low tumor cytoplasmic MKK7 expression ($P = .139$, log-rank test). AUC, area under curve.

Table 2 Univariate and multivariate analyses for prognostic factors of PDAC

Variables	n	Univariate			Multivariate		
		HR	95% CI	*P*	HR	95% CI	*P*
Age (y)				.935			
≤61	141	1					
>61	146	0.988	0.731-1.334				
Sex				.887			
Male	152	1					
Female	135	1.022	0.757-1.380				
Tumor location				.886			
Head	164	1					
Nonhead	112	0.977	0.715-1.336				
Tumor size (cm)				.247			
<3.25 *	142	1					
≥3.25	119	1.202	0.880-1.643				
CA19-9				.423			
<34	46	1					
≥34	199	1.189	0.778-1.817				
Histologic grade				.004 *			.015 *
G1-2	156	1			1		
G3	85	1.650	1.176-2.316		1.564	1.092-2.239	
pT stage				.692			
T1-2	17	1					
T3	266	1.138	0.600-2.158				
pN stage				.002 *			.014 *
N0	121	1			1		
N1	156	1.663	1.212-2.282		1.584	1.100-2.282	
MKK4 expression [a]				.034 *			.202
Low	164	1			1		
High	110	0.705	0.510-0.974		0.788	0.546-1.137	
MKK4 expression [b]				.942			
Low	162	1					
High	111	0.988	0.713-1.369				
MKK7 expression				.149			
Low	184	1					
High	96	0.778	0.554-1.094				

Abbreviations: G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated; pT stage, pathologic T stage; pN stage, pathologic N stage.

[a] MKK4 expression the cytoplasm.

[b] MKK4 expression in the nucleus.

* $P < .05$.

significantly higher than that in nontumor tissues ($P < .001$, Fig. 1D-F). MKK-7-positive staining was mainly located in the cytoplasm. By the Mann-Whitney *U* test, the H-score in nontumor tissues was significantly higher than that in tumor tissues ($P < .001$, Fig. 1G-I).

3.2. Cutoff values for MKK4/MKK7 expressions

We identified the H-score cutoff values with the largest YI for survival status by ROC curves. The cutoff values for cytoplasmic MKK4 expression, nuclear MKK4 expression, and MKK7 expression were 7.75 (Fig. 2A), 38.75 (Fig. 2C), and 7.5 (Fig. 2E), respectively.

3.3. Correlations of MKK4/MKK7 with clinicopathological parameters of PDAC

Table 1 summarizes the data for the correlations between MKK4/MKK7 expression and clinicopathological features of PDAC. High tumor cytoplasmic MKK4 expression was more frequent in G1-2 patients ($P = .040$, χ^2 test) and in accordance with MKK7 expression ($P = .023$, χ^2 test). High CA19-9 level was detected more often with low MKK4 expression ($P = .003$, χ^2 test). There were no significant correlations between nuclear MKK4, cytoplasmic MKK4, MKK7 expression, and the other clinicopathological parameters.

