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ORIGINAL RESEARCH—CLINICAL

Expression of Corticotropin-Releasing Hormone and Its Receptors May Be Associated With Survival Rate in Pancreatic Cancer

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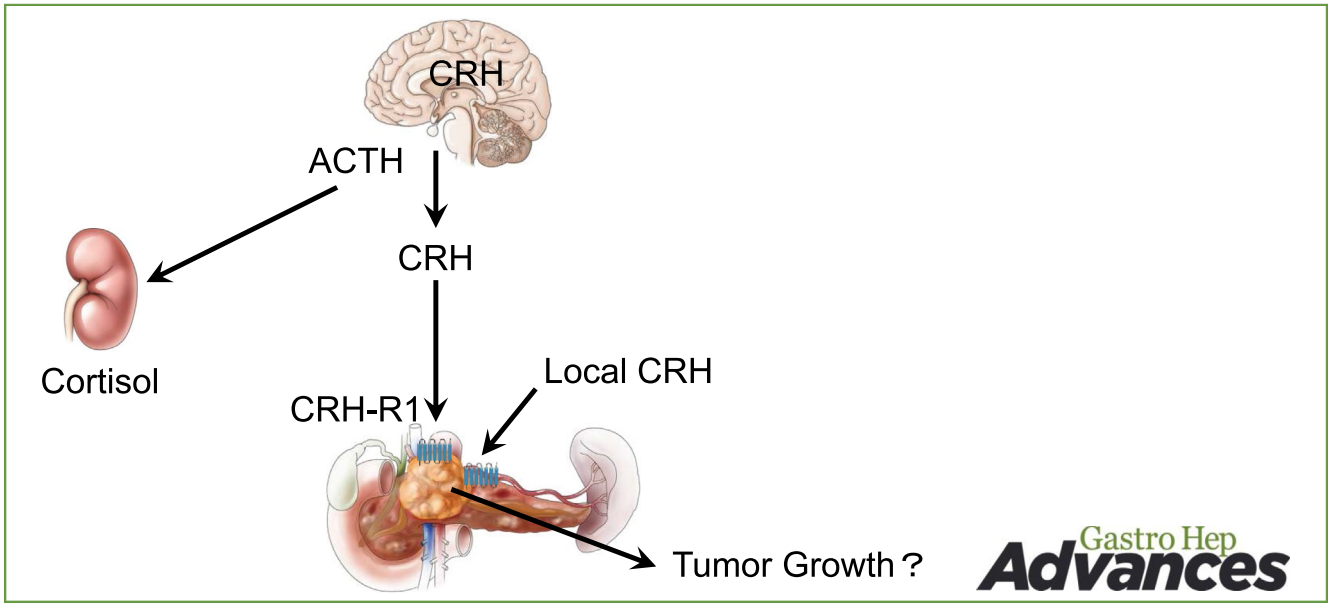

BACKGROUND AND AIMS: Corticotropin-releasing hormone (CRH) is a major regulator of the stress response to internal and external factors. CRH and its receptors (CRHR1 and CRHR2) are expressed in the central nervous system and some cancer cells, suggesting the importance of CRH signaling in pancreatic cancers. However, the clinicopathological significance of CRH remains unknown because the immunolocalization of CRH, CRHR1, and CRHR2 has not been examined in pancreatic carcinoma tissues. We clarified the correlation of the expression of CRH and its receptors with overall survival in pancreatic cancer. **METHODS:** This study evaluated 96 patients with pancreatic cancer who underwent microscopic complete resection (R0) but not neoadjuvant chemotherapy from 1988 to 2007 at Tohoku University Hospital, Japan. CRH, CRHR1, and CRHR2 immunoreactivity were detected in the pancreatic carcinoma cells. Overall survival curves were generated according to the Kaplan–Meier method. **RESULTS:** CRHR1 immunoreactivity was significantly associated with an increased risk of poorer prognosis in all patients ($P = .038$) and the adjuvant therapy group ($P = .022$). Overall survival was worse in the CRHR1-positive group than in the CRHR1-negative group among the 62 patients treated with gemcitabine hydrochloride ($P = .046$) and the 22 patients treated with other drugs ($P = .047$). CRHR1 expression was correlated with survival in univariate analysis but not in multivariate analysis. **CONCLUSION:** This study is the first to immunolocalize CRH,

Abbreviations used in this paper: **CRH, corticotropin-releasing hormone; CRHR1, CRH receptor 1; CRHR2, CRH receptor 2; GEM, gemcitabine hydrochloride.**

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CRHR1, and CRHR2 in pancreatic carcinoma tissues and to examine the biological prognosis. This study revealed that survival in patients with pancreatic cancer was significantly associated with expression of CRHR1 by assessing biological progression according to CRH and the expression of its receptors. However, CRHR1 expression was correlated with survival in univariate analysis but not in multivariate analysis.

