Plasminogen Activator Inhibitor 1 as a Poor Prognostic Indicator in Resectable Pancreatic Ductal Adenocarcinoma

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Abstract

Background: Plasminogen activator inhibitor 1 (PAI-1) was previously established to impact several phenotypes in many kinds of cancer, including pancreatic cancer. However, its prognostic significance in pancreatic ductal adenocarcinoma (PDAC) needs support of further evidence. This study was designed to address the issue.

Methods: PAI-1 expression was detected by tissue microarray-based immunohistochemical staining in formalin-fixed paraffin-embedded specimens from 93 PDAC patients with surgical resection from September 2004 to December 2008. Its relationships with clinicopathologic variables and tumor-specific survival (TSS) were further evaluated using Chi-square, Kaplan-Meier, log-rank, as well as Cox regression analyses.

Results: Expression of PAI-1 was much higher in tumor than that in nontumor tissues, based on comparison of all samples and 74 matched ones (95 [47.5, 180] vs. 80 [45, 95], Z = −2.439, P = 0.015 and 100 [46.9, 182.5] vs. 80 [45, 95], Z = −2.594, P = 0.009, respectively). In addition, tumoral PAI-1 expression was positively associated with N stage (22/35 for N1 vs. 21/51 for N0, χ² = 3.903, P = 0.048). Univariate analyses showed that TSS of patients with high PAI-1 tumors was significantly poorer than that of those with low PAI-1 tumors (log rank value = 19.00, P < 0.0001). In multivariate Cox regression test, PAI-1 expression was identified as an independent predictor for long-term prognosis of resectable PDAC (hazard ratio = 2.559, 95% confidence interval = 1.499–4.367, P = 0.001).

Conclusion: These results suggest that expression of PAI-1 is upregulated in PDAC and might serve as a poor prognostic indicator.

Key words: Pancreatic Ductal Adenocarcinoma; Plasminogen Activator Inhibitor 1; Prognosis

Introduction

Pancreatic cancer (PC), especially its main histological type, pancreatic ductal adenocarcinoma (PDAC), has long been well known as one of the most lethal malignant neoplasms worldwide.[1] In China, its incidence and mortality rate have also been reported to be remarkably raised in recent years.[2] Up to now, its overall long-term prognosis remains dismal, although curative resection and adjuvant therapy have achieved some favorable effects in highly selected patients.[3–5] Therefore, survival-associated variables in patients with this malignancy caught much attention. The main identified ones were crucial clinicopathologic and surgical factors, including lymph node metastasis, tumor histology grade, peri-neural invasion, and resection margin.[6–9] On the other hand, data on prognostic biomarkers in PC, particularly for resectable patients, were also accumulated.[10,11] However, further candidates need to be supplemented.

In view of the fact that approximately 70% of PDAC patients died from extensive metastatic disease,[1] invasion-related molecules in this malignancy, such as those previously reported,[12–14] were of particular importance. Plasminogen activator inhibitor-1 (PAI-1), encoded by SERPINE1, is a
specific inhibitory member of plasminogen activation (PA) system.\[^{[15]}\] Except for its key role in acute thrombotic events, PAI-1 was also expected to prevent cancer invasion and metastasis through its inhibition of urokinase-type plasminogen activator, which was demonstrated to be involved in malignant dissemination.\[^{[15]}\] Indeed, there were some published articles showing this metastasis inhibitory effect.\[^{[16‑18]}\] However, more articles suggested its pro-oncogenic roles.\[^{[19‑24]}\] In PC/PDAC, different in vitro and in vivo experiments also got inconsistent, even opposite results.\[^{[17,19,23]}\] However, those for its prognostic significance in PDAC seem to be more consistent. It was shown that PAI-1 mRNA/protein overexpression tended to predict poor patient survival, but not being statistically significant.\[^{[25,26]}\] The smaller sample size and incomplete staining evaluation criteria (without staining intensity) might be the main limits of these investigations.

The aim of this study was to discover the clinicopathologic and prognostic implications of PAI-1 expression in PDAC, through improvements of aforementioned limits.

**Methods**

**Ethical approval**

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Peking Union Medical College Hospital (No. JS1178). Being a retrospective study and since data analysis was performed anonymously, this study was exempted from the informed consent from patients.

**Patients**

This study enrolled 93 resected patients with PDAC from September 2004 to December 2008. The inclusion criteria included: (1) without chemo-/chemo-radiation therapy before surgery; (2) underwent curative resection; and (3) histologically confirmed. Fifty-eight patients were male and 35 were female. The age ranged from 34 to 85 years (median: 62 years). The main clinical and pathologic variables of patients are summarized in Table 1.

