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The Expression Levels of Vinculin in Pancreatic Cancer Tissues Significantly Correlates With Patient Survival

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Abstract. *Background/Aim: Proteomics is an approach that can detect differentially expressed proteins between cancerous and non-cancerous tissue samples. Previously, we found that vinculin was predominantly expressed in pancreatic cancerous tissues compared to adjacent non-cancerous tissues by performing proteomic differential display analysis. However, the clinicopathological significance of vinculin in pancreatic cancer has not yet been documented. Materials and Methods: The GEPIA2 and the Human Protein Atlas databases were used to analyze vinculin expression levels in cancerous tissue samples and investigate whether its expression level is clinically associated with patient survival. Results: Vinculin mRNA expression levels were solely increased in pancreatic cancer tissues, and increased expression was inversely related to patient survival. Higher levels of vinculin protein were found in pancreatic cancer tissues. In contrast, faint staining of vinculin was observed throughout the normal pancreatic tissues. Conclusion: Vinculin may be an unfavorable prognostic indicator for patients with pancreatic cancer.*

Pancreatic cancer is one of the most aggressive forms of malignancy with an abysmal patient prognosis. Most cases of pancreatic cancer are often diagnosed at advanced stages with distant organ metastases. In addition to the anatomical location of the pancreas, the lack of precise diagnostic and prognostic biomarkers underlies this poor prognosis (1, 2). Several experimental approaches have been undertaken in recent decades to discover strong biomarkers for pancreatic cancer to improve treatment outcomes and patient survival (2). Proteomics is a technique for studying cellular protein expression and has been widely used to identify biomarkers for various human malignancies. Two-dimensional gel electrophoresis (2-DE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) are two major methods for proteomics. The combination of 2-DE and LC-MS/MS with western blotting has high throughput and accuracy (3, 4). We previously performed proteomics differential display analysis using 2-DE and LC-MS/MS between pancreatic cancer and adjacent non-cancerous tissue samples. Six differentially expressed proteins were identified and, by western blotting, we confirmed that vinculin was predominantly expressed in pancreatic cancer tissues compared to non-cancerous tissues (4). Our results suggest that up-regulated vinculin expression may influence the clinicopathological status of patients with pancreatic cancer. However, a recent report described opposite effects and suggested that vinculin may attenuate pancreatic cancer progression (5). Owing to these contradictory findings, the involvement of vinculin in pancreatic cancer needs further elucidation. Our present study aimed to investigate the expression level and clinicopathological significance of vinculin in pancreatic cancer tissue samples by using bioinformatics platforms. In addition, the bioinformatics platform was also used to analyze vinculin expression level and its clinical significance in other cancer tissue samples. Finally, the expression and cellular origin of vinculin in pancreatic cancer tissue samples were investigated by using the Human Protein Atlas database.

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Materials and Methods

mRNA expression of vinculin in pancreatic cancer and survival analysis. To study the mRNA expression levels of vinculin in cancerous tissue samples, including pancreatic cancer, the bioinformatics platform Gene Expression Profiling Interactive Analysis (GEPIA2) was used (6). The cut-off criteria for mRNA expression were chosen as follows: LogFC cut-off=2.0; *p*-value cut-off=0.05; datasets=all; and matched normal data=match Cancer Genome Atlas (TCGA), the TCGA normal, and the Genotype-Tissue

Expression (GTEx). The individual cancer dataset was considered in statistical analysis when the number of samples was higher than 50 in each group (either control or tumor). Similarly, GEPIA2 was also used to analyze the association of vinculin gene expression in cancerous tissues with overall survival and disease-free survival status using the TCGA and GTEx databases. The survival plots were considered significant when shown in both overall survival and disease-free survival states. p=0.05 was considered to indicate a statistically significant difference.

Expression and cellular origin of vinculin in pancreatic cancer. Expression of the protein encoded by the vinculin gene in pancreatic cancer and normal pancreas tissue samples was validated by using the website Human Protein Atlas, based on spatial proteomics data and quantitative transcriptomics data (RNA-Seq) obtained from immunohistochemical analysis of tissue microarrays (7).