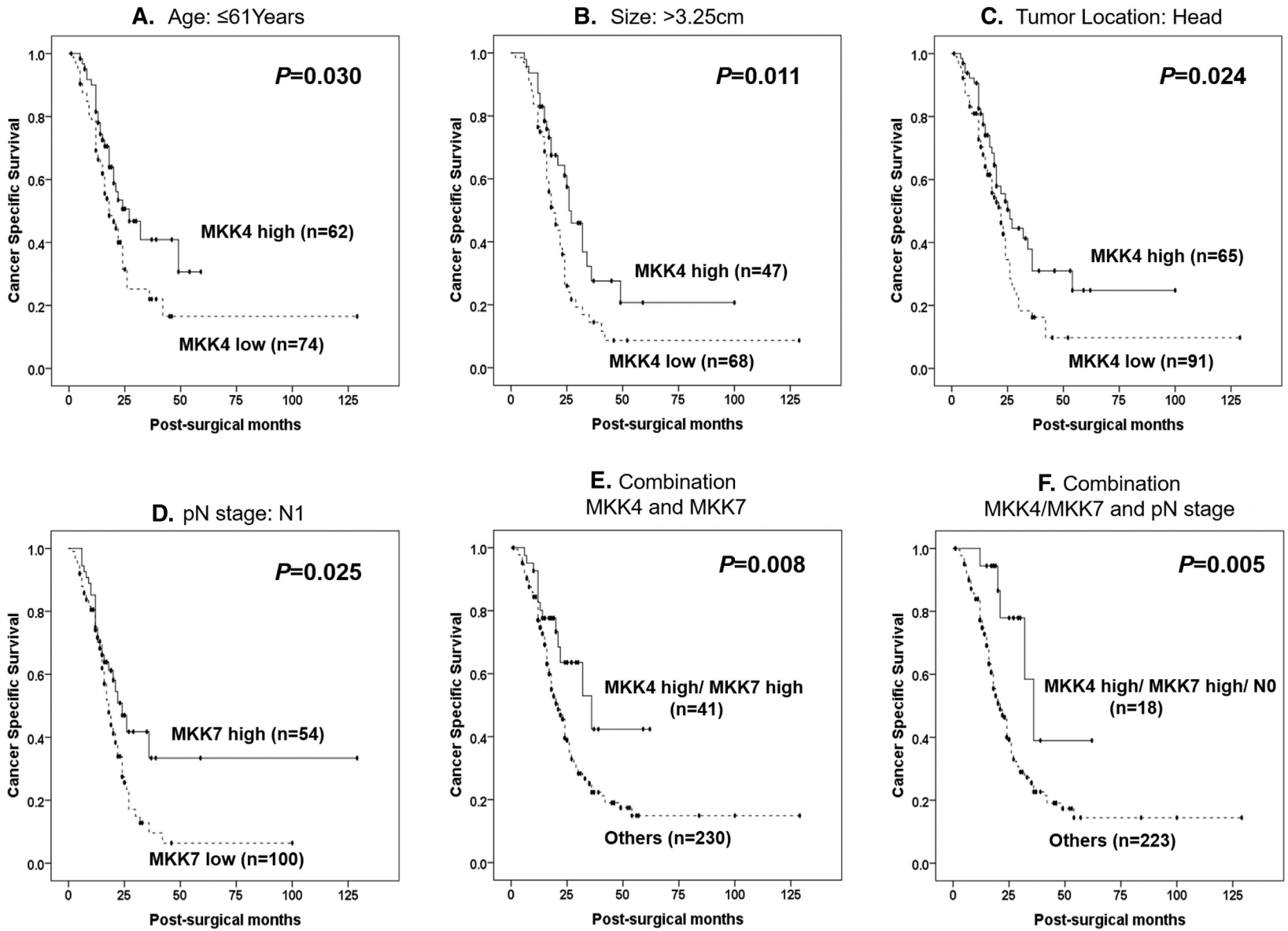

Fig. 3 Kaplan-Meier survival analysis of PDAC patients after surgical resection based on MKK4[†]/MKK7 expression in tumor tissues for different subgroups and combinations. A, Patients aged ≤61 years (MKK4: $P = .030$, log-rank test). B, Patients with tumor size >0.25 cm (MKK4: $P = .011$, log-rank test). C, Patients with carcinoma of the head of the pancreas (MKK4: $P = .024$, log-rank test). D, N1 tumors (MKK7: $P = .025$, log-rank test). E, MKK4 combined with MKK7 ($P = .008$, log-rank test). F, MKK4-MKK7 combined with pN stage ($P = .005$, log-rank test). pN stage, pathologic N stage. [†]MKK4 expression in the cytoplasm.

Table 3 Prognostic value of MKK4 [a]-MKK7 combined with main clinicopathological variables in univariate and multivariate analyses for PDAC

Variables	n	Univariate			Multivariate		
		HR	95% CI	*P*	HR	95% CI	*P*
MKK4/MKK7				.011 *			.153
High/High	41	0.493	0.285-0.852		0.656	0.368-1.170	
Others	230	1			1		
MKK4/MKK7/histologic grade				.270			
High/high/G1-2	28	0.706	0.380-1.312				
Others	199	1					
MKK4/MKK7/pN stage				.010 *			.031 *
High/high/N0	18	0.308	0.126-0.752		0.373	0.152-0.916	
Others	223	1			1		

Abbreviations: G1, well differentiated; G2, moderately differentiated; pN stage, pathologic N stage.

