Research Article

Cancer Epidemiology, Biomarkers & Prevention

Expression of Kallikrein-Related Peptidase 7 Predicts Poor Prognosis in Patients with Unresectable Pancreatic Ductal Adenocarcinoma

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Abstract

Background: Kallikrein-related peptidase 7 (KLK7) is overexpressed in pancreatic ductal adenocarcinomas (PDAC). The aims of this study were to examine the expression of KLK7 during progression of pancreatic intraepithelial neoplasia (PanIN) to invasive PDAC and to assess its prognostic significance for PDAC.

Methods: Immunohistochemistry was used to assess KLK7 expression using a tissue microarray (TMA) and full sections of pancreatic tissue containing normal tissue, PanIN, and invasive adenocarcinoma, and the association between KLK7 expression and prognosis was examined by a population-based pancreatic cancer TMA.

Results: Normal pancreatic epithelium was negative for KLK7 in either TMAs or full sections. Analysis by TMAs showed that 91% of cases showed KLK7 positivity in the adenocarcinoma component, which was significantly higher than PanIN 2/3. In full tissue sections of PDAC, KLK7 expression was detected in less than 1% of cells among PanIN 1A lesions, and increased with grade among PanIN 1B and PanIN2/3 lesions before reaching 69% in the invasive PDAC. In patients with unresected PDAC, KLK7 positivity was significantly associated with shorter overall survival.

Conclusions: Aberrant KLK7 expression starts in intermediate-to-late stages of PanIN progression, and KLK7-positive staining is associated with almost a three-fold increase in mortality rate of patients with unresected PDAC.

Impact: The association of KLK7 expression and poor outcome of patients with unresectable PDAC suggests that inhibiting either KLK7 expression and/or activity could be a therapeutic strategy. Because the vast majority of patients present with unresectable disease, such an intervention could have a significant impact upon the overall survival of this patient population. *Cancer Epidemiol Biomarkers Prev; 21(7); 1135–42. ©2012 AACR.*

Introduction

Pancreatic cancer is the fourth most common cause of cancer-related deaths in men and women in the United States (1). Similar to other epithelial cancers, the development of pancreatic ductal adenocarcinomas (PDAC) is multistep, with intermediate steps being defined by a series of morphologic changes and molecular alterations during progression from intraepithelial lesions to invasive carcinoma (reviewed in refs. 2, 3). Although our understanding of the genetic changes that accumulate during progression of low-grade pancreatic intraepithelial neoplasms (PanIN) to infiltrating adenocarcinomas has increased substantially in the past decade, prognosis of patients upon diagnosis remains extremely poor. Most patients present with inoperable, locally advanced or metastatic disease; thus, median survival times for patients diagnosed with locally advanced disease ranges between 6 and 10 months, compared with only 3 to 6 months for patients with metastatic disease (4). Even patients who undergo resection of localized adenocarcinoma of the head of pancreas without metastatic spread have a median survival of only 13 to 15 months and a 15% to 20% rate of long-term survival (5). Thus, numerous prognostic factors have been assessed for their impact on survival following surgical resection of pancreatic adenocarcinoma, and several have been shown to correlate with patient outcome, including clinicopathologic staging (e.g., tumor size, lymph node status), tumor biology, intraoperative factors, adjuvant chemoradiation therapy, and immunohistochemical markers such as VEGF, Bcl-2, p16, and p53 (6–9).

We previously generated an expression profile of serine proteases expressed in both normal and

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doi: 10.1158/1055-9965.EPI-11-1079

adenocarcinoma pancreatic tissues and found that kallikrein-related peptidase 7 (KLK7) is overexpressed in PDACs (10). KLK7, a chymotryptic-like serine protease, was initially purified and characterized from human skin extracts and is thought to play a role in desquamation of human skin (11, 12). Increasingly, however, the *KLK7* transcript and/or KLK7 protein have been found to be overexpressed in human cancers. Other laboratories have showed that it is overexpressed in ovarian (13, 14), squamous cervical (15), and breast cancers (16). In the current study, we used immunohistochemistry to examine the expression of KLK7 in lesions representing stages of PDAC development and to determine whether there is a correlation between KLK7 expression and overall survival (OS) in patients with pancreatic adenocarcinoma.

