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# Association of prion protein expression with pancreatic adenocarcinoma survival in the SEER residual tissue repository

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**Abstract**. Pancreatic ductal adenocarcinoma (PDAC) is an important cause of cancer death with no clear prognostic biomarker. Expression of prion (PrP) has been reported to be a marker of poor prognosis in a series of Caucasian PDAC cases. We determined the prognostic value of PrP in a racially and geographically diverse population-based series of PDAC cases. PrP expression was examined in 142 PDAC cases from three cancer registries. Cases included 71 Caucasian, 54 Asian/Pacific Islanders and 17 Blacks diagnosed from 1983–2000, and followed through 2008. Hazard ratios (HR) and 95% confidence intervals (CIs) for the association of PrP expression with survival were computed after adjustment for case attributes. The risk of death was about four times higher (HR = 3.8; 95% CI: 2.2, 6.5) among 108 PDAC cases with $PrP^+$ tumors (median survival 5 months) compared to the 34 cases with $PrP^-$ tumors (median survival 20 months). Of 51 cases with resected, localized PDAC median survival was 74 months for 17 cases with $PrP^-$ tumors versus 14 months for 34 cases with $PrP^+$ tumors (HR = 6.7; 95% CI: 2.6, 17.4). All 6 surviving cases had $PrP^-$ negative tumors (median survival, $>$ 10 years). PrP may have potential as a prognostic biomarker in PDAC patient management.

Keywords: Pancreas, adenocarcinoma, prion, prp, prognosis, immunohistochemistry, biomarker

**Abbreviations**

| | |
|---|---|
| CI | confidence interval; |
| FLNA | filamin A; |
| GPI | glycosylphosphatidylinositol; |
| HR | hazard ratio; |
| PDAC | pancreatic ductal cell adenocarcinoma; |
| PrP | prion protein; |
| PI | Pacific Islanders; |
| REMARK | recommendations for tumor marker prognostic studies |
| RTR | Residual Tissue Repository; |
| SEER | Surveillance, Epidemiology, and End-Results; |
| TMA | tissue microarray. |

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## 1. Background

Pancreatic cancer is the fourth leading cause of cancer death in the United States [1], and pancreatic ductal

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adenocarcinoma (PDAC) is the most common histologic type of pancreatic cancer. While both incidence and mortality trends are increasing, causes remain unknown, and progress is needed in the identification of valid early detection and prognostic markers for this fatal disease [2–4]. In 2012, pancreatic cancer was the cause of approximately 37,390 deaths [5], and the most recent 5-year relative survival was 5.6% [1]. Promising biomarkers of survival include BAX, Bcl-2, survivin, Ki-67, COX-2, E-cadherin and S100 calcium-binding proteins, but molecular marker assessment remains unincorporated within PDAC management algorithms because of lack of validation [4,6].

Prion protein has been shown to be expressed in human pancreatic cancer cell lines [7] and was associated with poor PDAC prognosis in a hospital-based study of PDAC cases at University Hospital, Cleveland [8]. Recent studies revealed that PrP was also detected in human melanoma, and may contribute to its progression [9]. Based on these findings, it has been proposed that PrP alters tumor biology, consequentially contributing to the aggressiveness of the tumor cells [8–10]. A limitation of the previous study of PrP and PDAC [7] was the homogeneous ethnic and racial composition of cases and its hospital-based design. The objective of the present report was to validate the prognostic significance of PrP in Residual Tissue Repository (RTR) specimens from three racially and geographically diverse Surveillance, Epidemiology, and End-Results (SEER) cancer registries covering Los Angeles County, Iowa, and Hawaii [11]. Formalin-fixed, paraffin-embedded tumor specimens were retrospectively obtained for all cases, along with SEER demographic, clinical and vital status information.

## 2. Methods

### 2.1. Tissue microarray

A single block tissue microarray (TMA) containing 161 pancreatic cancer tissues from three SEER registries was built by the NCI Tissue Array Research Program in Bethesda, MD [12]. These cases were a subset of 13,650 cases reported by source registries. The inclusion criterion was epithelial tumor of the pancreas, with sufficient material for construction of the TMA. The design, construction, histological evaluation and selection criteria of the TMA have been described in great detail [12]. The TMA also contains 15 cores of normal tissues from the matching pancreatic tumors as well as a selection of 15 adjacent benign tissues at other site. This study focused on 142 confirmed PDAC cases that were alive at time of diagnosis and were diagnosed with only one primary cancer. Cases were diagnosed between 1983 and 2000. This included 71 Whites (50%), of which five were Hispanic, 54 Asian/Pacific Islanders (38%) and 17 Blacks (12%). Based on active follow-up, 128 cases (90%) died of cancer, 8 died of other or unspecified causes, and the remaining 6 were alive in 2008.

