Original Article

Upregulated HSPA2 predicts early relapse of pancreatic cancer after surgery

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Background: Heat shock protein A2 (HSPA2) is known to relate to the pathogenesis and progress of cancer. This study aimed to investigate the connection between HSPA2 and early postsurgical relapse of pancreatic cancer (PC).

Methods: Expression of HSPA2 in 85 pairs of cancerous and matched noncancerous samples was determined by immunostaining method. The relationship between HSPA2 expression and early postsurgical recurrence was assessed using logistic regression. The performance and potential application of HSPA2 expression to predict early postsurgical recurrence was evaluated by receiver operating characteristic (ROC) curve analysis and decision curve analysis (DCA).

Results: HSPA2 expression in tumor specimens was markedly elevated compared with non-tumor specimens. Logistic regression analysis indicated that HSPA2 upregulation was an independent risk marker for early postsurgical recurrence of PC. ROC curve analysis and DCA demonstrated that both the area under the curve (AUC) and the net benefit of HSPA2 expression were higher than those of other clinicopathologic features in predicting early postsurgical relapse of PC. The combination of HSPA2 expression with other malignant clinicopathologic characteristics had greater AUC and net benefit relative to them alone in predicting early postsurgical recurrence.

Conclusions: Upregulated HSPA2 independently predicts early postsurgical recurrence of PC and has superior predictive performance and potential application value when combined with malignant clinicopathologic features. Our findings reveal that HSPA2 is a promising predictor for early postoperative relapse of PC.

Keywords: Heat shock protein A2 (HSPA2); pancreatic cancer (PC); surgery; recurrence; risk factor

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Introduction

Pancreatic cancer (PC) is an extremely invasive and quickly progressive human digestive system malignant tumor, which is the seventh main reason for cancer-associated deaths globally (1). Statistic data from the American Cancer Society indicate that the disease has jumped to the fourth main reason for malignancy-associated deaths domestically, with a five-year relative survival rate of approximately 9% for all stages and races from 2009 to 2015 (2). Although radical surgery is currently the most effective treatment for PC, the incidence of early tumor recurrence after surgery is high, which leads to a reduction in long-term survival. Therefore, the identification of specific biomarkers to predict early postoperative recurrence is of great value in improving the prognosis of PC.

Heat shock-related 70-kDa proteins (HSP70s) are a class of hot shock proteins (HSPs) with a molecular weight of about 70-kD, they usually play the role of molecular chaperones, and also have antioxidant, antiapoptotic and immunomodulatory effects (3). HSPA2, also named HSP70-2, is one of the significant members of the HSP70s family, originally found in male germ cells and described as a testicle-specific protein that is essential in spermatogenesis (4-6). The *HSPA2* gene is located on chromosome 14 (14q24.1), and its abnormal expression in the testis causes meiosis to be unsuccessful, leading to male sterility (4,7). Previous studies have reported that HSPA2 was overexpressed in several human solid neoplasms, including cervical malignancies (8), bladder urothelial cancer (9), esophageal squamous cell cancer (ESCC) (10), non-small cell lung cancer (NSCLC) (11), hepatocellular cancer (HCC) (12,13), colorectal cancer (14), breast cancer (15-17), and PC (18,19). These reports show that HSPA2 overexpression acts as a vital function in the genesis and progress of carcinoma.

It has been demonstrated that the upregulation of HSPA2 in cancerous tissues is tightly interrelated with the shortened postoperative survival of PC (18,19). However, the relationship between HSPA2 and early postoperative recurrence of PC remains unclear. Therefore, this study assessed HSPA2 expression in PC tissues by immunohistochemistry and analyzed its correlation with early postoperative recurrence of PC.

We present the following article in accordance with the STARD reporting checklist (available at https://dx.doi.org/10.21037/gs-21-262).

Methods

Tissue samples and clinicopathologic data

The cancerous and adjacent paracancerous specimens were gathered from 85 PC cases that achieved radical excision between January 2008 and December 2011. All patients were confirmed as PC by pathology and had not received presurgical anticancer treatment, including chemotherapy, radiotherapy, chemoradiotherapy, biologically targeted treatment, and/or immunotherapy. All patients included in the study had the entire tumor removed without residual tumor cells (R0 resection). The samples were immersed in formaldehyde solution within 30 minutes after excision for fixation and stored in the Biobank after embedding in paraffine. Paracancerous tissues refer to normal pancreas tissues which situate 2 cm or more away from the edge of the confirmed neoplasms. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics board of Renmin Hospital of Wuhan University (NO. WDRY2019-K070) and individual consent for this retrospective analysis was waived.