Materials and Methods

Tissue samples

The study was approved by the Research Ethics Board at the University Health Network and Institutional Review Board at the University of Arkansas for Medical Sciences. For the initial analysis of KLK7 expression during pancreatic adenocarcinoma tumorigenesis, tissue microarrays (TMA) assembled by sampling of paraffin blocks of 36 cases of resected PDACs were used (17). Normal, PanIN, and adenocarcinoma components were identified in TMA spots and assessed for KLK7 staining. Because of the nature of TMAs, individual spots contained one, several, or no targeted components (normal, PanIN, or adenocarcinoma); thus, 49 spots from 22 cases contained adenocarcinoma component, 17 spots from 17 cases contained PanIN 1 lesions, 46 spots from 35 cases contained PanIN 2/3 lesions, and 32 spots contained normal ducts from 21 cases. After the initial analysis of the TMAs, full sections were prepared from PDAC tumor blocks obtained following surgical resection and 22 cases were found to contain the targeted components: normal pancreatic ducts, invasive cancer, and at least one grade of PanIN in the same section. These full sections of 22 cases were evaluated for KLK7 immunoreactivity quantitatively. The full sections were chosen to reduce the biases of the TMA, such as small areas subject to heterogeneity effects and unbalanced representation of cases by a different number of cores due to technical reasons. The next step was to examine the association between KLK7 expression and prognosis. The Surveillance Epidemiology and End Results (SEER) Residual Tissue Repository (RTR) population-based pancreatic TMA was examined for KLK7 expression (generously provided by National Cancer Institute's SEER program, Bethesda, MD; ref. 18). The tissues within the TMA are linked to cases in cancer registries in Hawaii, Iowa, and Los Angeles, for which there are additional clinical and demographic data. The cases with tissues in the TMA can thus be compared with all cases in the population for representation and potential biases (18).

Immunohistochemical staining

Immunohistochemical staining was carried out as previously described (10). Briefly, formalin-fixed, paraffin-embedded sections were deparaffinized and rehydrated in xylene followed by graded ethanol. Following antigen retrieval, endogenous peroxidase activity was quenched by hydrogen peroxide treatment followed by a protein block (Dako serum-free protein block). Sections were incubated with a goat anti-hKLK7 antibody (R&D Systems, diluted 1:800) overnight in a humidified chamber at 4°C. Immunoreactive staining was detected using a DAKO LSAB$^+$ peroxidase system followed by hematoxylin counterstain.

Evaluation of immunohistochemical analysis

In the initial analysis of the TMAs, each spot was assessed for the presence of the following components: normal pancreatic ducts, 3 grades of PanIN, or invasive adenocarcinoma. KLK7 staining was recorded as positive in each component when there was unequivocal staining in any number of cells. The number and percentage of spots with positivity in each component was calculated. This TMA analysis showed higher frequency of KLK7 positivity in more advanced lesions, so our next step was to quantify KLK7 expression using larger samples. Full tissue sections of the blocks used for the TMA sampling were visually assessed for the percentage of KLK7-positive cells in each component. Then, after confirming that KLK7 expression correlates with morphologic steps of disease progression, population-based TMAs were analyzed for associations between KLK7 positivity and clinical outcome. These TMAs were analyzed digitally using previously described techniques (19, 20). Briefly, the KLK7-stained slide was scanned using an Aperio ScanScope scanning system (Aperio Technologies). Each TMA spot was saved as a separate file, then all parts of the tissue except for the component of interest were erased using "Photoshop CS3" (Adobe Systems). Then, the images were processed to generate 2 sets of files: tissue masks to limit analysis within the selected areas and signal images to assess KLK7 staining intensity. The tissue masks were obtained by thresholding through a value separating the clear background of histologic slides from the tissue. The signal images were generated by collecting the range of KLK7 brown color, erasing nonbrown pixels, and converting images into grayscale files. This step was standardized by recording an action (Photoshop CS3) and processing all images in one batch. Intensity of KLK7 signal (mean optical density, MOD) was measured within the mask areas using an image analysis program based on language IDL 6.3 (ITT Visual Information Solutions), which has been used in earlier studies (20–22).