Keywords: Pancreatic Cancer Cells; Biological Prognosis; Corticotropin-Releasing Factor; Hypothalamic Pituitary Adrenocortical Axis

Background

Pancreatic cancer diagnoses have doubled globally in the past 2 decades. In 2017, there were 441,000 new cases of pancreatic cancer worldwide.[1] In Japan, the annual number of pancreatic cancer deaths has been increasing year after year, reaching 34,224 in 2017. In the same year, pancreatic cancer was the fifth- and third-leading cause of cancer death in men and women, respectively, in Japan.[2] Because pancreatic cancer has very few signs and symptoms that can be noticed and the pancreas is difficult to visualize on medical imaging, pancreatic cancer is seldom detected early and generally has a poor prognosis, with 5-year survival as low as 7.9%.[3,4]

Corticotropin-releasing hormone (CRH) is a major regulator of the stress response to internal and external factors. CRH is a peptide hormone composed of 41 amino acids and was first purified from a sheep hypothalamus by Vale et al[5] in 1981. CRH promotes the synthesis and secretion of adrenocorticotropin in the pituitary gland and plays a central role in regulating stress responses in the hypothalamus–pituitary–adrenal axis as well as stress-induced changes in the autonomic nervous system, suppression of immune function, suppression of sexual behavior, and negative emotion.[6] Stress responses are considered to be abnormal when they are hyperactive, hypoactive, or sustained, or have no acclimation.[7] When the human body is exposed to stressors, CRH is released from the paraventricular nucleus of the hypothalamus[5] and stimulates CRH receptor 1 (CRHR1) and CRH receptor 2 (CRHR2) in the pituitary gland.[8] This induces the secretion of adrenocorticotropic hormone, which works to release cortisol from the adrenal cortex.[8,9] Recent studies have shown that CRH is expressed not only in the central nervous system but also in various parts of the peripheral nervous system and immune cells.[10]

Expression of CRH and CRH receptors has been reported in several cancers.[11,12] In previous studies, CRH and its receptors were detected in many primary human tumors. For example, CRH is expressed in melanoma[13] as well as adrenal,[14] ovarian,[15] and breast cancers.[16] CRHR1-immunoreactive cells have been detected in several human carcinomas, including those of the adrenal cortex,[17] breast,[18] ovaries,[15] endometrium[19] and central and peripheral nervous,[11] as well as in melanoma.[20] Research into the tumorigenic and genetic role of CRH–CRHR1 signaling in other cancer tumors has clarified that CRH contributes to cell proliferation by inducing cell proliferation and colony formation.[21] CRH binds to both CRHR1 and CRHR2 but its affinity is about 10 times greater for CRHR1 than CRHR2.[22] Therefore, CRH–CRHR1 signaling regulates various biological functions and may play an important role in the malignancy of pancreatic cancer. The pancreas is an important organ that governs endocrine function and may be deeply associated with the function of CRH. In addition, clarifying the effect of CRH on cancer growth in pancreatic tissues has the potential to contribute to the treatment of pancreatic cancer and improve survival. However, the clinicopathological significance of CRH remains to be clarified because immunolocalization of CRH, CRHR1, and CRHR2 has not been examined in pancreatic carcinoma tissues.

Therefore, the purpose of this study was to determine the expression rate of CRH and CRH receptors in pancreatic cancer tissues and to investigate the relation between this expression and survival prognosis.

Methods

Patients and Tissues

Of the 156 cases diagnosed as invasive ductal carcinoma of the pancreas after pancreatic resection at Tohoku University Hospital in Sendai, Japan from 1998 to 2007, the 144 that were traceable were extracted. Of these, 112 cases were histologically diagnosed as having no residual cancer (R0), and after excluding those that underwent preoperative chemotherapy, 96 (62 men, 34 women; age 64.3 $\pm$ 10.1 years) were analyzed[23] (Figure 1). Clinical outputs were evaluated based on overall survival, which was calculated by taking into account either recurrence, death, or the date of the last contact after the first operation. None of the patients examined had received radiotherapy, hormonal therapy, or chemotherapy prior to surgery.

Specimens were fixed in 10% formalin and embedded in paraffin wax. A review of the patient charts revealed that 84 patients received adjuvant chemotherapy, and 62 of them received gemcitabine hydrochloride (GEM) following surgery. Resectable vs borderline resectable was diagnosed based on preoperative images. Clinical outcome was evaluated based on overall survival status, which was calculated from the time of initial surgery to death or the date of the last contact. The mean follow-up time was 58 months (range, 8–136 months).