### 2.2. Immunohistochemistry

The anti-PrP monoclonal antibody, 8H4, used in the study has been extensively characterized [8,13,14]. In normal human pancreas, 8H4 reacts with islet cells but does not react with either ductal cells or acinar cells [7]. Immunohistochemical staining (Mab 8H4, 2.5 $\mu$g/ml) was performed in a Leica Bond-III® system (Bannockburn, IL). The two slides comprising the TMA were stained on different days. A citrate buffer (pH 5.5) was used for antigen retrieval. The TMA slides were blindly evaluated by two independent pathologists (W.X and L.Z.), and scored as follows: 0, between 0–5% tumor cell stained (negative); 1, 6–25% tumor cells stained (marginal to weak); 2, 26–50% tumor cells stained (moderate); 3, 51–75% tumor cells stained (strong); and 4, $>$ 76% tumor cells stained (intense). When differences occurred in scoring between pathologists, cases were re-evaluated. The consensus score was the mean of two readings. Out of the 142 PDAC cases, only scores from 6 cases needed re-evaluation.

### 2.3. Statistical analysis

Kaplan-Meier survival curves were used to compare survival months from diagnosis (Proc Lifetest, SAS v.9, Cary, NC) [15]. The log-rank test was used to compare Kaplan-Meier survival for tumors by biomarker and history of resection. Cox proportional hazards models that examined survival in months from diagnosis were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for death from pancreatic cancer associated with PrP expression (Proc Phreg, SAS v.9, Cary, NC) [16]. PrP scores were assigned into two categories, scores of 0 (no staining, $n = 14$) and 1 (weak, $n = 20$) were classified as negative while scores of 2 (moderate, $n = 42$), 3 (strong, $n = 42$) and 4 (very strong, $n = 24$) were classified as positive. Stepwise model selection was used to build a parsimonious model consisting of sex, age ($<$ 75 versus

75+ years), Asians/Pacific Islanders versus other racial groups, SEER summary stage at diagnosis (local, regional, distant, unstaged or unknown), calendar period at diagnosis (1983–1991 versus 1992–2000), cancer registry source, primary or metastatic site and reported surgical resection. The interaction of PrP with various demographic and clinical variables on the risk of death was assessed through cross product terms. Final models excluded interaction terms for PrP and Asian/Pacific Islander race ($P = 0.08$), PrP and stage ($P = 0.73$) or PrP and resection ($P = 0.15$) because these terms did not substantially improve the fit of the data.

### 2.4. Assurances

The study (IRB# EM-10-13) was approved by the Center for Clinical Research and Technology at the University Hospital, Case Medical Center and the University of Hawaii Committee on Human Studies.

## 3. Results

### 3.1. Case characteristics

Demographic and clinical data for the 142 PDAC cases in the tissue microarray are summarized in Table 1. Hawaii contributed 52% of cases, Iowa provided 22% of cases and Los Angeles accounted for 26% of cases. Most cases were diagnosed from 1992 through 2000 and were about evenly distributed by sex. One half of the cases were Caucasian, with the remainder Asian/Pacific Islander or Black. The majority of cases were diagnosed at localized or regional stage, but 20% were missing stage or unstaged at diagnosis. More than a third of cases contributed metastatic tumor tissues, and 42% underwent tumor resection. Overall, 108 of 142 (76%) cases had PrP scores greater than 1, referred to hereafter as positive. The median survival time from diagnosis to death was 7 months.