The highest intensity value of background staining was measured in the visually negative pancreatic and nonpancreatic tissue included in the same TMA (subjected to the same methodologic variables). This value, a MOD of 15, was used as the cutoff point to dichotomize intensities as positive (if $\geq$15) or negative (if <15) for KLK7 expression.

Statistical analysis

TMAs were assessed via pairwise χ^2 tests for differences in the percentage of KLK7-positive samples between PDAC, PanIN 2/3, and PanIN 1. Full sections were compared for trend with disease severity in the percentage of KLK7-positive cells using a case-stratified version of Spearman correlation analysis that uses a generalized Cochran–Mantel–Haenszel (CMH) test with rank scores (23) to adjust for the expected dependency among different components from the same sample. For the population-based TMA, samples were categorized into groups based on the characteristics from the accompanying clinical data: (i) whether the sampled tumor was "primary" or "metastatic" and (ii) whether the patient underwent surgical resection (resectable tumor at presentation) or the tumor was unresectable (sampled at autopsy). Four samples classified as "resected metastatic" were excluded from the analysis, since these were sampled at the time of presentation by resection, but were found to have a disease stage that was designated as unresectable for other cases with the same stage. Stagewise, these cases were "unresectable," but were sampled as "resectable" at presentation and by resection, thus raising questions of potential misclassification for members of this group. The remaining samples were stratified into 3 groups, "resected primary," "unresected primary," and "unresected metastatic." These were construed to be the 3 levels of a variable called disease group. KLK7 positivity was tested via χ^2 test for associations with disease group, age ($\leq$54, 55–64, 65–74, and $\geq$75), gender (male vs. female), tumor grade (poorly differentiated vs. moderately or well-differentiated), and clinical stage. The effect of KLK7 positivity on OS was explored in 3 phases. In the first phase, "subgroup analyses," the effect of KLK7 positivity within each disease group was visualized via Kaplan–Meier curves and assessed quantitatively via univariate Cox regressions. In the second phase, "two-factor analysis," the effect of KLK7 positivity was reassessed by fitting to the entire data set a 2-factor model consisting of Cox regression with KLK7, disease group, and their interaction as the predictors, to determine whether the notable variation in KLK7 effect between disease groups was statistically significant. In the third phase, "multivariate analysis," the 2-factor model was expanded to include stage and age as additional predictors. Two-way interactions in addition to the KLK7-by-disease group term were screened via the Backward Elimination algorithm at $P < 0.05$, but none qualified for incorporation into the final multivariate model.

Results

KLK7 expression increases during progression from premalignant ductal lesions to invasive adenocarcinoma

Results of immunohistochemical analyses of KLK7 on the TMA composed of preinvasive and invasive ductal lesions were consistent with those of previous analyses (10). Figure 1 shows visual examples of how each component stained for KLK7, whereas Fig. 2A quantifies the proportion of cases showing positivity in each component. No staining was observed for KLK7 in normal pancreatic acinar or ductal epithelium sampled by the TMAs, whereas 91% (20 of 22) of the ductal adenocarcinoma cases were positive (i.e., unequivocal staining above the background level). There was clearly a significant difference in KLK7 expression between PDAC and PanIN 2/3, where the latter were positive in only 49% (17 of 35) of cases ($P = 0.0011$), but minimal difference in KLK7 expression between the PanIN 2/3 compared with PanIN 1 lesions, for which 47% (8 of 17) cases were positive ($P = 0.92$; Fig. 2A).