Immunohistochemistry

Pancreatic cancer samples of 96 Japanese patients who received surgical treatment were collected and fixed in 10% formalin, and tissues embedded in paraffin wax were used for immuno-tissue staining.

Goat polyclonal antibodies for CRH (C-20), CRHR1 (V-14), and CRHR2 (N-20) were purchased from Santa Cruz Biotechnology (Dallas, TX).

A Histofine Kit (Nichirei Biosciences, Tokyo, Japan), which employs the streptavidin-biotin amplification method, was used in this study. Antigen retrieval was performed by heating the slides in a microwave for 20 minutes in citric acid buffer (2 mM citric acid and 9 mM trisodium citrate dehydrate [pH 6.0]) for staining with anti-CRH and CRHR1. No

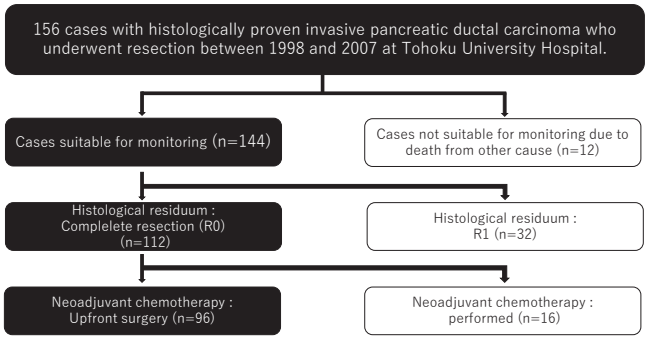

Figure 1. Survey target selection flow.

antigen retrieval was performed for CRHR2 immunostaining. The primary antibodies used in this study were diluted as follows: CRH, 1/50; CRHR1, 1/50; and CRHR2, 1/50.[24] The antigen–antibody complex was visualized with 3,3′-diaminobenzidine solution (1 mM 3,3′-diaminobenzidine, 50 mM Tris-HCl buffer [pH 7.6], and 0.006% H_2O_2) and counterstained with hematoxylin. Human placental tissue was used as a positive control for CRH, CRHR1, and CRHR2 immunostaining.[19] As a negative control, normal goat or mouse IgG was used instead of the primary antibody.

When CRH, CRHR1, and CRHR2 immunoreactivity was detected in the cytoplasm of the pancreatic carcinoma cells, cases with more than 10% positive carcinoma cells were considered positive for CRH, CRHR1, and CRHR2, respectively, and the percentage of immunoreactivity (labeling index) was determined. Cases with labeling index >10% were considered positive in this study.[25,26]

Statistical Analysis

The association of CRH, CRHR1, and CRHR2 immunohistochemical status with clinicopathological factors was evaluated using Student's *t*-test or cross-tabulation with the chi-squared test. Overall survival curves according to adjuvant therapy status were generated using the Kaplan–Meier method, and statistical significance was assessed using the log-rank test. Univariate and multivariate analyses were performed according to Cox's proportional hazards model. *P* values <.05 were considered significant. All statistical analyses were performed using SPSS 21.0J software (IBM Corp, Armonk, NY).

Ethical Considerations

Written informed consent for pathological examinations of resected tissue in past cases of pancreatic cancer was obtained from participants prior to the start of the study, and notice of their use in this study was posted on the websites of the relevant organizations. The research protocols for this study were approved by the Ethics Committee of Tohoku University School of Medicine (2012-1-204). All authors had access to the study data and reviewed and approved the final manuscript.

Results

Immunolocalization of CRH, CRHR1, and CRHR2 in Pancreatic Cancer

Figure 2 shows representative photos of positive cases for CRH, CRHR1, and CRHR2 immunostaining. The immunolocalization of CRH, CRHR1, and CRHR2 in pancreatic carcinoma tissues was the same as that in the previous report on endometrial carcinoma.[26] Immunostaining of pancreatic cancer tissue specimens obtained from 96 patients revealed that CRH, CRHR1, and CRHR2 were positive in 50 (52%), 47 (49%), and 43 (45%) cases, respectively. Table 1 shows the relationship between the immunohistochemical status of CRH and the clinicopathological factors of patients.