Table 1
Demographic and clinical attributes of 142 pancreatic ductal adenocarcinoma cases

| Attribute | Category | Number | Percent |
|---|---|---|---|
| Registry | Hawaii | 74 | 52% |
| | Iowa | 31 | 22% |
| | Los Angeles | 37 | 26% |
| Diagnosis years | 1983–1991 | 44 | 31% |
| | 1992–2000 | 98 | 69% |
| Gender | Male | 72 | 51% |
| | Female | 70 | 49% |
| Race | White | 71 | 50% |
| | Asian/PI† | 54 | 38% |
| | Black | 17 | 12% |
| Age at diagnosis | < 55 years | 24 | 17% |
| | 55–64 years | 39 | 27% |
| | 65–74 years | 43 | 30% |
| | 75–84 years | 32 | 23% |
| | 85+ years | 4 | 3% |
| Stage | Localized | 15 | 11% |
| | Regional | 58 | 41% |
| | Distant | 41 | 29% |
| | Unstaged/Missing | 28 | 20% |
| Tumor source | Primary site | 89 | 63% |
| | Metastasis | 53 | 37% |
| Reported resection | Yes | 59 | 42% |
| | No | 83 | 58% |
| Median survival | Months | 7 | 100% |
| All cases | Total | 142 | 100% |

†PI = Pacific Islander.

### 3.2. PrP expression and prognosis

Representative images of PrP immunohistochemical staining results with different scores are shown in Fig. 1. The PrP positive group had a median survival time of 5 months while the PrP negative group had a median survival time of 20 months (Table 2). In a proportional hazard model that adjusted for age, resection, registry and tumor sources, gender, race, stage and diagnosis year (Table 2), a statistically significant elevation in risk of death (HR = 3.8, 95% CI: 2.2 to 6.5) was observed among cases with tumors showing strong PrP expression compared to cases with tumors expressing weak or no PrP. A Kaplan-Meier survival curve comparing these two groups is shown in Fig. 2 (Log-Rank test, equality over strata, $P < 0.0001$).

### 3.3. Stratification by resection and primary versus metastatic origin

In total, 59 cores (42%) were from reported resected tumors, while the remaining 83 cores were from cases without reported resected tumors (Table 3). When the adjusted proportional hazard model described above was restricted to cases with resected tumors, the risk of death among the 42 cases with PrP-positive resected tumors was 7.1 (95% CI: 2.7 to 18.9) compared to the 17 cases with PrP-negative resected tumor (Table 3). By contrast, the risk of death among the 66 PrP-positive cases without reported tumor resection was 2.8 (95% CI: 1.4 to 5.5) compared to the 17 cases with PrP-negative tumors. The test for heterogeneity of HRs by resection status was statistically significant ($P < 0.0001$).

The 51 cases that were diagnosed with localized or regional stage PDAC with reported tumor resections

Table 2
Proportional hazard model, 142 pancreatic duct adenocarcinoma cases (main effect)

| Variable | Category | No. | Survival (mos) | Hazard ratio** | 95% CI† |
|---|---|---|---|---|---|
| PrP expression* | Positive | 108 | 5 | 3.8 | (2.2, 6.5) |
| | Negative | 34 | 20 | 1.0 | Referent |
| Age group | < 75 | 106 | 9 | 0.7 | (0.4, 1.0) |
| | 75+ | 36 | 5 | 1.0 | Referent |
| Reported resection | Yes | 59 | 21 | 0.3 | (0.2, 0.5) |
| | No | 83 | 5 | 1.0 | Referent |
| Registry source | Hawaii | 74 | 15 | 0.3 | (0.1, 0.6) |
| | Iowa | 31 | 5 | 0.9 | (0.4, 1.8) |
| | Los Angeles | 37 | 3 | 1.0 | Referent |
| Tumor source | Primary Site | 89 | 12 | 0.7 | (0.4, 1.1) |
| | Metastasis | 53 | 4 | 1.0 | Referent |
| Gender | Female | 70 | 9 | 0.9 | (0.6, 1.3) |
| | Male | 72 | 5 | 1.0 | Referent |
| Race | Asian/PI‡ | 54 | 11 | 1.4 | (0.9, 2.2) |
| | Other Races | 88 | 5 | 1.0 | Referent |
| Stage | Unstaged/Blank | 28 | 4.5 | 0.7 | (0.2, 2.0) |
| | Distant | 41 | 3 | 1.2 | (0.6, 2.7) |
| | Regional | 58 | 13.5 | 1.0 | (0.5, 1.9) |
| | Localized | 15 | 20 | 1.0 | Referent |
| Diagnosis year | 1992–2000 | 98 | 10 | 0.6 | (0.4, 1.1) |
| | 1983–1991 | 44 | 5 | 1.0 | Referent |

*Negative was defined as no or weak PrP expression, scores of 0 and 1. Positive was defined as moderate to strong PrP expression, scores of 2, 3, and 4.
**This model adjusted for sex, age, race, stage, diagnosis period, registry, primary or metastatic source and reported resection.
†CI=confidence interval; ‡PI=Pacific Islander.