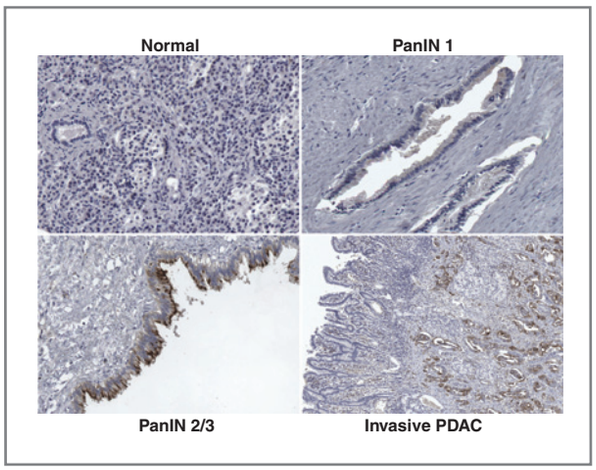


Figure 1. Representative immunohistochemical staining of KLK7 in human pancreatic tissues. Normal pancreas ×25, PanIN 1 and 2/3 ×40, invasive PDAC ×10. Note negativity in all components of normal pancreatic tissue: ductal, acinar, and endocrine cells. The invasive adenocarcinoma shown is infiltrating into the duodenal mucosa.

To provide a more quantitative assessment of KLK7 expression during progression from premalignant ductal lesions to invasive adenocarcinoma, KLK7 immunohistochemistry was carried out on full tissue sections containing 5 epithelial components: normal pancreatic ducts, 3 grades of PanIN, and PDAC. There was no staining of normal epithelial, ductal, or acinar cells. KLK7 expression was detected in PanIN and invasive carcinoma, where staining of the cells was cytoplasmic membranous, involving segments of the ductal lining epithelium in PanIN and heterogeneous areas of staining in invasive carcinoma. In the latter, KLK7 positivity was pronounced at the invasive front (Fig. 1). The epithelial components were assessed for percentage of KLK7-positive cells; Table 1 shows the results by case number, whereas Fig. 2B shows the increasing trend with lesion severity. The mean (median) percentages of KLK7-positive cells were 0% (0%) among 22 normal duct epithelia, <1% (0%) among 13 PanIN 1A lesions, 8% (5%) among 19 PanIN 1B, 41% (40%) among 8 PanIN 2/3, and 69% (80%) among 22 PDAC. This trend was subjected to generalized CMH test with the cases as strata to yield a correlation χ^2 of 41.78 [degrees of freedom (df) = 1; $P < 0.0001$] and

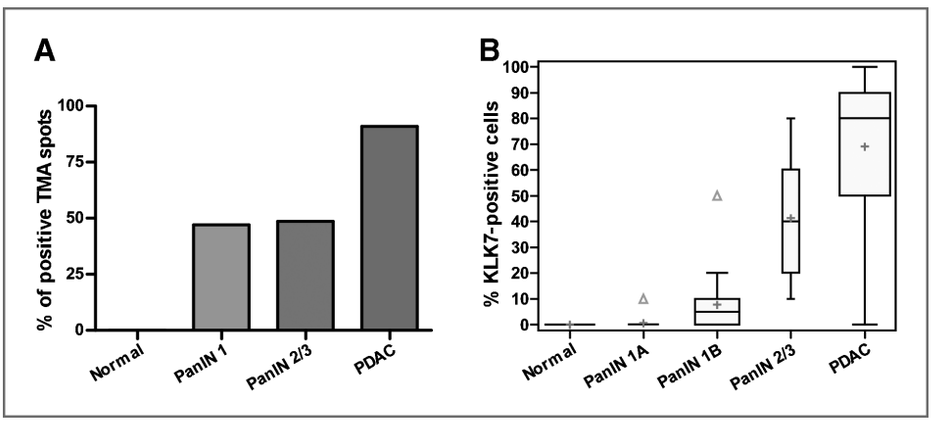


Figure 2. Immunohistochemical analysis of KLK7 expression in human pancreatic tissues. TMAs (A) and full sections (B) were stained for KLK7 and the staining was assessed in each component (normal pancreatic ducts, PanIN, PDAC). A, initial assessment by TMA, percentage of cases showing unequivocal KLK7 staining in each component and (B) analysis of full tissue sections, box plot of percentage of KLK7-positive cells in each epithelial component. The cases were assessed to select full sections containing normal ductal epithelium, invasive carcinoma, and at least one grade of PanIN (see Table 1) in the same section. Twenty-two cases had at least one section fulfilling these criteria and one section from each case was used for the analysis. Box widths vary with number of samples in each class. Each box spans the group's first to third quartiles, with the horizontal line denoting its median and the whiskers denoting nonoutlier values outside the quartiles. Triangles, outliers; plus signs, group means.

corresponding value of 82.1% for the Spearman correlation between percentage of KLK7-positive cells and disease severity of the pancreatic epithelial component.