CRH immunoreactivity was significantly correlated with CRHR1 status, CRHR2 status, and lymph node metastasis ($P < .001$, $P < .001$, and $P = .032$, respectively). Furthermore, in the CRH-positive group, CRHR1-positive and CRHR2-positive expression was frequently observed, indicating associations among CHR, CRHR1, and CRHR2. We found no significant association of CRH status with age, sex, stage, postoperative chemotherapy status, and other factors such as CA19-9 levels (Table 1). CRH immunoreactivity in specimens obtained from the 84 patients who had received adjuvant chemotherapy (Table 2) as well as the 62 patients who received GEM as their adjuvant therapy (Table A1) showed significant associations with both CRHR1 and CRHR2 status ($P < .001$).

Association of CRH, CRHR1, and CRHR2 Status With Clinical Outcome in Pancreatic Cancer

Survival analysis using the Kaplan–Meier method revealed that overall survival was significantly shorter in the CRHR1-positive group than in the CRHR1-negative group ($P = .038$, $\chi^2 = 4.311$) (Figure 3A-2). In contrast, no significant difference was seen according to CRH or CRHR2 status. Of the 96 patients analyzed, 84 received adjuvant therapy, and a significant reduction in survival was seen in the CRHR1-positive group compared with the CRHR1-negative group ($P = .022$, $\chi^2 = 5.174$) (Figure 3B-2), but there was no significant difference according to CRH or CRHR2 status. GEM was the most frequently used adjuvant therapy (62 patients), and the analysis of those who received GEM showed that the CRHR1-positive group had a poorer prognosis compared with the CRHR1-negative group ($P = .046$, $\chi^2 = 3.991$), but there was no significant difference according to CRH or CRHR2 status. In the group where drugs other than GEM were used, the CRH-positive group had a poorer prognosis compared with the CRH-negative group ($P = .017$, $\chi^2 = 5.728$). There was no significant difference in postoperative survival according to CRH, CRHR1, or CRHR2 status in the 12 patients who did not undergo adjuvant therapy. In the analysis of all patients (Table 1), there was a significant association with the presence or absence of lymph node metastasis on CRH. Kaplan–Meier analysis was performed as a subset analysis, and the results showed that lymph node metastasis had a significantly worse prognosis. ($P = .007$, $\chi^2 = 7.291$).

Univariate analysis of overall survival using the Cox model showed that stage ($P < .001$), CA19-9 levels ($P =$

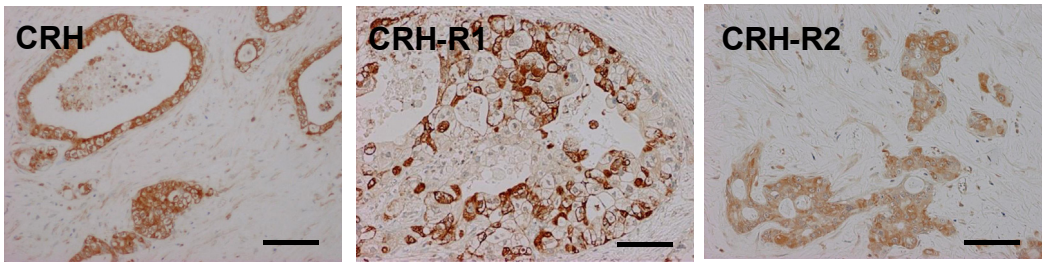

Figure 2. Representative photos of positive cases for CRH, CRHR1, and CRHR2 immunoreactivity in pancreatic cancer patients. Bar, 100 μm.

Table 1. Association Between CRH and Its Receptors Based on Immunoreactivity and Clinicopathological Parameters in 96 Pancreatic Cancer Patients