The clinicopathologic parameters of PC were collected retrospectively from the medical records (Electronic Medical Record), including age, sex, tumor site, tumor size, presurgical serum carbohydrate antigen 19-9 (CA19-9) measurement values, tumor differentiation grade, lymph node metastasis (LNM) pattern, peripheral nerve infiltration (PNI) pattern, tumor staging status and early postsurgical relapse state. The tumor differentiation was histologically categorized into moderate-poor and well differentiation. The tumor staging was determined with the 8th edition of the AJCC TNM staging system (20). All cases were followed up continuously after radical resection. Early postsurgical recurrence of PC was designated as the neoplasm recurred within one year following radical surgery (21). Postoperative recurrence includes local recurrence (e.g., residual pancreas, anastomosis, etc.) and distant metastases (e.g., liver, peritoneum, abdominal lymph nodes, lungs, bone, etc.).

Immunohistochemical analysis

The expression of HSPA2 in clinical samples was detected by immunohistochemical staining, as formerly reported (19). The paraffin-embedded specimens were made into 4-micron contiguous tissues and attached to glass slides.

Next, the sections with tissues were placed in a heater at 60 ℃ for 60 min to melt the paraffin. The tissue sections were then treated with xylene to dewax and with different concentrations of alcohol to hydrate. After that, the tissue slices were immerged into sodium citrate-hydrochloric acid buffer solution (0.01 mol/L, pH 6.0) and heated by microwave at 80 ℃ for 20 min to achieve antigenic retrieval. After quenching the endogenetic peroxidase function with 3% deionized H_2O_2 solution, the tissue sections were incubated with monoclonal rabbit anti-human HSPA2 primary antibody (Abcam Company, Cambridge, England, UK) at 4 ℃ overnight. After rewarming, the sections were cleaned in phosphate-buffered saline (PBS) and then incubated with goat anti-rabbit HRP-labeled IgG secondary antibody (Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China) at room temperature for 30 minutes. Finally, the sections were sequentially subjected to chromogenic reaction, counterstaining, dehydration, cleaning, and mounting. The section was not incubated with the HSPA2 antibody served as a negative control.

Immunostaining results were scored independently and semi-quantitatively by two researchers participating in the experiment based on the percent of staining cells and the degree of color, as mentioned formerly (19). The scoring range for the percent of staining cells was as follows: 0 marks means nonstained cells, 1 mark means 1–10% staining cells, 2 marks means 10–30% staining cells, and 3 marks means >30% staining cells (19). The scoring range of immunostaining degree was recorded as: 0 for colorless, 1 for weak coloring, 2 for middle coloring, 3 for strong coloring (19). The final total score for each section was the staining percentage score multiplied by the staining degree score. For statistical purpose, a total point of less than 3 was designated as low expression and no less than 3 as high expression. The difference in scores was resolved through a joint evaluation.

Statistical analysis

The statistical analyses were conducted with SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA), Microsoft Office Excel 2007 (Microsoft, Redmond, WA, USA), and R software version 4.0.3. Categorical variables were expressed as the number of cases and percentages, and the statistic analysis between the groups was estimated by Pearson's chi-square test or Fisher's exact test. Logistic regression analysis was applied to determine risk variables related to early postoperative recurrence. Variables that showed statistically significant in univariate logistic regression analyses were incorporated into a multivariate logistic regression model to identify independent predictors of early postoperative relapse. The Hosmer-Lemeshow test was used to estimate the goodness of fit of the multivariate logistic regression model. Receiver operating characteristic (ROC) curve analysis was applied to assess the predictive power of risk factors for early relapse of PC after surgery. The clinical utility evaluation of risk factors was performed by decision curve analysis (DCA). A two-tailed P value of less than 0.05 was suggested to be statistically significant.