Associations between KLK7 expression, tumor characteristics, and demographics

To determine whether KLK7 expression in PDAC could be used as a prognostic indicator, we used a population-based pancreatic cancer TMA assembled from tumor tissue in 3 cancer registries as part of the SEER Program of the National Cancer Institute (18) for immunohistochemical analysis of KLK7. To digitally analyze the staining for KLK7 on the TMA, the slide was scanned using an Aperio ScanScope scanning system (Aperio Technologies). All intensity measurements were done digitally in epithelial cells using the brown color selection measurement. Intensity values were dichotomized as described earlier, and then assessed for association with age ($\leq$54, 55–64, 65–74, and $\geq$75), gender (male vs. female), grade (poorly vs. moderately or well-differentiated), tumor stage (localized vs. metastatic disease), and disease group (unresected metastatic, unresected primary, and resected primary). Four samples from patients denoted as having resected metastatic disease were excluded from the analyses as they did not fall into the treatment and sampling protocols used for the other patients of the same disease stage and the number was too small to form a separate group. All 4 were negative for KLK7.

Of the remaining 124 PDAC cases examined, 70 (56%) were classified as positive and 54 (44%) as negative for KLK7 expression (Table 2). Significant associations were observed between positive KLK7 staining and more favorable grade and stage. KLK7-positive samples constituted 61% of tumors graded as moderately or well-differentiated compared with only 35% of those graded as poorly differentiated ($P = 0.035$). Likewise, KLK7 positivity was seen in 68% of tumors designated as localized (clinical stage I) compared with only 45% of those with metastatic spread, regional, or distant ($P = 0.011$). Among the 3 disease groups (Table 2), rates of KLK7 positivity were significantly different ($P = 0.0002$), being much lower in metastases (32%) than in primary tumors, both unresected (72%) and resected (69%). No significant associations were found between KLK7-positive expression and patient age or gender (Table 2).

Association of KLK7 expression with OS

One hundred nineteen deaths occurred among the 124 study subjects during 185.75 person-years of follow-up, yielding a crude mortality rate [95% confidence interval (CI)] of 0.641 (0.531–0.761) deaths year^{-1} person^{-1}. OS among subjects with KLK7-positive tumors had a median (quartiles) of 6.0 (2.0–19.0) months compared with 7.5 (3.0–18.0) months for subjects with KLK7-negative tumors (log-rank $P = 0.74$), suggesting initially that KLK7 expression may have little influence on survival. However, when subjects were grouped by resection status and whether disease was primary or metastatic, Kaplan–Meier survival curves in conjunction with Cox regression results (Fig. 3) disclosed that the effect of KLK7 expression depended on whether the tumor had been resected. Among subjects whose primary tumors had been resected, KLK7 expression had little impact on OS (Fig. 3A). But among subjects whose primary tumors had not been resected, those with KLK7-positive tumors had a significantly shorter survival than those with KLK7-negative tumors (Fig. 3B). Likewise, patients with unresected metastatic disease had a significantly shorter survival if their tumors stained positive (compared with negative) for KLK7 (Fig. 3C). Moreover, the differences among these 3 groups in the strength of the KLK7 effect was statistically significant ($P = 0.041$) under 2-factor analysis adjusting for disease group, and