[a] MKK4 expression the cytoplasm.

* $P < .05$.

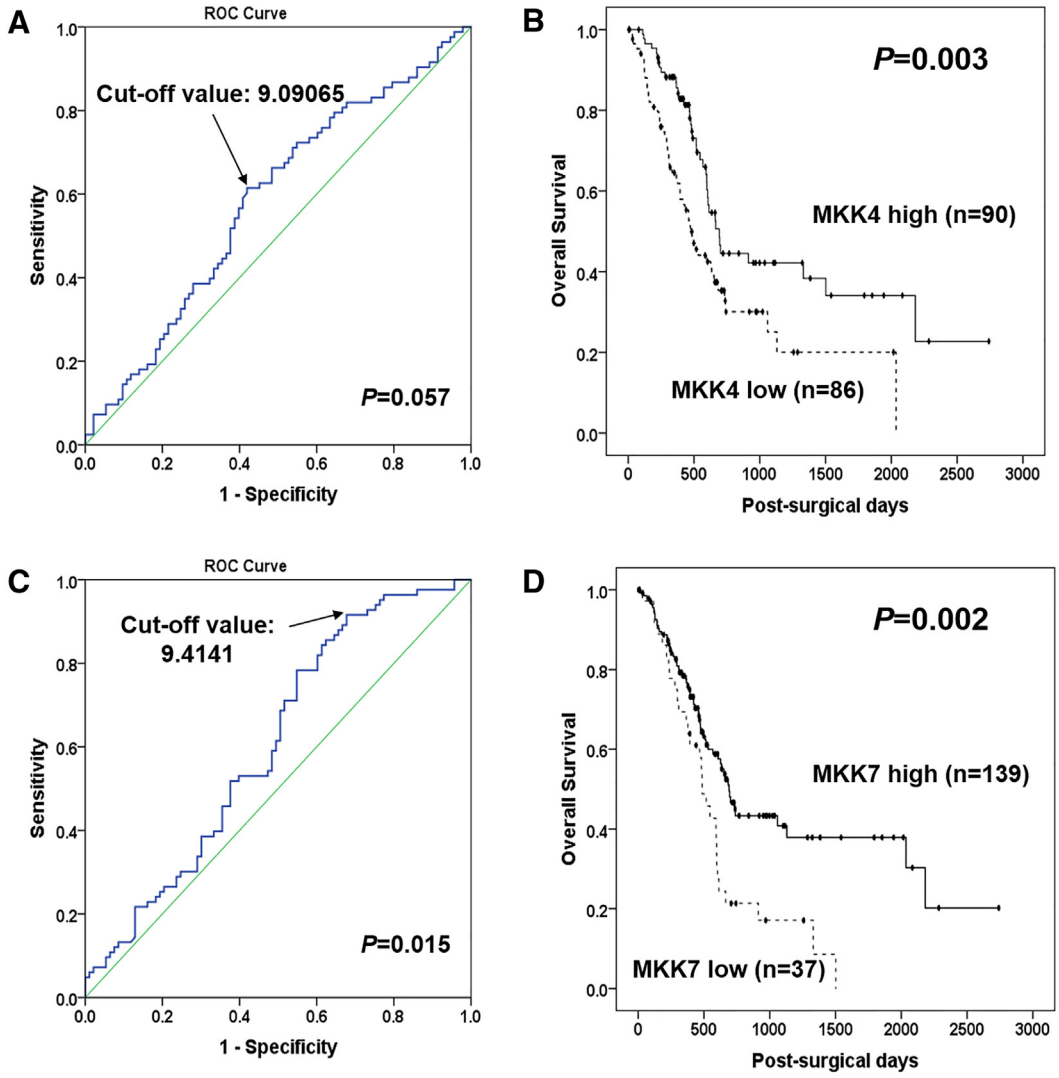

Fig. 4 Prognostic impacts of MKK4 and MKK7 mRNA expression in PDAC in the TCGA database. A, ROC curve for MKK4 expression and overall survival (AUC, 0.583). B, Overall survival curves for patients with high or low MKK4 expression ($P = .003$, log-rank test). C, ROC curve for MKK7 expression and overall survival (AUC, 0.606). D, Overall survival curves for patients with high or low MKK7 expression ($P = .002$, log-rank test). AUC, area under curve.