Results

Vinculin mRNA expression level was exclusively up-regulated in pancreatic cancer tissues. To identify the mRNA expression levels of vinculin in cancerous tissue samples, TCGA datasets were analyzed using the GEPIA2 platform. Vinculin expression profiles in cancerous tissues were downloaded from GEPIA2. The results showed that the mRNA expression levels of vinculin were down-regulated in uterine corpus endometrial carcinoma (UESC) and uterine carcinosarcoma (UCS). Interestingly, the mRNA expression levels of vinculin were exclusively up-regulated in pancreatic cancer tissues compared to normal pancreatic tissues (p=0.05) (Figure 1).

Vinculin mRNA expression is inversely related to the survival of patients with pancreatic cancer. To investigate whether the increased mRNA expression levels of vinculin are clinically related to the survival of patients with pancreatic cancer, the Kaplan–Meier survival plots were constructed using the GEPIA2 platform. It was found that elevated vinculin expression levels were inversely correlated with the survival of patients with pancreatic cancer. Meanwhile, the clinical significance of down-regulated vinculin expression in UESC and USC was also investigated using the GEPIA2 platform. However, no significant association was found regarding vinculin expression (p=0.05) (Figure 2).

Protein expression of vinculin is increased in pancreatic cancer tissues. The expression levels of the protein encoded by the vinculin gene were obtained from the database Human Protein Atlas. The protein expression profile in clinical samples of pancreatic cancer is shown in Figure 3. High vinculin expression was observed in pancreatic cancer tissues compared to normal pancreatic tissues. Further observations showed that vinculin was strongly expressed by pancreatic cancer cells. In contrast, weak staining of vinculin was observed throughout the region of normal pancreatic tissues.

Discussion

In the present study, we analyzed the mRNA expression and clinicopathological involvement of vinculin in pancreatic cancer tissues by using bioinformatics platforms. We found that vinculin was exclusively up-regulated in pancreatic cancer tissues and that elevated vinculin expression levels were inversely correlated with patient survival. In addition, expression and cellular origin of vinculin in pancreatic cancer tissues was investigated using the Human Protein Atlas database. Protein expression of vinculin was higher in pancreatic cancer tissues than in normal pancreatic tissue samples. Overall, our results suggest that vinculin may represent a novel potential prognostic biomarker in pancreatic cancer patients.

Previously, by using the proteomics approach in combination with western blotting, we showed that vinculin was significantly up-regulated in pancreatic cancer tissues compared with adjacent non-cancerous tissue samples (4). However, the underlying mechanisms of vinculin in pancreatic cancer are still unknown. Vinculin, an actin cytoskeletal protein, is localized at cell-cell and cell-extracellular matrix (ECM) junctions. The localization of vinculin suggests that it may be involved in the formation of complexes that can couple members of the cadherin and integrin families of cell adhesion molecules to the actin cytoskeleton and modulate ECM composition (8). The remodeling of ECM promotes cell transformation and enhances tumorigenesis by affecting stromal cell behavior, resulting in extensive desmoplasia with ECM deposition, and desmoplasia is a characteristic feature of pancreatic cancer, accounting for up to 90% of tumor volume (9). Therefore, changes in vinculin expression may trigger pancreatic cancer progression by altering ECM