Table 1. Percentages of KLK7-positive cells in full sections

Case number	Normal epithelium[a]	PanIN 1A[a]	PanIN 1B[a]	PanIN 2/3[a]	PDAC[a]
1	0	—	5	—	80
2	0	0	50	—	90
3	0	10	10	20	90
4	0	0	10	80	50
5	0	—	0	—	90
6	0	0	5	—	80
7	0	0	5	—	90
8	0	—	20	40	0
9	0	0	0	—	70
10	0	0	—	—	90
11	0	0	0	20	80
12	0	0	5	50	70
13	0	0	0	—	80
14	0	—	—	70	50
15	0	0	—	—	90
16	0	—	0	—	100
17	0	—	10	40	50
18	0	—	0	—	50
19	0	0	5	10	80
20	0	—	0	—	30
21	0	0	20	—	60
22	0	—	0	—	50
Mean (SD)	0 (0)	<1 (3)	8 (12)	41 (25)	69 (24)
Median (quartiles)	0 (0–0)	0 (0–0)	5 (0–10)	40 (20–55)	80 (50–90)

[a]Numbers are percentages of KLK7-positive cells. Numbers in the same row are from the same full section (identified by case number). Every full section had a normal epithelium, a PDAC, and at least one of the 3 severity gradings of PanIN indicated by the column headings.

also significant ($P = 0.032$) under multivariate analysis adjusting for disease group, age, and stage (Fig. 3D).

Discussion

Human KLKs comprise a group of 15 structurally homologous trypsin or chymotrypsin-like serine proteases encoded by a cluster of protease genes on chromosome 19q13.4 (24). Kallikreins have been implicated in a wide range of normal physiologic processes including regulation of blood pressure, tissue remodeling, prohormone processing, and skin desquamation. In many cancer types, aberrant KLK expression is thought to play a role in cancer cell growth, angiogenesis, invasion, and metastasis (reviewed in refs. 25, 26). Because of their widespread expression in various cancers, the potential role of KLKs as biomarkers for screening, diagnosis, prognosis, or monitoring response to cancer treatment has been intensively investigated in recent years (27, 28). The use of KLKs as biomarkers is highlighted by the use of KLK3, commonly known as prostate-specific antigen, for prostate cancer management (29).

Our previous observation of KLK7 expression in ductal adenocarcinomas prompted us to assess in this report the expression of KLK7 during the progression from intraepithelial neoplasia toward invasive cancer. KLK7 expression was detected with increasing frequency in lesions from PanIN 1 to invasive adenocarcinoma and the percentage of KLK7-positive cells increased with disease severity. There were no KLK7-positive cells observed in the normal epithelium and very few KLK7-positive PanIN 1 lesions. KLK7-positive cells, however, became readily apparent in many PanIN 1B lesions and increased to 41% of PanIN 2/3 lesions and 69% of PDAC examined. It is noteworthy that similarly increased KLK7 staining was observed by Termini and colleagues in more advanced cervical carcinomas, particularly among adenocarcinoma specimens (30). Compared with other molecular abnormalities analyzed in PanINs (31), expression of KLK7 could be classified as an intermediate-to-late change with a pattern of expression reminiscent of cyclin D1, MUC1, or 14-3-3σ. In a series of *in vitro* experiments, we have previously shown that E-cadherin can be cleaved from the surface of cultured pancreatic cancer cells by KLK7 (10). β-Catenin is sequestered at the plasma membrane through its interaction with E-cadherin and cleavage of the extracellular domain of E-cadherin has been

Table 2. Characteristics of 124 patients with PDAC analyzed from SEER TMA

Characteristic and component groups	Group subtotals, N (%)[a]	KLK7-positive tumors, N (%)[b]	KLK7-negative tumors, N (%)[b]	χ^2 *P*[c]	Median (quartiles) OS[d], mo	Log-rank *P*[e]
Age						
<55	22 (18)	12 (55)	10 (45)	0.67[§]	10.5 (2–53)	0.043[§]
55–64	31 (25)	16 (52)	15 (48)		8 (3–16)	
65–74	37 (30)	24 (65)	13 (35)		9 (5–20)	
≥75	34 (27)	18 (53)	16 (47)		3.5 (2–10)	
Gender						
Male	58 (47)	37 (64)	21 (36)	0.12[†]	5 (2–14)	0.042[†]
Female	66 (53)	33 (50)	33 (50)		9.5 (4–21)	
Histopathologic grade						
Poorly differentiated	20 (16)	7 (35)	13 (65)	0.035[†]	7.5 (2–13)	0.10[†]
Moderately/well-differentiated	104 (84)	63 (61)	41 (39)		6.5 (3–21)	
Stage						
Localized	62 (50)	42 (68)	20 (32)	0.011[†]	14 (7–28)	<0.0001[†]
With metastases, regional or distant	62 (50)	28 (45)	34 (55)		3 (1–6)	
Disease group						
Resected primary	48 (39)	33 (69)	15 (31)	0.0002[‡]	21 (8.5–33.5)	<0.0001[‡]
Unresected[f] primary	32 (26)	23 (72)	9 (28)		6 (3–11)	
Unresected[f] metastatic	44 (35)	14 (32)	30 (68)		3 (1–6.5)	