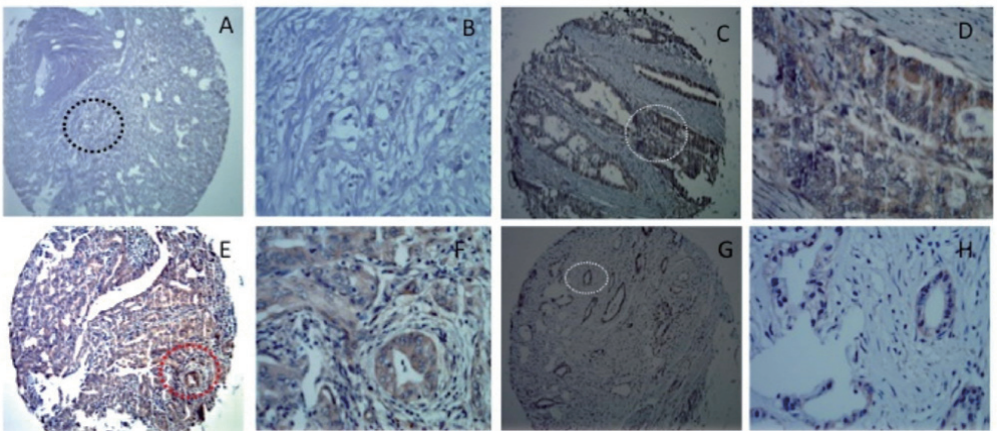


Fig. 1. Immunohistochemical detection of PrP in the pancreatic cancer TMA. A. A representative image of a PrP negative case. × 20. B. A higher magnification image of the circled area in image A. × 40. C. A representative image showing PrP staining with a score of 4. × 20. D. A higher magnification image of the circled area in image C. × 40. E. A representative image showing PrP staining with a score of 3. × 20. F. A higher magnification image of the circled area in image E. × 40. G. A representative image showing PrP staining with a score of 2. × 20. H. A higher magnification image of the circled area in image G. × 40.

were further examined. In this group, the 17 cases (33%) who were PrP negative had a median survival of at least 74 months versus 14 months for the 34 cases (67%) with PrP positive tumors (adjusted HR = 6.7, 95% CI: 2.6 to 17.4; Table 3). All 6 surviving cases had PrP negative tumors resected at localized or regional stage (median survival as of 2008: 10 years). A Kaplan-Meier survival curve comparing the two groups is shown in Fig. 3 (Log-Rank test, $P < 0.0001$). Eight cases with distant or unstaged PDAC were reported to have received resections. None of these cases had tumors with weak or no PrP expression.

Sensitivity analyses were performed with 89 non-metastatic tumors and 53 tumors of metastatic origin.

Table 3
Stratified proportional hazard models for pancreatic ductal adenocarcinoma cases

| Stratum | PrP expression* | No | Survival (Months) | Adjusted hazard ratio† | 95% CI† |
|---|---|---|---|---|---|
| Resected tumors | Positive | 42 | 13 | 7.1 | (2.7, 18.9) |
| | Negative | 17 | 74‡ | 1.0 | Referent |
| Non-resected tumors | Positive | 66 | 3 | 2.8 | (1.4, 5.5) |
| | Negative | 17 | 10 | 1.0 | Referent |
| Resected, Local/Regional stage | Positive | 34 | 14 | 6.7 | (2.6, 17.4) |
| | Negative | 17 | 74‡ | 1.0 | Referent |
| Resected, distant stage | Positive | 8 | 10 | – | – |
| | Negative | 0 | – | Undefined | Referent |
| Non-metastatic tumors | Positive | 67 | 8 | 3.8 | (1.9, 7.7) |
| | Negative | 22 | 28 | 1.0 | Referent |
| Metastatic tumors | Positive | 41 | 3 | 4.6 | (1.7, 12.3) |
| | Negative | 12 | 9 | 1.0 | Referent |

*Negative was defined as no or weak PrP expression, scores of 0 and 1. Positive was defined as stronger PrP expression, scores of 2, 3, and 4;
†Models adjusted for sex, age, race, stage, diagnosis period, registry, primary or metastatic source and reported resection;
‡All 6 surviving cases had PrP negative tumor resected at localized or regional stage (median survival as of 2008: 10 years).