	CRH status			CRHR1 status			CRHR2 status		
	Positive	Negative		Positive	Negative		Positive	Negative	
Variables	n = 50	n = 46	*P*	n = 47	n = 49	*P*	n = 43	n = 53	*P*
Age, y	64.4 (9.8)	64.3 (10.6)	.95	66.5 (9.9)	62.3 (10.0)	.42	63.0 (12.3)	65.4 (7.9)	.28
Sex			.20			.39			.29
Male	29 (47%)	33 (53%)		28 (45%)	34 (55%)		25 (40%)	37 (60%)	
Female	21 (62%)	13 (38%)		19 (56%)	15 (44%)		18 (53%)	16 (47%)	
Diagnostic imaging			.11			.29			.19
Resectable	18 (42.9%)	24 (57.1%)		18 (42.9%)	24 (57.1%)		22 (52.4%)	20 (47.6%)	
Borderline resectable	32 (59.3%)	22 (40.7%)		29 (53.7%)	25 (46.3%)		21 (38.9%)	33 (61.1%)	
Histological stage			.10			.70			.88
IA	0 (0%)	2 (100%)		0 (0%)	2 (100%)		1 (150%)	1 (50%)	
IB	1 (20%)	4 (80%)		2 (40%)	3 (60%)		2 (40%)	3 (60%)	
IIA	10 (40%)	15 (60%)		13 (52%)	12 (48%)		9 (36%)	16 (64%)	
IIB	38 (61%)	24 (39%)		31 (50%)	31 (50%)		30 (48%)	32 (52%)	
III	1 (50%)	1 (50%)		1 (50%)	1 (50%)		1 (50%)	1 (50%)	
Adjuvant			.54			.59			.10
Received	45 (53%)	39 (47%)		42 (50%)	42 (50%)		37 (44%)	47 (56%)	
Not received	5 (42%)	7 (58%)		5 (42%)	7 (58%)		6 (50%)	6 (50%)	
Lymph node metastasis			**.03**			.67			.28
Present	38 (60%)	25 (40%)		32 (51%)	31 (49%)		31 (49%)	32 (51%)	
Absent	12 (36%)	21 (64%)		15 (45%)	18 (55%)		12 (36%)	21 (64%)	
CA19-9 preoperatively			.28			.79			1.00
≥37 U/mL	44 (55%)	36 (45%)		40 (50%)	40 (50%)		36 (45%)	44 (55%)	
<37 U/mL	6 (37.5%)	10 (62.5%)		7 (43.8%)	9 (56.3%)		7 (43.8%)	9 (56.3%)	
CA19-9 postoperatively (n = 92)	(n = 48)	(n = 44)	.53	(n = 45)	(n = 47)	1.00	(n = 40)	(n = 52)	.68
≥37 U/mL	28 (56%)	22 (44%)		24 (48%)	26 (52%)		23 (46%)	27 (54%)	
<37 U/mL	20 (47.6%)	22 (52.4%)		21(50%)	21 (50%)		17 (40.5%)	25 (59.5%)	
CRHR1			**<.001**						
Positive	39 (83%)	8 (17%)							
Negative	11 (22%)	38 (78%)							
CRHR2			**<.001**			**<.001**			
Positive	33 (77%)	10 (23%)		32 (74%)	11 (26%)				
Negative	17 (40%)	36 (40%)		15 (28%)	38 (72%)				

Association of CRH, CRHR1, and CRHR2 immunoreactivity with clinicopathological parameters was assessed by *t*-test, Pearson's chi-square test, or Fisher's exact test. $P < .05$ was considered significant and is indicated in boldface.
CA19-9 was considered positive above 37 U/mL. Data are presented as the mean (standard deviation) or n (%). All other values represent the number of cases and their percentage of positive and negative cases.

.004), and CRHR1 status ($P = .038$) were significant factors, and the subsequent multivariate analysis revealed that of these, stage and postoperative CA19-9 levels were borderline significant factors ($P = .02$ and .001, respectively) without multicollinearity (Table 3). Similar analyses were conducted of 84 patients in the adjuvant therapy group (Table 3) and the 62 patients who received GEM as their adjuvant group (Table A2). In the adjuvant therapy group,

Table 2. Association of Immunohistochemical CRH, CRHR1, and CRHR2 Status With Clinicopathological Parameters in 84 Pancreatic Cancer Patients Who Received Adjuvant Chemotherapy Postoperatively