**Tissue microarray construction**

The tissue microarray was constructed using formalin-fixed paraffin-embedded blocks. The construction method was same as our previous report.\[^{[27]}\]

**Immunohistochemistry**

A mouse monoclonal antibody for human PAI-1 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and a two-step staining kit (EnVision™ plus kit, Dako, Denmark) were used for staining. First, 4-µm-thick slides were mounted, deparaffinized, and rehydrated. After antigen retrieval in an autoclave, sections were incubated with 3% hydrogen peroxide (10 min) to block endogenous peroxidase. Then, slides were sequentially incubated with the primary antibody (dilution: 1: 50) overnight at 4°C and horseradish peroxidase-labeled secondary antibody (30 min). Diaminobenzidine was applied as a chromogen. Finally, those were counterstained with hematoxylin. Nonimmune mouse serum at the same dilution was used as the negative control.

**Staining evaluation**

Two experienced pathologists who had no clinicopathologic and follow-up information evaluated the sections, according to the H-score,\[^{[28]}\] a widely used immunostaining evaluation criterion.\[^{[29,30]}\] Similar to a previous study,\[^{[31]}\] the H-score with the largest Youden index (YI) within the receiver operating characteristic (ROC) curve for survival status was adopted as the cutoff value.

**Follow-up**

All patients accepted the postsurgical follow-up (range: 2–87 months; median: 11 months). A total of 61 patients (65.6%) died, and the other 32 (34.4%) were alive.

**Statistical analysis**

The H-scores of PAI-1 in tumor and nontumor tissues were compared using Mann-Whitney U-test. Chi-square test was applied to determine the relationships between PAI-1 expression and clinicopathologic variables. The prognostic significance of PAI-1 was explored by Kaplan-Meier method and log rank test. Univariate and multivariate

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**Table 1: Relationships between PAI-1 expression and clinicopathologic variables of resectable PDAC**

| Variables       | n   | PAI-1 expression, n | χ²   | P   |
|-----------------|-----|---------------------|------|-----|
|                 |     | High                | Low  |     |
| Gender          |     |                     |      |     |
| Male            | 58  | 30                   | 28   | 0.001 0.978 |
| Female          | 35  | 17                   | 18   |     |
| Age (years)     |     |                     |      |     |
| ≥62             | 47  | 24                   | 23   | 0.011 0.915 |
| <62             | 46  | 24                   | 22   |     |
| Tumor site      |     |                     |      |     |
| Head            | 57  | 30                   | 27   | 0.061 0.805 |
| Nonhead         | 36  | 18                   | 18   |     |
| Tumor size (cm) |     |                     |      |     |
| >4              | 54  | 27                   | 27   | 0.016 0.899 |
| ≤4              | 37  | 19                   | 18   |     |
| Histological grade |  |                    |      |     |
| G1–G2           | 64  | 33                   | 31   | <0.001 0.988 |
| G3–G4           | 29  | 15                   | 14   |     |
| T stage         |     |                     |      |     |
| T1–T2           | 71  | 39                   | 32   | 2.479 0.115 |
| T3              | 20  | 7                    | 13   |     |
| N stage         |     |                     |      |     |
| N0              | 51  | 21                   | 30   | 3.903 0.048 |
| N1              | 35  | 22                   | 13   |     |

Partial data were not available, and statistics were based on available data. P values were derived from the Pearson’s Chi-square test (two tailed).
Cox regression (proportional hazard model) analysis was adopted for prognostic factor identification. Statistical software package Statistical Package for the Social Sciences version 11.5 (SPSS Inc., Chicago, IL, USA) was employed for all the analyses. A statistical significance was defined when $P < 0.05$.

**RESULTS**

**Expression pattern of plasminogen activator inhibitor 1 in resectable pancreatic ductal adenocarcinoma**

As shown in Figure 1a and 1b, the positive staining of PAI-1 was mainly located in the cytoplasm of both tumor and nontumor tissues. In all patients and 74 with matched tumor and nontumor samples, the H-scores in tumor tissues were much higher than those in nontumor ones (95 [47.5, 180] vs. 80 [45, 95], $Z = -2.439, P = 0.015$ and 100 [46.9, 182.5] vs. 80 [45, 95], $Z = -2.594, P = 0.009$, respectively) [Figure 1c and 1d].

**Prognostic value of plasminogen activator inhibitor 1 in resectable pancreatic ductal adenocarcinoma**

In the first, the H-score value of PAI-1 (cutoff value: 94.375) with the largest Y1 in the ROC curve for survival status was selected as the cutoff one [Figure 2a]. In contrast to patients’ low tumoral PAI-1 expression, those with high PAI-1 expression carried significantly poorer tumor-specific survival (TSS) [$\chi^2 = 19.00, P < 0.0001$].

**Relationships of plasminogen activator inhibitor 1 with clinicopathologic variables of resectable pancreatic ductal adenocarcinoma**

Using Chi-square analysis, high tumoral PAI-1 expression, based on the same cutoff value, was associated with N1 stage [22/35 for N1 vs. 21/51 for N0, $\chi^2 = 3.903, P = 0.048$, Table 1]. No significant associations between PAI-1 expression and other clinicopathologic parameters were found [$P > 0.05$, Table 1].