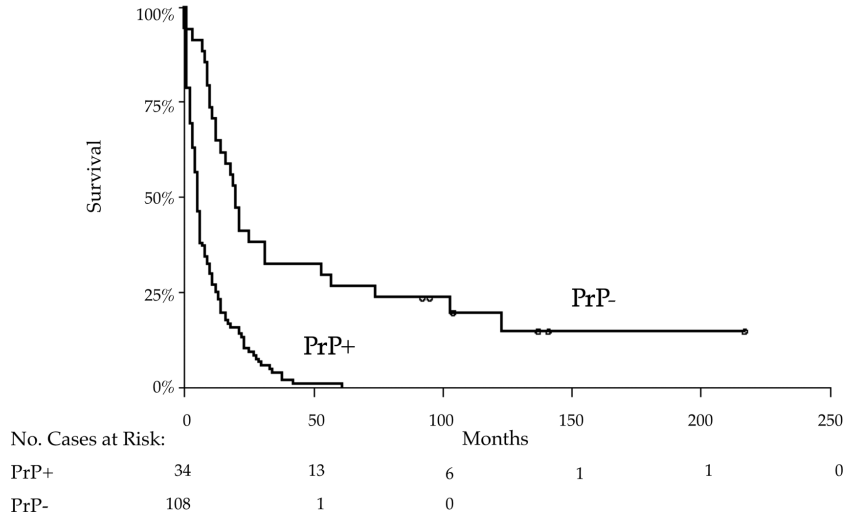


Fig. 2. Kaplan-Meier Survival Curves for 142 PDAC by PrP expression. Comparing PrP negative group (0 and 1, $n = 34$) with PrP positive group (2, 3, and 4; $n = 108$) of all PDAC cases in the TMA. Survival time for six cases alive at last follow-up in 2008 depicted by circles. (Log-Rank test, $P < 0.0001$).

When the proportional hazard models were restricted to cases with non-metastatic tumors, the adjusted HR was 3.8 (95% CI: 1.9 to 7.7) while that for metastatic tumors only was 4.6 (95% CI: 1.7 to 12.3). The test for heterogeneity of HRs by primary versus metastatic tumor site was not statistically significant ($P = 0.14$).

## 4. Discussion

Data from the SEER Residual Tissue Repository validate our previous observation that PrP expression is a marker of poor prognosis in PDAC [8]. In the present study, more than half the PDAC cases from the three population-based registries were non-white, supporting the broad utility of PrP as a prognostic tool. Confirmation of the prognostic value of PrP expression is noteworthy because pancreatic cancer is a leading and increasing cause of cancer death in the United States [1,5]. In addition, few biomarkers have demonstrated prognostic value for this highly fatal cancer [2–4].

In the present study, PrP negative resected tumors carried a more favorable prognosis than non-resected tumors. Because non-resected tumors are more likely to be diagnosed at a later stage than resected tumors, PrP expression could have been a proxy for tumor progression. Indeed, we observed a higher proportion of

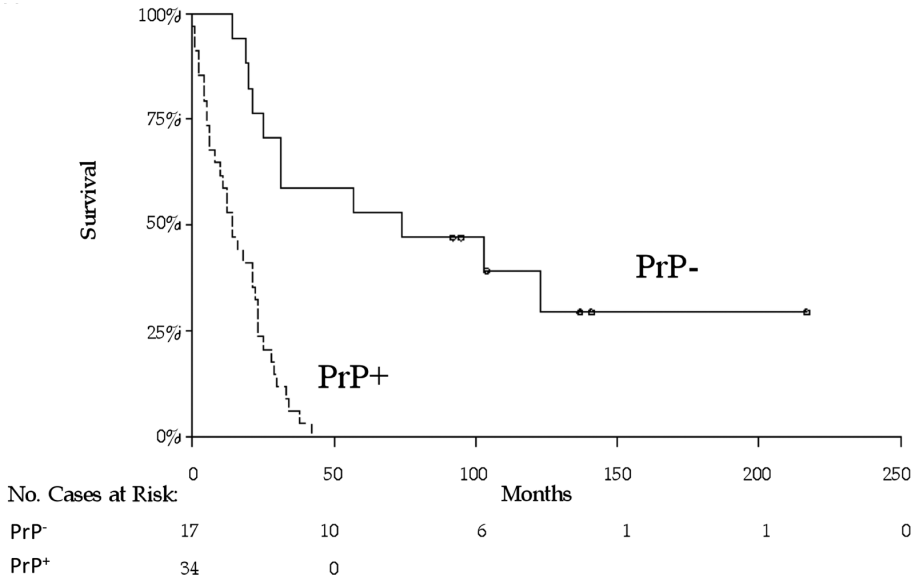


Fig. 3. Kaplan-Meier Survival Curves, 51 cases with resected, localized and regional stage pancreatic tumors. Dashed line represents PrP positive tumors, solid line represent PrP negative tumors. Circular markers denote latest follow-up time of six cases that were alive at last follow-up in 2008. (Log-Rank test, $P < 0.0001$).