	CRH status			CRHR1 status			CRHR2 status		
	Positive	Negative		Positive	Negative		Positive	Negative	
	n = 45	n = 39	*P*	n = 42	n = 42	*P*	n = 37	n = 47	*P*
Age, y	64.7 (9.7)	63.6 (10.3)	.62	66.4 (10.3)	62.0 (9.2)	**.04**	63.0 (12.7)	65.1 (7.0)	.32
Sex			.36			1.00			1.00
Male	27 (49.1%)	28 (50.9%)		27 (49.1%)	28 (50.9%)		24 (43.6%)	31 (56.4%)	
Female	18 (62.1%)	11 (37.9%)		15 (51.7%)	14 (48.3%)		13 (44.8%)	16 (55.2%)	
Diagnostic imaging			.15			.19			.16
Resectable	16 (44.4%)	20 (55.6%)		15 (41.7%)	21 (58.3%)		19 (52.8%)	17 (47.2%)	
Borderline resectable	29 (60.4%)	19 (39.6%)		27 (56.3%)	21 (43.8%)		18 (37.5%)	30 (62.5%)	
Histological stage			.18			.71			.53
IA	0 (0%)	1 (100%)		0 (0%)	1 (100%)		0 (0%)	1 (100%)	
IB	1 (25%)	3 (75%)		1 (25%)	3 (75%)		1 (25%)	3 (75%)	
IIA	8 (38.1%)	13 (61.9%)		13 (52.4%)	10 (47.6%)		7 (33.3%)	14 (66.7%)	
IIB	35 (62.5%)	21 (37.5%)		29 (51.8%)	27 (48.2%)		28 (50%)	28 (50%)	
III	1 (50%)	1 (50%)		1 (50%)	1 (50%)		1 (50%)	1 (50%)	
Lymph node metastasis			.06			.64			.10
Present	35 (61.4%)	22 (38.6%)		30 (52.6%)	27 (47.4%)		29 (50.9%)	28 (49.1%)	
Absent	10 (37.0%)	17 (63.0%)		12 (44.4%)	15 (55.6%)		8 (29.6%)	19 (70.4%)	
CA19-9 preoperatively			.10			.57			.78
≥37 U/mL	40 (58.0%)	29 (42.0%)		36 (52.2%)	33 (47.8%)		31 (44.9%)	38 (55.1%)	
<37 U/mL	5 (33.3%)	10 (66.7%)		6 (40.0%)	9 (60.0%)		6 (40%)	9 (60%)	
CA19-9 postoperatively (n = 80)	(n = 43)	(n = 37)	.50	(n = 40)	(n = 40)	1.00	(n = 34)	(n = 46)	.50
≥37 U/mL	25 (58.1%)	18 (41.9%)		21 (48.8%)	22 (51.2%)		20 (46.5%)	23 (53.5%)	
<37 U/mL	18 (48.6%)	19 (51.4%)		19(51.4%)	18 (48.6%)		14 (37.8%)	23 (62.2%)	
CRHR1			**<.001**						
Positive	35 (83.3%)	7 (16.7%)							
Negative	10 (23.8%)	32 (76.2%)							
CRHR2			**<.001**			**<.001**			
Positive	29 (78.4%)	8 (21.6%)		28 (75.7%)	9 (24.3%)				
Negative	16 (34.0%)	31 (66.0%)		14 (29.8%)	33 (70.2%)				

Association of CRH, CRHR1, and CRHR2 immunoreactivity with clinicopathological parameters was assessed by *t*-test, Pearson's chi-square test, or Fisher's exact test. $P < .05$ was considered significant and is indicated in boldface.
CA19-9 was considered positive above 37 U/mL. Data are presented as the mean (standard deviation) or n (%). All other values represent the number of cases and their percentage of positive and negative cases.

univariate analysis showed that CRHR1 status ($P < .022$), stage ($P = .008$), preoperative CA19-9 ($P = .048$), and postoperative CA19-9 levels ($P = .035$) were significant. In the GEM group, univariate analysis indicated that CRHR1 status ($P = .046$) and CA19-9 levels ($P = .035$) were significant, but none of these was shown to be an independent prognostic factor with a relative risk greater than 1.0 by multivariate analysis.

Discussion

This study is the first to immunolocalize CRH, CRHR1, and CRHR2 in pancreatic carcinoma tissues and to examine the biological prognosis. Although a previous report[11] stated that CRHR1 and CRHR2 are not expressed in pancreatic cancer tissues, there is another report[27] of immunohistostaining in pancreatic tumor tissue. The results of the present study demonstrate the expression of CRH, CRHR1, and CRHR2 in pancreatic carcinoma tissues, which suggests the importance of the CRH pathway in aggressive growth. This study also clarified the directionality of action for CRH in pancreatic cancer cells by assessing biological progression according to CRH expression. Immunohistological CRH status was positively associated with CRHR1 and CRHR2 status, and CRHR1 status was significantly associated with poor clinical outcomes. In endometrial cancer[28] and breast cancer,[29,30] CRH might promote proliferation of cancer cells via CRHR1. Inhibition of cell proliferation by CRH was counteracted in a concentration-dependent manner by the nonselective CRH receptor antagonist astressin as well as by the CRH-R1 selective receptor antagonist antalarmin in breast cancer cells.[29] Therefore, the effect of CRH via CRHR1 may be considered to be associated with an increased risk of recurrence and poor prognosis in patients with pancreatic cancer.