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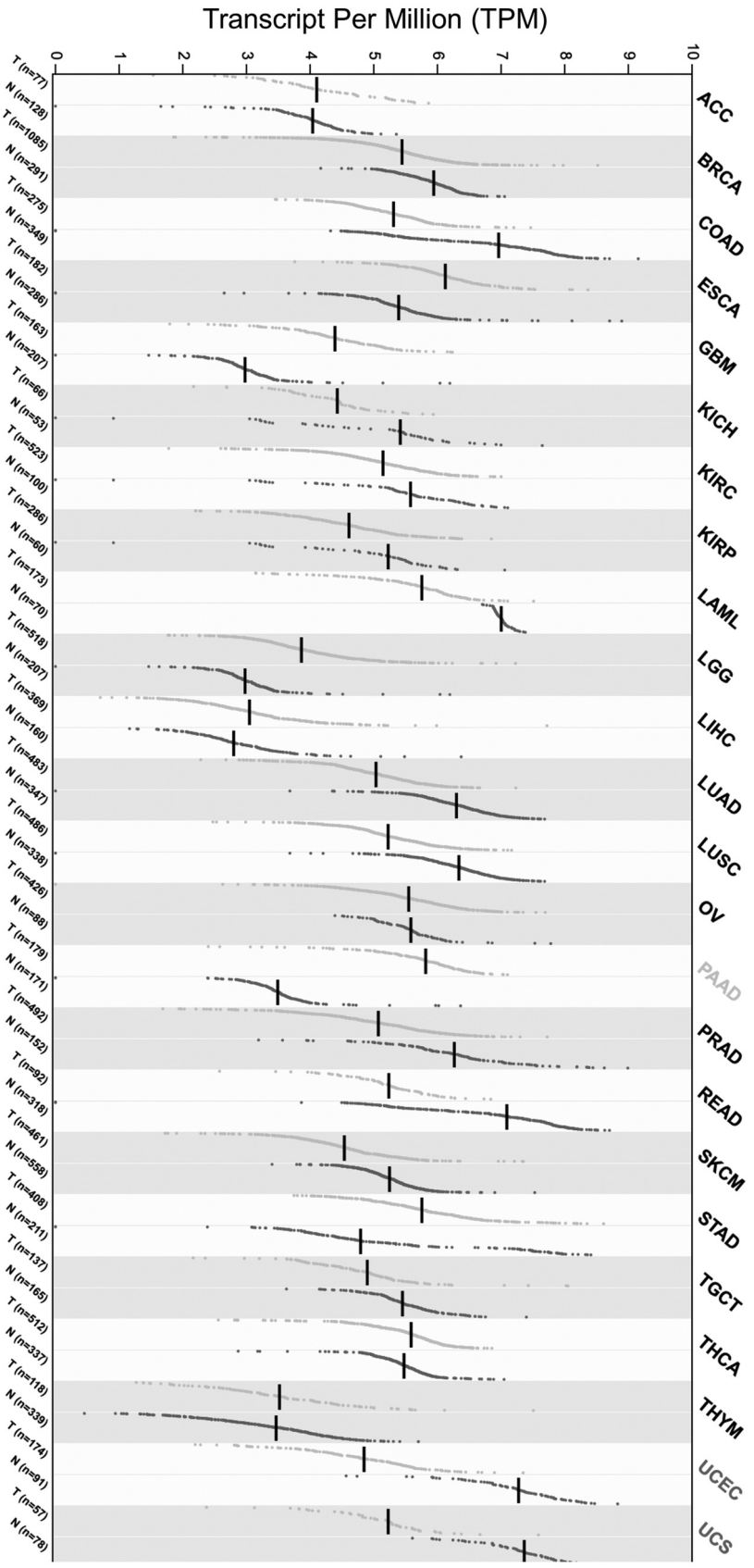
Figure 1. *The analysis of mRNA expression level of vinculin in cancerous tissues: vinculin gene expression profile was downloaded by using the online platform. The gray dots represent the expression levels in cancer tissues. The black dots represent the expression levels in normal tissues. The expression of vinculin was up-regulated in PAAD, whereas down-regulated expression was observed in UCEC and UCS cancers. p=0.05 was regarded as statistically significant. ACC: Adrenocortical carcinoma; BRCA: breast invasive carcinoma; COAD: colon adenocarcinoma; ESCA: esophageal carcinoma; GBM: glioblastoma multiforme; KICH: kidney chromophobe; KIRC: kidney renal clear cell carcinoma; KIRP: kidney renal papillary cell carcinoma; LAML: acute myeloid leukemia; LGG: brain lower grade glioma; LIHC: liver hepatocellular carcinoma; LUAD: lung adenocarcinoma; LUSC: lung squamous cell carcinoma; OV: ovarian serous cystadenocarcinoma; PAAD: pancreatic ductal adenocarcinoma; PRAD: prostate adenocarcinoma; READ: rectum adenocarcinoma; SKCM: skin cutaneous melanoma; STAD: stomach adenocarcinoma; TGCT: testicular germ cell tumors; THCA: thyroid carcinoma; THYM: thymoma; UCEC: uterine corpus endometrial carcinoma; UCS: uterine carcinosarcoma.*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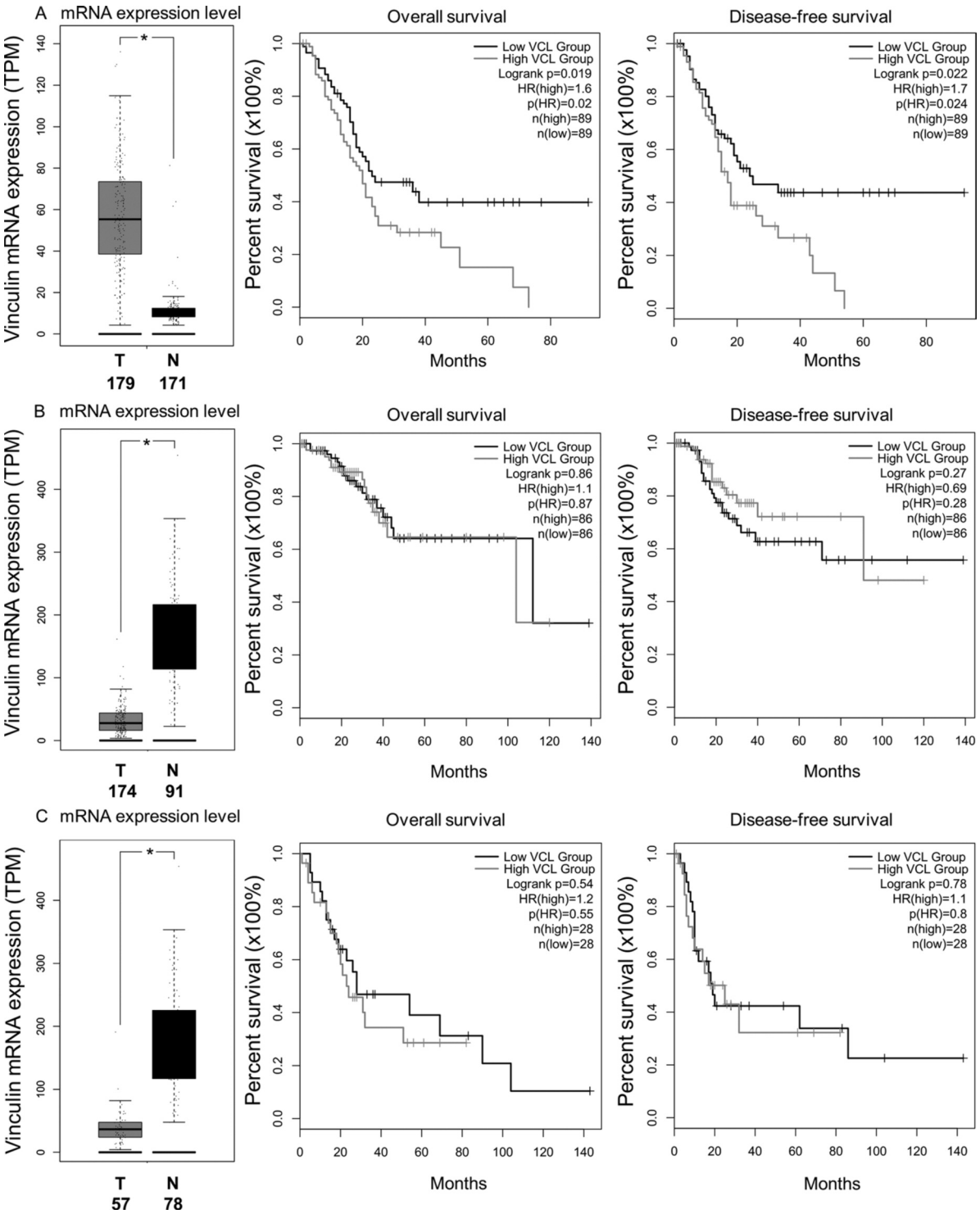