Results

Clinicopathologic features of PC patients

A total of 85 PC patients were included in this study. The average age of these PC patients was 56.9±6.5 years (ranging from 44 to 68 years), including 45 men and 40 women. There were 41 patients with tumor size less than 20 mm, 44 patients with tumor size greater than or equal to 20 mm, 71 patients with pancreatic head cancer, 14 patients with pancreatic body/tail carcinoma, 38 patients with serum CA19-9 ≤37 U/mL, 47 patients with >37 U/mL. Postoperative pathological examination confirmed that 29 patients with moderate/poor tumor differentiation, 56 patients with well tumor differentiation, 26 patients with non-metastatic lymph nodes, 59 patients with metastatic lymph nodes, 49 patients without PNI, 36 patients with PNI, 23 patients with stage I, and 62 patients with stage II. Thirty-five cases without early postsurgical recurrence and 50 cases with early postsurgical recurrence. Among the patients with early postoperative relapse, 14 presented with local recurrence, 29 with liver metastases, 3 with peritoneal metastases, 3 with abdominal lymph node metastases, and 1 with pulmonary metastases. A summary of clinicopathologic parameters is shown in *Table 1*.

Expression of HSPA2 in PC tissues

Immunostaining indicated that HSPA2 was largely distributed in the cytoplasmic space, as shown in *Figure 1A*. Of the 85 PC specimens, overexpressed HSPA2 was observed in 70.6% (60/85). However, only 25.9% (22/85) of HSPA2 overexpression was found in the matched non-tumor specimens. Statistical analysis revealed that HSPA2 expression was signally increased in PC specimens relative to surrounding para-carcinoma specimens (P=0.003; *Figure 1B*

Table 1 Clinicopathologic data of 85 PC patients

Clinicopathologic data	Group	Number	%
Age (years)	<60	51	60.0
	≥60	34	40.0
Sex	Male	45	52.9
	Female	40	47.1
Tumor size (mm)	<20	41	48.2
	≥20	44	51.8
Tumor site	Head	71	83.5
	Body/tail	14	16.5
Serum CA19-9 (U/mL)	≤37	38	44.7
	>37	47	55.3
Tumor differentiation	Well	29	34.1
	Moderate/poor	56	65.9
LNM	Absent	26	30.6
	Present	59	69.4
PNI	Absent	49	57.6
	Present	36	42.4
Tumor stage	I	23	27.1
	II	62	72.9
Early postoperative recurrence	No	35	41.2
	Yes	50	58.8

PC, pancreatic cancer; CA19-9, carbohydrate antigen19-9; LNM, lymph node metastasis; PNI, peripheral nerves infiltration. Neoplasm recurrence within 12 months after surgery was defined as early postoperative relapse.

and *Table 2*). HSPA2 expression was observably higher in tumors with early postsurgical relapse compared with those without early postsurgical relapse (P<0.001; *Figure 1C* and *Table 3*). Moreover, HSPA2 expression was markedly linked to malignant clinicopathologic features such as preoperative serum CA19-9, tumour differentiation, LNM, PNI and tumour stage (*Table 4*).

Logistic regression analysis of risk variables potentially associated with early postsurgical relapse of PC

To ascertain the variates that pre-estimate the early postsurgical recurrence of PC, we carried out univariate and multivariate analyses with logistic regression models. Univariate logistic regression analysis revealed that early neoplasm relapse was observably correlated with high serum CA19-9 levels before surgery (P=0.019), moderate/poor tumor differentiation (P=0.001), the presence of LNM (P<0.001), the presence of PNI (P=0.011), high tumor staging (P<0.001), and overexpressed HSPA2 (P<0.001) in PC patients after resection (*Figure 2A* and Table S1).

In the multivariate logistic regression model, upregulated HSPA2 proved to be an independent hazard variate for early recidivation of PC following surgery [odds ratio (OR) =6.601; 95% confidence interval (CI): 1.635–26.652; P=0.008]. The high tumor stage was also shown to be an independent variable for predicting early relapse of PC after the operation (OR =8.049; 95% CI: 1.783–36.334; P=0.007) (*Figure 2B* and Table S1). The Hosmer-Lemeshow test showed a P value of 0.642 (greater than 0.05), indicating that this multivariate logistic regression model had good goodness of fit.