3.4. MKK4/MKK7 expression in PDAC and cancer-specific survival

High cytoplasmic MKK4 expression was associated with significantly longer cancer-specific survival (P = .029, Fig. 2B). There were no significant associations of MKK7 expression (P = .139, Fig. 2F) and nuclear MKK4 expression (P = .941, Fig. 2D) with cancer-specific survival.

3.5. Identification of prognostic factors in resectable PDAC

Table 2 presents the results for MKK4/MKK7 expression and clinicopathological factors in relation to cancer-specific survival by univariate and multivariate analyses. Poor differentiation (P = .004) and presence of lymphatic invasion (P = .002) were significantly correlated with shorter survival. Furthermore, multivariate analysis demonstrated that both parameters were independent prognostic factors for cancer-specific survival (P = .015 and P = .014, respectively). Subgroup analyses were also conducted for different clinicopathological factors. Cytoplasmic MKK4 expression in tumor tissues was a favorable factor in different subgroups (patients aged ≤61 years, P = .030, Fig. 3A; patients with tumor size >3.25 cm [cutoff value defined by largest YI in ROC curve for survival, Supplementary Fig. S1], P = .011, Fig. 3B; patients with carcinoma of the head of the pancreas, P = .024, Fig. 3C). In addition, high MKK7 expression was significantly associated with longer survival in patients with N1 tumors (P = .025, Fig. 3D).

3.6. MKK4-MKK7 combination with main clinicopathological variables and cancer-specific survival

We examined the association of combined cytoplasmic MKK4 and MKK7 expression with cancer-specific survival in PDAC patients after resection. As shown in Table 3, combined high expression of MKK4 and MKK7 (Fig. 3E) had a significant advantage over individual high expression for prognosis prediction (MKK4-MKK7 versus MKK4: hazard ratio [HR], 0.493 versus 0.705). For combinations with main clinicopathological variables, histologic grade did not improve the prognostic efficacy (P = .258, Supplementary Fig. S2). Meanwhile, high MKK4-MKK7 expression combined with pN0 stage was significantly correlated with favorable prognosis (HR, 0.308; 95% confidence interval [CI], 0.126-0.752; Fig. 3F) in both univariate and multivariate analyses. Moreover, with the addition of pN stage to the combination of MKK4 and MKK7 in univariate Cox regression, the new