Figure 2. *The mRNA expression and clinicopathological involvement of vinculin in cancer tissues: boxplots were downloaded from the online platform. The gray boxes represent the expression levels in cancer tissues. The black boxes represent the expression levels in normal tissues. Kaplan–Meier survival plots were generated using the online platform, and the overall survival and disease-free survival graphs compared a high-expression group (in gray) and a low-expression group (in black) in cancer tissues. (A) The mRNA expression and Kaplan–Meier survival analysis of vinculin expression in pancreatic cancer tissues. (B) The mRNA expression and Kaplan–Meier survival analysis of vinculin expression in endometrial carcinoma tissues of uterine corpus. (C) The mRNA expression and Kaplan–Meier survival analysis of vinculin expression in uterine cancer tissues. p=0.05 was regarded as statistically significant. T: tumor tissues; N: normal tissues; TPM: transcripts per million.*

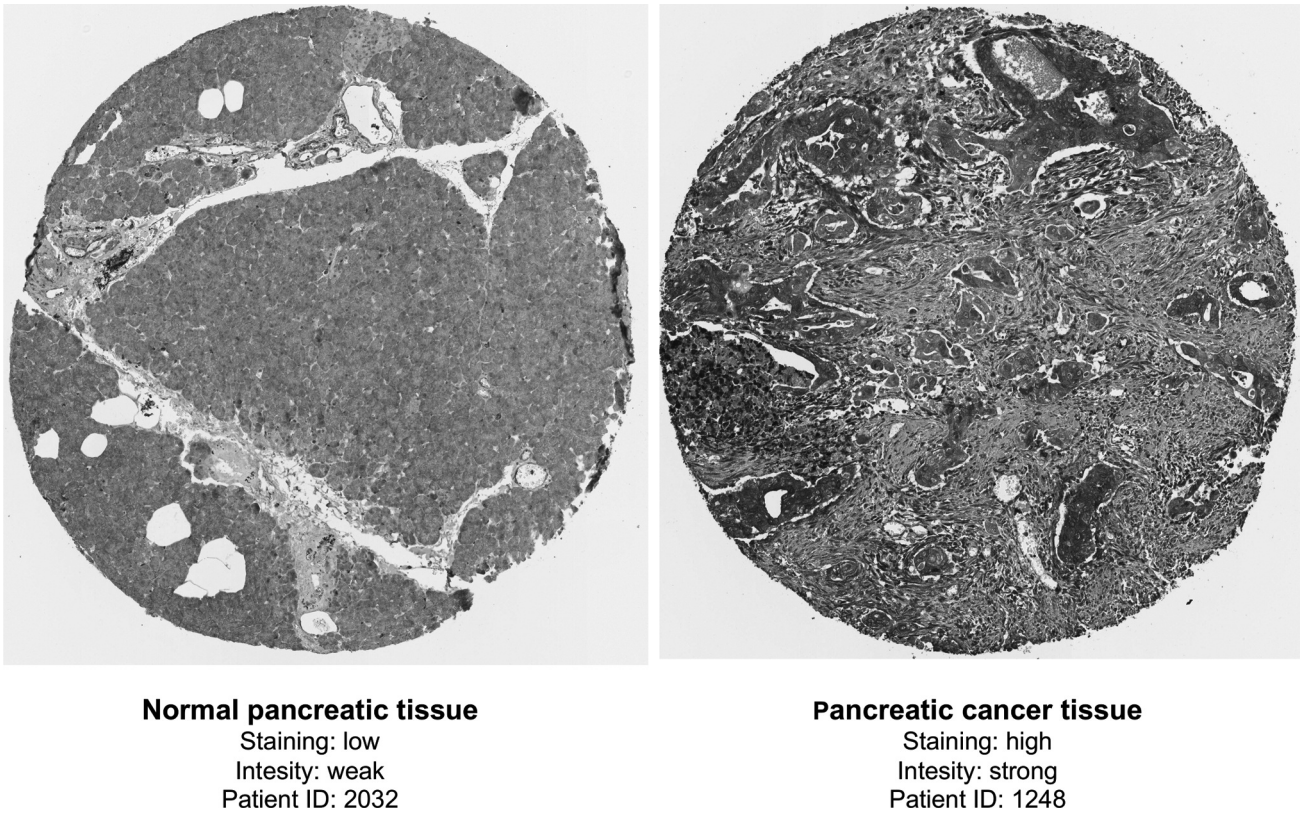

Figure 3. *Immunohistochemical expression of vinculin in human pancreatic cancer specimens: immunohistochemical data were taken from the Human Protein Atlas database. Staining showed that vinculin protein expression was higher in pancreatic cancer tissues than in normal pancreatic tissue samples. Image courtesy: Human Protein Atlas (http://www.proteinatlas.org).*

composition (4). However, other possible molecular mechanisms cannot be ruled out. Our previous and current data suggest the possible involvement of vinculin in pancreatic cancer. However, there are also contradictory results regarding the involvement of vinculin in this cancer. Indeed, a recent study has shown that increased vinculin expression inhibits proliferation, invasion, and metastasis of pancreatic cancer cells by attenuating the focal adhesion kinase signaling pathway (5). Owing to these conflicting observations, an independent online bioinformatics tool was used to clarify the expression and prognostic significance of vinculin in pancreatic cancer patients using the TCGA databases (6). Bioinformatics analysis confirmed that vinculin was significantly upregulated in pancreatic cancer tissues, whereas its downregulation was observed in other human malignancies. We then analyzed whether the upregulated expression of vinculin was related to the survival of patients with pancreatic cancer. By using the Kaplan–Meier survival plot, we found that high vinculin