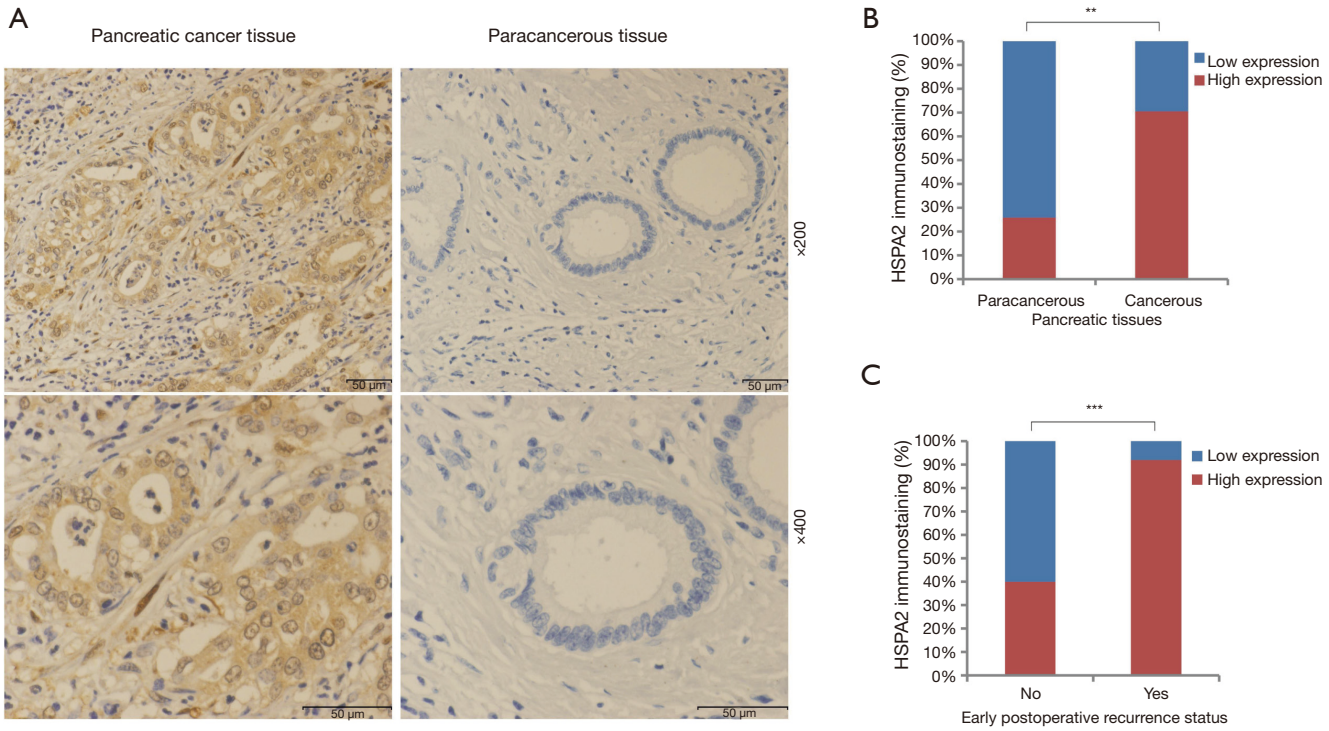


Figure 1 Expression of HSPA2 in PC tissues. (A) Representative photographs of HSPA2 immunostaining in cancer and paracancerous tissues were taken at different magnifications (top panel ×200, bottom panel ×400). High expression of HSPA2 was observed in cancer tissues, mainly in the cytoplasm, while the expression of HSPA2 was low in paracancerous tissues. (B) High expression rate of HSPA2 in cancer tissues was significantly higher than that in adjacent paracancerous tissues. (C) High expression rate of HSPA2 in tumors with early postoperative relapse was significantly higher than that of tumors without early postoperative relapse. HSPA2, heat shock protein A2; PC, pancreatic cancer. **P<0.01, ***P<0.001.

Table 2 Expression of HSPA2 in 85 pairs of cancerous and adjacent paracancerous clinical specimens based on immunohistochemistry

Pancreatic tissues	Number	HSPA2 expression		P value
		Low	High	
Cancerous	85	25	60	0.003
Paracancerous	85	63	22	

The semi-quantitative score value 3 of immunohistochemistry was used as the cut-off value for high and low expression of HSPA2. Data were analyzed by chi-squared test. P<0.05 indicates statistical significance of differences. HSPA2, heat shock protein A2.