**Identification of prognostic indicators in resectable pancreatic ductal adenocarcinoma**

It was revealed by univariate Cox regression analysis that histological grade, N stage, and PAI-1 expression were predictors of TSS [$P < 0.05$, Table 2]. Multivariately, these factors remained to be significant, thus being independent prognostic determinants [$P < 0.05$, Table 2].

**DISCUSSION**

It was long unexpectedly found that PAI-1 promoted many malignant behaviors of several kinds of cancer cells,[19-24] although it is one of the specific inhibitory members of PA system and was thus thought to inhibit cancer invasion/metastasis.[16-18] Therefore, its biological roles in cancer might vary largely. In PC/PDAC, previous articles revealed that high PAI-1 expression at mRNA and protein levels all tended to be associated with poor patient survival,[25,26] unlike inconsistent data from in vitro and in vivo experiments.[17,19,23] The possible reasons of imperfect statistical results might be attributed to smaller sample size (46 cases)[26] and defective staining evaluation criteria (absence of staining intensity).[25] In the present study, we included relatively more patients and used the H-score that considered both positive cell ratio and staining intensity[20] and wished to make it more comprehensive and reliable than aforementioned ones.[25,26] Our data showed that PAI-1 expression was remarkably higher in tumor than that in nontumor tissues of PDAC, based on the comparison of all samples and 74 matched ones. Moreover, PAI-1 expression positively correlated with N stage, an important factor that reflects tumor cell dissemination and predicts dismal prognosis in PC.[6,7] similar with evidence from other cancers.[32,33] Thus, the data preliminarily provide the histological evidence of PAI-1 as a proto-oncogene in PDAC. However, detailed mechanistic explorations remain to be in need.

More importantly, the prognostic value of PAI-1 expression remains to be elucidated in PDAC, on the basis of previous clues.[25,26] Using H-score and reasonable cutoff value determination method,[25,28] we showed that high tumoral PAI-1 expression was significantly related to poor TSS [Figure 2b]. Furthermore, PAI-1 expression plus the key clinicopathologic parameters of PDAC, histological grade and N stage, were significant prognostic factors, estimated by univariate Cox regression analysis. In view of its positive correlation with N stage, its influence on patient survival might easily be understood. Subsequent multivariate Cox regression analysis identified PAI-1 expression as one of the independent predictors of postsurgical survival of PDAC. These findings, which are largely consistent with those from other tumor types,[32-39] suggested that PAI-1 might be a strong biomarker for
long-term prognosis of PDAC. All our data support its promoting role in PDAC invasion. In the future, combined evaluation of this molecule and other variables might be of interest. On the other hand, relative molecular mechanisms are also worth further investigation.

However, this work has some limitations, such as its retrospective design and single detection method. Therefore, subsequent prospective and more comprehensive validations might be necessary.

In conclusion, our data indicate that PAI-1 expression is upregulated in PDAC and might serve as a poor prognostic marker.

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**Conflicts of interest**
There are no conflicts of interest.
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可切除胰腺导管腺癌中纤溶酶原激活物抑制物1作为不良预后指标

摘要

背景：之前研究发现纤溶酶原激活物抑制物1（PAI-1）在多种癌症，包括胰腺癌中影响许多表型。然而，其在胰腺导管腺癌(PDAC)中的预后意义需要进一步证据支持。本研究意在阐述这个问题。

方法：采用基于组织芯片的免疫组化染色检测2004年9月至2008年12月行外科切除的胰腺导管腺癌福尔马林固定石蜡包埋标本中PAI-1的表达。其与临床病理参数和肿瘤特异性生存(TSS)的关系以卡方检验、Kaplan-Meier和Log-rank检验以及Cox回归进行评价。

结果：在所有标本和74例配对标本中，癌组织PAI-1表达显著高于非癌组织(95 [47.5, 180] vs 80 [45, 95], Z=-2.439, P=0.015和100 [46.875, 182.5] vs 80 [45, 95], Z=-2.594, P=0.009)。而且，肿瘤组织PAI-1表达与N分期呈正相关（N1组22/35比N0组21/51, \chi^2=3.903, P=0.048）。单因素分析显示癌组织PAI-1高表达患者肿瘤特异性生存显著差于低表达者（Log-rank值=19.00, P<0.0001）。在多因素Cox回归中，PAI-1表达被鉴定为可切除胰腺导管腺癌长期预后的独立预测因素（相对危险度[HR]=2.559, 95%可信区间[CI]=1.499–4.367, P=0.001）。

结论：这些结果提示胰腺导管腺癌中PAI-1表达上调，并可能作为不良预后的指标。