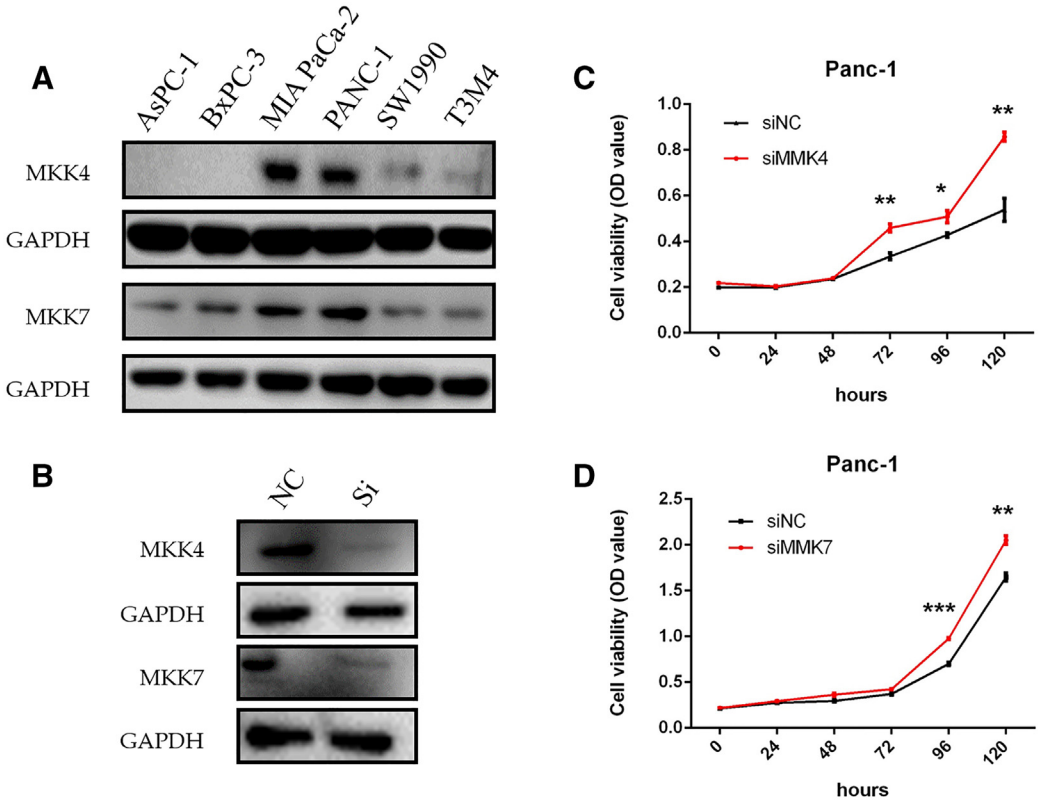

Fig. 5 Effects of downregulation of MKK4 and MKK7 on pancreatic cancer cells. A, Protein expression levels of MKK4 and MKK7 in different pancreatic cancer cell lines. B, Protein expression levels of MKK4 and MKK7 after treatment with specific siRNAs in PANC-1 cells. C, Effects of MKK4-siRNA on cell viability in PANC-1 cells (*P < .05; **P < .01; ***P < .001). D, Effects of MKK7-siRNA on cell viability in PANC-1 cells (**P < .01; ***P < .001). pT stage, pathologic T stage; NC, normal control group.

combination had the lowest HR for cancer-specific survival (HR, 0.308 versus 0.493).

3.7. Prognostic value of MKK4-MKK7 in The Cancer Genome Atlas database

We further analyzed 176 patients in The Cancer Genome Atlas (TCGA) database. The patients comprised 97 men and 79 women, with a median age of 65 years (range, 35-85 years). The optimal cutoff values for MKK4 (Fig. 4A, Supplementary Fig. S3A) and MKK7 (Fig. 4C, Supplementary Fig. S3C) messenger RNA (mRNA) expression were respectively determined based on the YI for overall survival and relapse-free survival in ROC curves. High MKK4 and MKK7 mRNA expressions were both significantly correlated with favorable overall survival (MKK4: $P = .003$, Fig. 4B; MKK7: $P = .002$, Fig. 4D). High MKK4 expression was also significantly associated with longer relapse-free survival ($P = .029$, Supplementary Fig. S3B). However, there was no significant association of MKK7 expression with relapse-free survival ($P = .206$, Supplementary Fig. S3D).

3.8. MKK4 and MKK7 suppress cell proliferation in PDAC

To validate the functions of MKK4 and MKK7, we first performed Western blotting to validate their expressions in different PDAC cell lines. The data revealed that the protein expression levels of MKK4 and MKK7 were significantly upregulated in PANC-1 cells compared with the other PDAC cell lines (Fig. 5A). Next, we inhibited MKK4 and MKK7 expressions in PANC-1 cells using specific small interfering RNAs (siRNAs) and confirmed their downregulation by Western blotting analysis (Fig. 5B). We then detected the actions of MKK4-siRNA and MKK7-siRNA on cell proliferation of PANC-1 cells and found that downregulation of MKK4 or MKK7 markedly increased the pancreatic cancer cell viability (Fig. 5C and D).

4. Discussion

MKK4 and MKK7 belong to the mitogen-activated protein kinase kinase family and activate c-Jun N-terminal kinase in a stress-activated cascade [13,14]. In many malignancies, MKK4 and MKK7 are considered to act as tumor suppressors [9,12,15-17]. However, Wu et al [25] reported that patients with MKK4 protein expression in gastric cancer had significantly poorer overall survival than did those without MKK4 expression. In PDAC, data for the biological roles of MKK4 and MKK7 have remained controversial. Davies et al [18] revealed that MKK4 and MKK7 were required for acinar regeneration, and that loss of MKK4 and MKK7 caused marked synergistic acceleration of PDAC development. However, Wang et al [19] showed that MKK4 promoted growth and invasion after ectopic expression in MKK4-negative pancreatic cancer cell lines. Hence, further cumulative studies are needed in PDAC.