Table 3 Differential expression of HSPA2 between patients with early postoperative recurrence and patients without early postoperative recurrence (n=85)

Early postoperative recurrence status	Number	HSPA2 expression		P value
		Low	High	
No	35	21	14	<0.001
Yes	50	4	46	

The semi-quantitative score value 3 of immunohistochemistry was used as the cut-off value for high and low expression of HSPA2. Data were analyzed by chi-squared test. P<0.05 indicates statistical significance of differences. HSPA2, heat shock protein A2.

Table 4 Correlations between HSPA2 expression and clinicopathologic characteristics of PC (n=85)

Clinicopathologic characteristics	Group	Number	HSPA2 expression		P value
			Low	High	
Age (years)	<60	51	14	37	0.627
	≥60	34	11	23	
Sex	Male	45	14	31	0.715
	Female	40	11	29	
Tumor size (mm)	<20	41	13	28	0.654
	≥20	44	12	32	
Tumor site	Head	71	23	48	0.174
	Body/tail	14	2	12	
Serum CA19-9 (U/mL)	≤37	38	20	18	<0.001
	>37	47	5	42	
Tumor differentiation	Well	29	19	10	<0.001
	Moderate/poor	56	6	50	
LNM	Absent	26	18	8	<0.001
	Present	59	7	52	
PNI	Absent	49	21	28	0.002
	Present	36	4	32	
Tumor stage	I	23	18	5	<0.001
	II	62	7	55	

The semi-quantitative score value 3 of immunohistochemistry was used as the cut-off value for high and low expression of HSPA2. Data were analyzed by chi-squared test. P<0.05 indicates statistical significance of differences. HSPA2, heat shock protein A2; PC, pancreatic cancer; CA19-9, carbohydrate antigen19-9; LNM, lymph node metastasis; PNI, peripheral nerves infiltration.

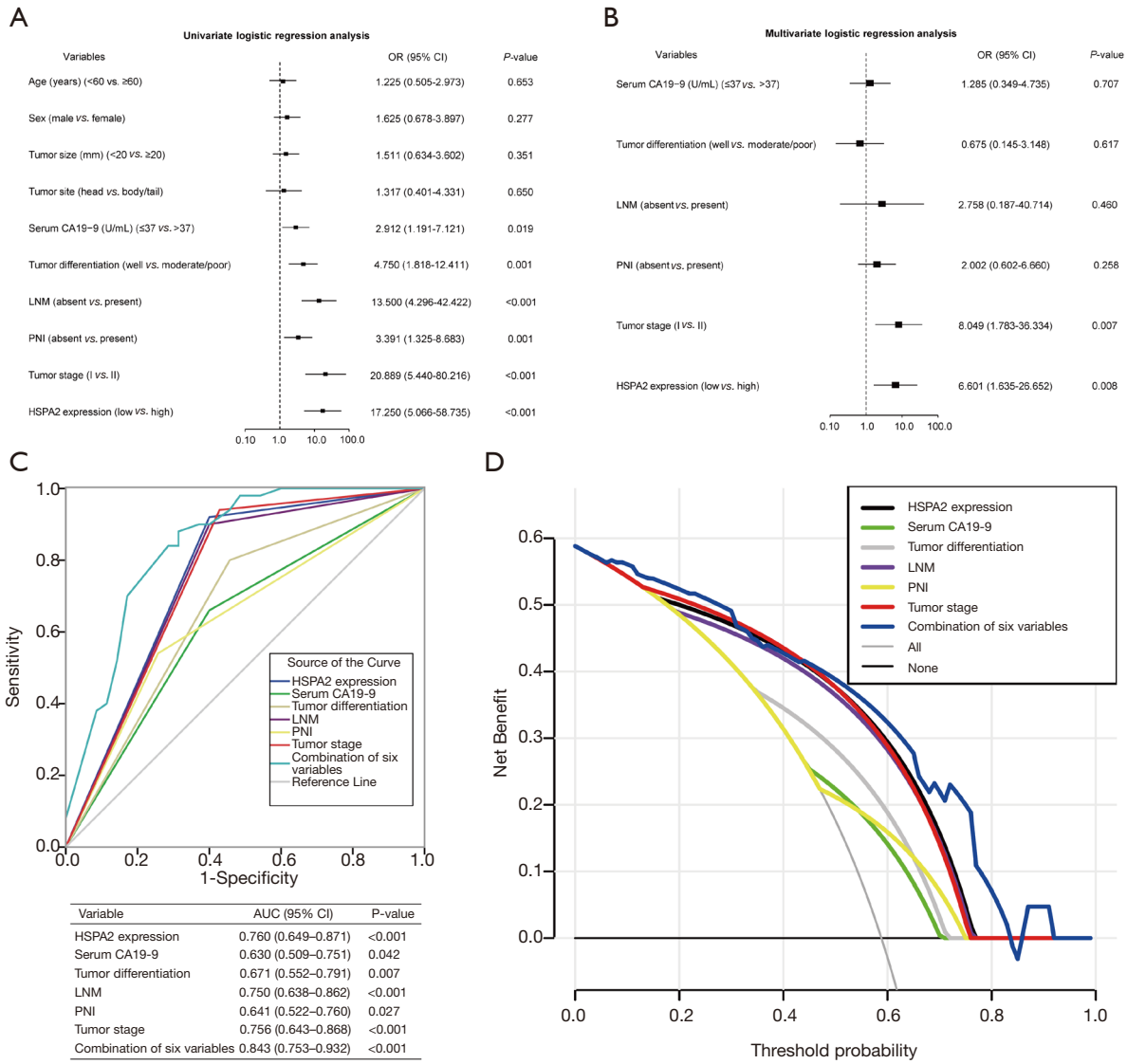
Predictive performance and potential application value of HSPA2 for early postsurgical relapse