PrP positive tumors among non-resected cases (80%) than among resected cases (71%). In a sub-analysis of cases with resected tumors who were diagnosed at either localized or regional stage we found that cases with PrP positive tumor had significantly shorter survival times (14 months) than cases with PrP negative tumors ($\geqslant$ 74 months), confirming the utility of PrP as a marker of poor PDAC prognosis independent of stage and resection status.

Ductal adenocarcinoma (PDAC) is by far the most common histological type of human pancreatic cancer [1,2]. We have previously reported that human PDAC cell lines express high levels of prion protein (PrP) without the normal glycosylphosphatidylinositol (GPI)-anchor [8–10]. In PDAC the PrP protein exists as pro-PrP, retaining the GPI-anchor peptide signal sequence, which contains a filamin A (FLNA)-binding motif. FLNA is an integrator of cell mechanics and signaling that links cell surface receptors, such as integrins to actin filaments to maintain membrane integrity, cell-cell, and cell-matrix interactions [10]. In normal human pancreas, only islet cells have detectable PrP. PrP is also undetectable in chronic pancreatitis, or in precancerous pancreatic intraepithelial (PanIN) lesions, such as PanIN-1, and PanIN-2. Expression of pro-PrP in tumors has been associated with a marked decrease in survival time [8]. Other studies also detected pro-PrP in human melanoma, with invasive melanoma expressing higher levels of PrP [9]. We have proposed that binding of pro-PrP to FLNA may perturb FLNA function, contributing to tumor cell aggressiveness [8–10].

The Residual Tissue Repository has several strengths including the population-based survival cohort design [11]. Cohort members are identified though the population-based registers of three Surveillance, Epidemiology, and End-Results (SEER) cancer registries covering Los Angeles County, Iowa, and Hawaii. The repository is both large and ethnically diverse, reflecting appreciable numbers of non-whites in the catchment area populations [4,12]. In the assessment of prognostic biomarkers, a population-based design has several advantages compared with hospital-based studies, not the least of which is the ready assessment of the representativeness of cases compared to the source population [11]. Cases included in the PDAC tissue microarray had a similar gender distribution to all cases from the three population-based registries, but were somewhat younger with a tendency to be diagnosed at a less advanced stage [12]. In adherence with reporting recommendations for tumor marker prognostic studies (REMARK) guidelines [6], assay methods were reported and multivariable analyses were performed with annotated tumors based on demographic and clinical annotation including survival time from diagnosis. Limitations included less than optimal statistical power, particularly in stratified analyses. In addition, the collection of paraffin-embedded tissue blocks that were obtained from pathology laboratories in registry

areas had little information regarding preservation and storage methods.

In conclusion, PrP expression is a promising prognostic marker for pancreatic adenocarcinoma, a malignancy with poor survival. Further studies that meet all REMARK reporting guidelines should evaluate PrP and other promising biomarkers of potential prognostic value from well-annotated tissue banks nested in defined populations, such as the RTR. Of particular interest is whether PrP expression in early stage PDAC could identify a subset of more aggressive tumors. Such information would be of important assistance to pathologists and oncologists in targeting personalized treatment for this highly fatal cancer.

## Conflicts of interest

No author declares a conflict of interest.

## Author contributions

M-S.S. conceived the experiment, participated in data analysis and interpretation, and wrote the first draft of the manuscript; S.A. participated in the creation the TMA database, statistical analysis, interpretation and presentation of results, writing and revision of the manuscript; C.Y, performed the immunohistochemical staining and data analysis; C.F.L., M.T.G., B.Y.H. and M.S.S participated in the creation of the TMA and associated data annotation, analyzing the data, and writing the manuscript; X.H and W. X. performed the immunohistochemical staining and scored the staining results; S.M.H. created the TMA and helped with preparation of the manuscript.

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