To date, there are limited articles for the prognostic significance of MKK4 in pancreatic cancer. Xin et al [20] found a trend toward worse survival in patients with loss of MKK4 expression. Handra-Luca et al [21] reported that cytoplasmic and nuclear MKK4 expression were not significant factors for survival. However, both studies were limited by their relatively small sample sizes. Moreover, no studies have investigated the prognostic value of MKK7 in pancreatic cancer. Therefore, we examined MKK4/MKK7 expression and their prognostic value in a large-scale retrospective cohort using TMA-based immunohistochemistry.

First, we found that cytoplasmic MKK4 and MKK7 expressions were significantly downregulated in tumor tissues compared with nontumor tissues in PDAC, whereas nuclear MKK4 expression had the opposite findings. Similarly, Sethi et al [26] found that nuclear MKK4 was required for expression of TNF-induced antiapoptotic proteins as well as TNF-induced phosphorylation and nuclear translocation of p65, indicating that MKK4 promoted cell survival. However, the differences arising from the localizations of MKK4 remained unclear, suggesting a need for detailed mechanistic studies. In normal prostate tissues, high levels of MKK4 expression were found in the epithelial, but not stromal, compartment [27], indicating that MKK4 may exert different functions in different conditions. Furthermore, other molecules like survivin exhibited different prognostic implications, molecular mechanisms, and bifunctional roles after nuclear and cytoplasmic accumulation [28-30].

Second, we found that lower MKK4/MKK7 expression in the cytoplasm was associated with higher histologic grade in PDAC, suggesting that MKK4 and MKK7 play crucial roles in cancer proliferation. Meanwhile, low cytoplasmic MKK4 expression was significantly linked with high CA19-9 level. In PDAC, tumor grade and CA19-9 have been repeatedly verified as independent negative predictors for prognosis [31,32], suggesting that MKK4 and MKK7 may be negatively associated with progression of PDAC. However, there were no significant correlations between nuclear MKK4 and clinicopathological parameters. The function of nuclear MKK4 in PDAC should be explored in future studies.

Third, our data revealed that high tumor cytoplasmic MKK4 H-score was significantly associated with favorable cancer-specific survival in univariate analyses. In addition, high MKK7 expression showed a trend toward favorable prognosis. In different subgroups, high MKK4/MKK7 expression was associated with significantly longer cancer-specific survival, broadly indicating that MKK4 and MKK7 may serve as tumor-suppressive genes in PDAC. In addition, univariate and multivariate analyses confirmed that histologic grade and pN stage were independent prognostic factors, consistent with a previous study [7]. More importantly, prognostic values of MKK4 and MKK7 were confirmed at the mRNA level in the TCGA database, making the results more reliable and persuasive.

Regretfully, MKK4 was not statistically significant in the multivariate analysis. Therefore, we further explored its prognostic value in combination with other factors. We found that combined high cytoplasmic MKK4 and MKK7 expression was associated with significantly longer cancer-specific survival in PDAC patients and distinguished their prognosis more effectively than either MKK4 or MKK7 alone. These findings suggested that MKK4 and MKK7 may cooperatively regulate progression in pancreatic cancer, in accordance with a previous study showing that absence of both MKK4 and MKK7 interacted synergistically with expression of activated Kras to promote invasive PDAC in mice [18].

To further enhance the prognostic efficiency, we attempted to combine the evaluation with significant clinicopathological variables. The combination of MKK4 and MKK7 plus pN stage was independently associated with prognosis with the highest predictive power and lowest HR. Accordingly and significantly, for postoperative PDAC patients with low cytoplasmic MKK4 and low MKK7 expression together with pN1 stage, intensive follow-up and intensified adjuvant strategies could be recommended to improve their survival.

We performed in vitro experiments to verify our findings for biological support. We found that downregulation of MKK4 or MKK7 promoted proliferation of pancreatic cancer cells, thereby validating the histologic findings in tissues.

In conclusion, both cytoplasmic MKK4 and MKK7 expressions were significantly downregulated in tumor tissues. High MKK4 H-score was significantly associated with favorable cancer-specific survival, whereas MKK7 expression and pN stage increased the predictive efficiency in resectable PDAC.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2018.11.026.

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