ROC curves were constructed to assess the reliability of these risk factors for predicting early relapse of PC following surgery. The area under curve (AUC) was 0.760 (95% CI: 0.649–0.871) for HSPA2, 0.630 (95% CI: 0.509–0.751) for serum CA19-9, 0.671 (95% CI: 0.552–0.791) for tumor differentiation, 0.750 (95% CI: 0.638–0.862) for LNM, 0.641 (95% CI: 0.522–0.760) for PNI, 0.756 (95% CI: 0.643–0.868) for tumor stage. The combination of HSPA2 and the above-mentioned malignant clinicopathologic parameters improved the specificity and sensitivity over that of HSPA2 or these clinicopathologic parameters alone for predicting early postsurgical relapse of PC, with an AUC of 0.843 (95% CI: 0.753–0.932) (*Figure 2C*).

DCA was employed to quantify the probability of net benefits of risk factors to further assess their predictive performance and potential clinical application value for early recurrence of PC after surgery. The results demonstrated that HSPA2 expression had a larger net benefit than other risk variables. The combination of all risk variables presented the highest net benefit relative to individual risk variables, indicating that HSPA2 expression combined with other malignant clinicopathologic characteristics had a great advantage in predicting the possibility of early postoperative relapse (*Figure 2D*).

Discussion

HSPA2 expression has been confirmed to be elevated in a variety of human solid malignancies, including PC (8-19). Although overexpression of HSPA2 in PC tissues has



Variable	AUC (95% CI)	P-value
HSPA2 expression	0.760 (0.649–0.871)	<0.001
Serum CA19-9	0.630 (0.509–0.751)	0.042
Tumor differentiation	0.671 (0.552–0.791)	0.007
LNM	0.750 (0.638–0.862)	<0.001
PNI	0.641 (0.522–0.760)	0.027
Tumor stage	0.756 (0.643–0.868)	<0.001
Combination of six variables	0.843 (0.753–0.932)	<0.001