CRH has been shown to increase the expression of the Fas-ligand of the tumor-necrosis factor family via its receptor CRHR1.[31] Fas-ligands are expressed on the surface of cytotoxic T cells and bind to Fas-receptors to induce

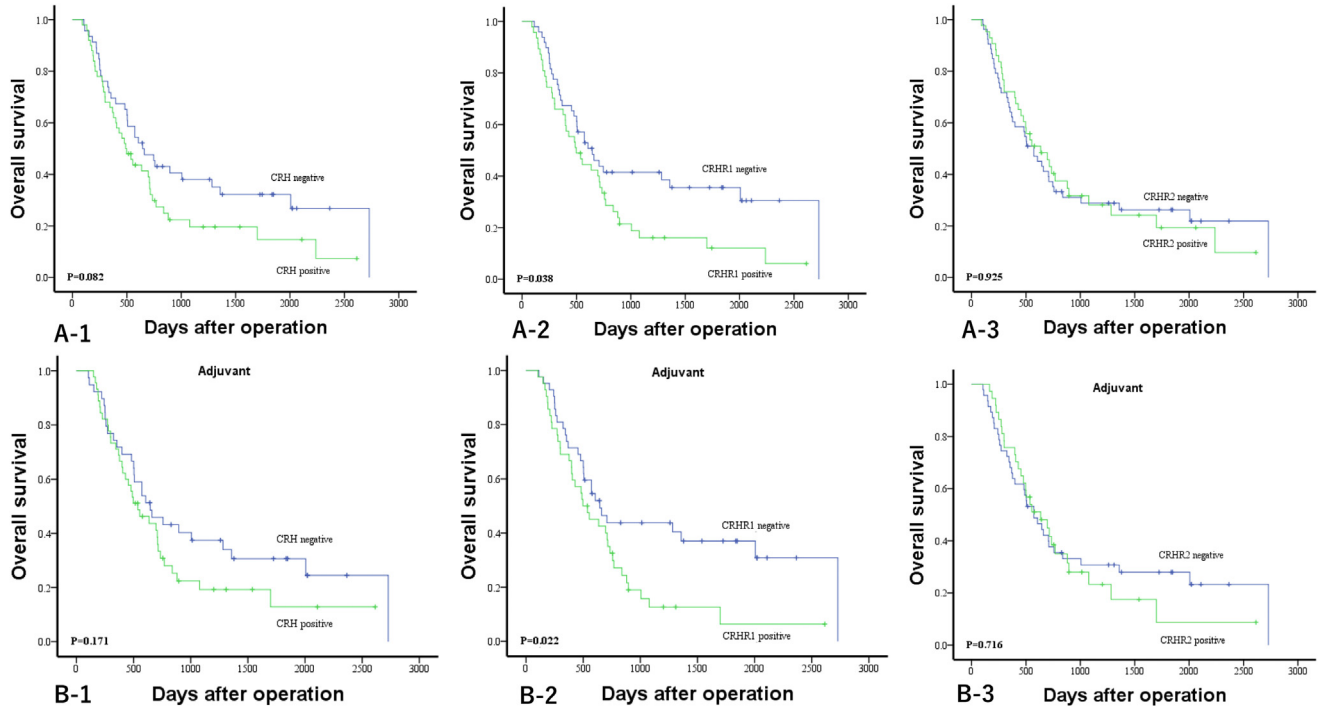

Figure 3. Overall survival of stage I–VI pancreatic cancer cases (Kaplan–Meier method). (A-1–A-3) Overall survival of 96 pancreatic cancer patients postoperatively, according to CRH, CRHR1, and CRHR2 immunoreactivity. (B-1–B-3) Overall survival of 84 pancreatic cancer patients who received adjuvant chemotherapy postoperatively, according to CRH, CRHR1, and CRHR2 immunoreactivity. CRHR1 immunoreactivity was significantly associated with an increased risk of poorer prognosis. Overall survival was worse in the CRHR1-positive group than in the CRHR1-negative group among all patients ($P = .038$) (A-2) and the adjuvant therapy group ($P = .022$) (B-2). Strong, circumscribed membrane staining of CRH, CRHR1, and CRHR1 in >10% carcinoma cells was considered positive. *P* values were obtained using the log-rank test.

apoptosis in cytotoxic T cells.[32] Fas-ligand receptor interactions promote cancer progression because cytotoxic T cells play a role in stopping cancer progression by injuring tumor cells. Minas reported that CRH–CRHR1 signaling might favor the survival and progression of the tumor in human ovarian cancer.[15] As a further possibility, CRH has been reported to promote the proliferation of the mouse breast cancer cell line 4T1 via the TGF-β signaling pathway in a time-dependent manner,[10] suggesting that CRH may be involved in the development and proliferation of cancer cells. The results of our previous study suggest that intratumoral CRH–CRHR1 signaling plays an important role in the progression of endometrial carcinoma and that CRHR1 is a potent prognostic factor in patients with this disease.[26] CRH-positive cases may have a poor prognosis because many in this population have positive lymph node metastases, a known factor for poor prognosis. According to the analyzed association of CRH, CRHR1, and PCRHR2 mRNA expression with overall survival in pancreatic cancer patients based on TCGA datasets,[33] the expression of CRHR1 is protective for survival. However, the TCGA datasets were derived from a disproportionate number of Caucasian patients with stage IIb cancer, and the treatments performed are unclear. The patients in our study were Japanese, did not undergo preoperative chemotherapy, and all cases were postoperative R0. We considered mainly the overall survival in patients treated with postoperative gemcitabine, and thus the conditions may not be the same as those in the TGCA data. Also, there are reports[10,12,26,29] that CRH acts on cancer growth, and the action of CRH-CRHR1 in living organisms remains unclear. Therefore, further studies are warranted.