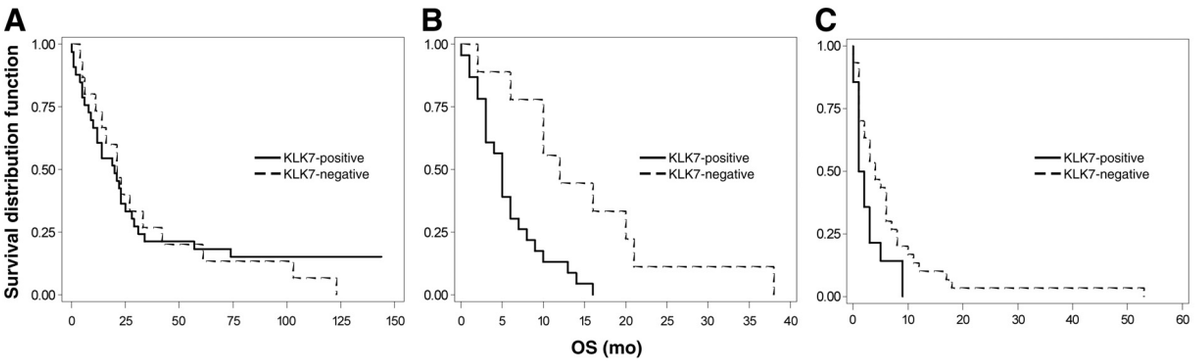
[a]Percentage of 124 total.
[b]Percentage of the group subtotal.
[c]*P* value from the χ^2 test with [†]1, [‡]2, or [§]3 degrees of freedom.
[d]OS in months after diagnosis.
[e]*P* value from the log-rank test with [†]1, [‡]2, or [§]3 degrees of freedom.
[f]Unresected or unknown.

associated with increased β-catenin nuclear accumulation and lymphocyte enhancer factor/T-cell factor (LEF/TCF)-mediated transcription, including cyclin D1 (32). Thus, expression of KLK7 in PanINs could play an indirect role in enhancing cell proliferation via shedding E-cadherin; however, the function of this protease in early preneoplastic events remains to be established.

When KLK7 expression was examined in 124 PDAC specimens, a significantly higher percentage of tumors graded as moderately or well-differentiated displayed KLK7-positive staining compared with those graded as poorly differentiated. Interestingly, when KLK7 mRNA expression was analyzed in a series of ovarian tumors, it was significantly higher in patients with high-grade (G3) tumors (13), whereas in patients with PDAC, increased KLK7 expression was associated with lower grade moderate/well-differentiated tumors.

Analysis of OS among patients with KLK7-positive tumors was significantly different compared with OS of patients with KLK7-negative tumors, except among patients whose primary tumors had been resected. Among patients with unresectable disease, those with KLK7-positive tumors had significantly shorter survival than those with KLK7-negative tumors. However, among patients with resected primary disease, there was very little difference in OS between KLK7 groups. A similar phenomenon of prognostic associations within a clinical subgroup has been reported for ovarian cancer, in which the OS of patients with KLK7-positive tumors was not significantly different compared with patients with KLK7-negative tumors overall, but significantly reduced when a subgroup of ovarian cancer patients, those with low-grade tumors, was analyzed (13). In studies of breast (16), colorectal (33–35), and intracranial (36) tumors, KLK7 expression was shown to be significantly associated with reduced OS. Similarly, in oral squamous cell carcinoma, patients with intense staining for KLK7 (as well as KLK4) had significantly shorter OS (37). Thus, although there are differences amongst the various cancer types, it is apparent that aberrant KLK7 expression contributes to a significant increase in patient mortality.