Figure 2 Prediction of risk variables for early postsurgical recurrence of PC. (A,B) Forest plots for univariate and multivariate logistic regression analysis. High HSPA2 expression, high preoperative serum CA19-9 levels, moderate/poor tumor differentiation, the presence of LNM, the presence of PNI, and late tumor stage were risk factors for early postoperative recurrence. High HSPA2 expression and late tumor stage were independent risk factors. (C) ROC curves analysis of risk variables for predicting the early postsurgical relapse. The AUC for HSPA2 expression, serum CA19-9, tumor differentiation, LNM, PNI, tumor stage and the combination of these variables (combination of six variables) were 0.760, 0.630, 0.671, 0.750, 0.641, 0.756, and 0.843, respectively. (D) DCA of risk variables for predicting the early postsurgical relapse. The Y-axis and X-axis represent the net benefit and the threshold probability, respectively. The two curves of "All" and "None" mean respectively assuming that all patients have an early postoperative relapse and that no patients have an early postsurgical recurrence. HSPA2 expression, serum CA19-9, tumor differentiation, LNM, PNI, tumor stage, and the combination of these variables (combination of six variables) had more net benefits than the two extreme cases (All and None) across the area of appropriate threshold probabilities. HSPA2 expression was superior to the other five clinicopathologic features in predicting early postoperative relapse. The combination of six variables was better than HSPA2 expression or clinicopathologic parameters alone. Net benefit = (number of true positives/overall number of cases) – (number of false positives/overall number of cases) × [threshold probability/(1-threshold probability)]. PC, pancreatic cancer; CA19-9, carbohydrate antigen19-9; LNM, lymph node metastasis; PNI, peripheral nerves infiltration; HSPA2, heat shock protein A2; OR, odds ratio; CI, confidence interval; ROC, receiver operating characteristic; AUC, area under the curve; DCA, decision curve analysis. P<0.05 indicates statistically significant.

been confirmed to be strongly correlative with shortened survival time, the relationship between HSPA2 and early postsurgical recurrence of PC remains unclear. Therefore, this study explored the associativity between HSPA2 expression and early reappearance of PC after surgery. The findings showed that HSPA2 expression in PC specimens was markedly enhanced relative to the nontumor specimens; which was in accord with the former results (18,19). Elevated HSPA2 expression served as an independent risk indicator for early postsurgical relapse of PC. ROC curve analysis showed that HSPA2 expression combined with other malignant clinicopathologic parameters had a larger AUC value. DCA indicated that HSPA2 expression combined with other malignant clinicopathologic features had a higher net benefit than HSPA2 or clinicopathologic parameters alone.

There is mounting evidence that HSPA2 expression is closely associative with the aggressive progression of tumors. In cervical cancer, bladder urothelial cancer, colorectal cancer, and breast cancer, the depletion of HSPA2 expression observably reduced the proliferation, invasion and migration abilities of carcinoma cells, initiated cell apoptosis, and restrained the increase in the volume of transplanted tumors (8,9,14-16,22). In kidney cancer, ablated HSPA2 in tumor cells caused the attenuation of vitality, colony formation and movement (23). In ovarian cancer cells, HSPA2 silencing induced by shRNA resulted in the decrease of cell proliferation, viability, colony formation and cell motility capacity, and the cell cycle was arrested in the G0-G1 phase (24). In lung adenocarcinoma, siRNA-mediated downregulation of HSPA2 caused the reduction of cell activity and cell cycle arrest in the G1-S stage (25). Additionally, some studies have shown that HSPA2 overexpression was prominently associated with malignant clinicopathologic characteristics of several human malignant neoplasms, including ESCC (10), NSCLC (11), HCC (12), breast cancer (17) and PC (18,19). Our data also confirmed that high expression of HSPA2 in neoplastic tissues was strongly linked to malignant clinicopathologic features such as poor tumor differentiation, tumor nerve infiltration, tumor lymphatic metastasis, and more advanced tumor staging. The current findings, together with previous data imply an important contribution of HSPA2 in the malignant progression of cancer.