expression was inversely associated with the survival of patients with pancreatic cancer. Remarkably, the results of bioinformatics analysis were consistent with our previous data, suggesting that upregulated vinculin expression is involved in carcinogenesis and malignant progression of pancreatic cancer. Meanwhile, in combination with carbohydrate antigen 19-9 (CA19-9), several proteomics biomarkers have already been described in the diagnosis of pancreatic cancer. CA19-9 is the only FDA-approved clinical biomarker, and measurement of serum CA19-9 levels is useful for evaluating response to treatment after surgery or chemotherapy in patients with pancreatic cancer (10). However, due to inadequate sensitivity and specificity, CA 19-9 is not recommended for screening, although it remains the most commonly used tumor marker for pancreatic cancer (11). Indeed, it has recently been suggested that CA19-9 in combination with other proteomics biomarkers could improve the sensitivity and specificity of early diagnosis of pancreatic cancer (12-14). Therefore, the biomarker identified in our study could be used as a combinatorial biomarker with CA19-9 in the diagnosis and prognosis of patients with pancreatic cancer. This type of combinatorial approach could usher in a new era of precision diagnosis and treatment in patients with pancreatic cancer. Kitamura *et al*. reported proteomic analysis

of malignant ascites from patients with PDAC. They identified 18 specific proteins in the ascites, but vinculin was not included there. To clarify the role of vinculin in pancreatic carcinogenesis, further investigation is necessary (15).

Taken together, our previous report and the current analysis suggest that vinculin may represent a novel potential prognostic biomarker for patients with pancreatic cancer. Further studies are warranted to explore the underlying mechanisms by which vinculin is involved in pancreatic cancer.

Conflicts of Interest

The Authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' Contributions

SI, TK, TA, and YK conceived and designed the study. SI and YK performed the bioinformatic analysis. SI, TK, TA, and YK analyzed and interpreted the data. SI wrote the initial draft of the manuscript. TK, TA, and YK contributed in the critical revision of the manuscript. All Authors read and approved the final manuscript. SI and YK confirm the authenticity of experimental data.

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