On the basis of the *in vitro* studies that have established direct and indirect involvement of KLK7 in processes that facilitate tumor cell invasion and metastasis in pancreatic adenocarcinoma, the association between KLK7 positivity and poor prognosis in patients with unresected pancreatic adenocarcinoma may be readily understood. It was noted in the analysis of full tissue



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Disease groups	Subgroup analyses[a]		Two-factor analysis[b]		Multivariate analysis[c]	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
A: Resected primary	0.94 (0.50–1.76)	0.8454	0.94 (0.50–1.78)	0.8486	0.88 (0.45–1.72)	0.7116
B: Unresected primary	4.08 (1.53–10.90)	0.0051	2.96 (1.29–6.80)	0.0104	3.52 (1.48–8.38)	0.0045
C: Unresected metastatic	1.99 (1.02–3.90)	0.0440	2.70 (1.28–5.73)	0.0093	2.48 (1.26–4.87)	0.0086

Figure 3. Effect of KLK7 positivity on OS in PDAC. Kaplan–Meier curves of KLK7 effect in (A) resected primary, (B) unresected primary, and (C) unresected metastatic disease. Solid curves, KLK7-positive; dashed curves, KLK7-negative. D, strength of KLK7 effect in disease groups. [a]Separate analyses of KLK7 association with OS subgrouped by disease group. HRs are the unadjusted HRs for the Kaplan–Meier curves shown in A–C. [b]Two-factor analysis of KLK7 association with OS adjusting for disease group. The change in the KLK7 association between disease groups was statistically significant (KLK7–disease interaction: $\chi^2 = 6.39$, df = 2; $P = 0.041$). [c]Multivariate analysis of KLK7 association with OS adjusting for disease group, stage, and age. The change in KLK7 association between disease groups was statistically significant (KLK7–disease interaction: $\chi^2 = 6.89$, df = 2; $P = 0.032$).

sections that KLK7 positivity was accentuated at the invasive adenocarcinoma front. The proteolytic activity of KLK7 may facilitate tumor cell invasion and metastasis by altering cell–cell interactions by cleaving E-cadherin (10), urokinase-type plasminogen-activator receptor (38), and desmoglein-2 (39); as well as by directly degrading components of the extracellular matrix (e.g., fibronectin; ref. 40) or activating other matrix-degrading proteases (e.g., matrix metalloproteinases-9; ref. 41). Thus, its aberrant expression in invasive adenocarcinomas can be readily appreciated, particularly in patients with more advanced, unresectable disease.

The association of KLK7 expression and poor outcome of patients with unresectable PDAC suggests that inhibiting either KLK7 expression and/or activity could be a potential therapeutic target. Because the vast majority of patients present with unresectable disease, such an intervention could thus have a significant impact upon the OS of this patient population.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: V. Iakovlev, R.S. Haun
Development of methodology: V. Iakovlev, M.-S. Tsao, R.S. Haun
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): V. Iakovlev, R.S. Haun
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): V. Iakovlev, E.R. Siegel, M.-S. Tsao
Writing, review, and/or revision of the manuscript: V. Iakovlev, E.R. Siegel, M.-S. Tsao, R.S. Haun
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M.-S. Tsao, R.S. Haun
Study supervision: M.-S. Tsao, R.S. Haun

Acknowledgments

The authors thank Drs. Sean Altekruse (NCI, SEER Program) and Stephen Hewitt (NCI, Tissue Array Research Program) for helpful discussions concerning the pancreatic TMA and analyses.

Grant Support

This work was supported in part by the Department of Veterans Affairs, Veterans Health Administration, Office of Research and Development, Biomedical Laboratory Research and Development, VA Merit Award 01BX000828-01A2 (to R.S. Haun), and grant #700809 from the Canadian Cancer Society (to M.-S. Tsao).

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Received November 16, 2011; revised April 17, 2012; accepted April 25, 2012; published OnlineFirst May 9, 2012.

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