Correlations between HSPA2 overexpression and clinical outcomes in several malignant tumors have been reported. For instance, Zhang *et al.* (10) reported that increased HSPA2 expression was apparently associated with a declining survival rate and independently forecasted the adverse outcome in ESCC individuals. Scieglinska *et al.* (11) demonstrated that HSPA2 upregulation was markedly relevant to shortened overall survival time in the NSCLC population with stages I and II. Fu *et al.* (12) suggested that HCC with upregulated HSPA2 presented a less overall survival time than those with downregulated HSPA2, and HSPA2 overexpression could be used as an independent adverse variable for prognosis prediction. Yang *et al.* (17) showed that overexpression of HSPA2 was clearly connected with reduced survival time of breast cancer patients. Moreover, previous studies have also shown that elevatory HSPA2 expression was significantly linked to the shortened recurrence-free survival and overall survival of PC cases (18,19). Nevertheless, the relationship between HSPA2 and early recurrence of PC after surgery is still unexplored. Although serum CA19-9 has been reported to be associated with early relapse of PC after resection, it still lacks sensitivity and specificity (26,27). Therefore, it would be of great benefit to seek additional tumor markers, such as HSPA2, as predictors of early postsurgical recurrence of PC. In the present work, we found that upregulated HSPA2 was evidently correlated with early postoperative relapse of PC and was an independent hazard indicator of early postoperative recurrence. Furthermore, HSPA2 expression combined with other malignant clinicopathologic characteristics significantly improved the reliability for the prediction of early postsurgical recurrence, with a great potential application value. These findings together suggest that HSPA2 overexpression is tightly connected with the unfavorable outcome of human malignant tumors, which further reveals the important role of its gene in the progress and recurrence of malignancies.

We have to acknowledge the deficiencies of this research. First, the establishment of logistic regression models that predict the risk factors for early postoperative relapse of PC was based on a retrospective analysis of a single institution with a relatively small sample capacity, so there is ineluctably a selection bias. Second, we did not investigate the effect of neoadjuvant therapy on HSPA2 expression, and further studies are needed to reveal the clinical relevance of HSPA2 to neoadjuvant therapy. Third, the current study did not determine the effect and mechanism of HSPA2 expression on the biological behavior of PC cells. Finally, this study did not explore the influence of HSPA2 on the biological behaviors of transplanted tumours in animals. Consequently, it is necessary to collect more clinical data, apply more experimental techniques, and design more

rigorous research protocols to further validate the current findings and reveal the biological role of HSPA2 in PC.

Conclusions

In conclusion, the current results indicate that high expression of intratumoral HSPA2 is strongly linked to early relapse of PC after resection and acts as an independent predictor of early postsurgical recurrence. The combination of HSPA2 expression and other malignant clinicopathologic parameters shows better performance and greater potential clinical value in predicting early postsurgical recurrence of PC. These data reveal that HSPA2 has a significant part in cancer progress and is a potential tumor marker for predicting early postoperative recurrence of PC.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics board of Renmin Hospital of Wuhan University (NO. WDRY2019-K070) and individual consent for this retrospective analysis was waived.

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Supplementary

Table S1 Logistic regression analysis of factors associated with early postoperative recurrence of PC

Variables	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
Age (years) (<60 *vs.* ≥60)	1.225 (0.505–2.973)	0.653		
Sex (male *vs.* female)	1.625 (0.678–3.897)	0.277		
Tumor size (mm) (<20 *vs.* ≥20)	1.511 (0.634–3.602)	0.351		
Tumor site (head *vs.* body/tail)	1.317 (0.401–4.331)	0.650		
Serum CA19-9 (U/mL) (≤37 *vs.* >37)	2.912 (1.191–7.121)	0.019	1.285 (0.349–4.735)	0.707
Tumor differentiation (well *vs.* moderate/poor)	4.750 (1.818–12.411)	0.001	0.675 (0.145–3.148)	0.617
LNM (absent *vs.* present)	13.500 (4.296–42.422)	<0.001	2.758 (0.187–40.714)	0.460
PNI (absent *vs.* present)	3.391 (1.325–8.683)	0.011	2.002 (0.602–6.660)	0.258
Tumor stage (I *vs.* II)	20.889 (5.440–80.216)	<0.001	8.049 (1.783–36.334)	0.007
HSPA2 expression (low *vs.* high)	17.250 (5.066–58.735)	<0.001	6.601 (1.635–26.652)	0.008

PC, pancreatic cancer; CA19-9, carbohydrate antigen19-9; LNM, lymph node metastasis; PNI, peripheral nerves infiltration; HSPA2, heat shock protein A2; OR, odds ratio; CI, confidence interval. The HSPA2 aggregate score <3 was defined as low expression, and ≥3 as high expression. P<0.05 indicates statistically significant.

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