It is worth noting that patients with positive CRH expression in pancreatic cancer cells had a higher distribution of lymph node metastasis compared with other patients in this study. However, this may not rule out the possibility that positive expression of CRH in pancreatic cancer tissues promotes tumor metastasis and consequently leads to a poor prognosis. Interestingly, Renz et al[34] reported that endogenous mouse models of pancreatic cancer showed prolonged survival when adrenalectomies were performed. Taken together with other related experiments, their findings suggest that stress-dependent sympathetic signaling can induce PDAC in preneoplastic lesions (ie, PanINs) and that the central nervous system may be involved in the influence of the macroenvironment on tumor biology. Thus, CRH–CRHR1 signaling may be related to several mechanisms of cancer cell proliferation and poor prognosis in pancreatic cancer.

The results of this study also suggest that the presence or absence of CRHR1 expression may be useful for predicting the prognosis of pancreatic cancer and that, in particular, it may suppress the effect of adjuvant

Table 3. Univariate and Multivariate Analysis of Overall Survival

	All patients (n = 96)			Received adjuvant chemotherapy postoperatively (n = 84)		
	Univariate	Multivariate		Univariate	Multivariate	
Variable	*P*	*P*	Relative risk (95% CI)	*P*	*P*	Relative risk (95% CI)
CRH status	.082	ND		.171	ND	
CRHR1 status	**.038**[a]	.071	1.55 (0.96–2.51)	**.022**[a]	.494	1.51 (0.46–4.92)
CRHR2 status	.925	ND		.716	ND	
Age (≥65/<64), y	.713	ND		.394	ND	
Sex (male/female)	.178	ND		.372	ND	
Lymph node metastasis (present/absent)	.053	ND		.232	ND	
Histological stage (1, 2/3)	.528	ND		.509	ND	
Diagnostic imaging (resectable/borderline resectable)	**.001**[a]	**.002**[a]	2.22 (1.34–3.68)	**.008**[a]	.678	1.27 (0.41–3.91)
CA19-9 (≥37 U/mL/<37 U/mL) preoperatively	.084	ND		**.048**[a]	.380	1.75 (0.50–6.159)
CA19-9 (≥37 U/mL/<37 U/mL) postoperatively	**.004**[a]	**.011**[a]	1.88 (1.15–3.06)	**.016**[a]	.268	1.78 (0.64–4.95)
Adjuvant therapy after surgery (received/not received)	.638	ND		–	–	–
GEM (received/not received)	.239	ND		.205	ND	

Data considered significant ($P < .05$) are in boldface.
Relative risks are presented as mean (95% CI).
CI, confidence interval; GEM, gemcitabine hydrochloride; ND, not determined.
[a]Significant ($P < .05$) and borderline-significant ($.05 < P < .10$) values were examined in the multivariate analyses in this study.

chemotherapy. The usefulness of neoadjuvant chemotherapy has been demonstrated in recent years in Japan,[35] and S-1 alone has been used as a first-line treatment.[36] However, at the time of sample collection in our study, adjuvant chemotherapy was the standard treatment; chemotherapy for pancreatic cancer was used to treat unresectable cases and as adjuvant therapy after resection. Therefore, the tissue samples used in this study were not affected by neoadjuvant chemotherapy. GEM is said to act on the cell nucleus and have a suppressive effect on cellular proliferation. Tadros et al[37] has reported that the lipid metabolism pathway is involved in the decreased responsiveness of pancreatic cancer to GEM. It has also been reported that in a mouse model of pancreatic cancer, increased expression of fatty acid synthase was associated with poor response to GEM and poor survival. In addition, the combined use of GEM and fatty acid synthase metabolic pathway inhibitors induced endoplasmic reticulum stress, which suppresses proliferation of cancer cells, resulting in increased response to GEM. For instance, activation of this pathway by CRH might accelerate the formation of macrophage foam cells and promote atherosclerosis, which is the main condition of cardiovascular disease.[38] Regarding the reactivity of GEM to pancreatic cancer, it may also be necessary to pay attention to the effect of lipid metabolism resulting from CRH expression.

This study has several limitations. First, due to its descriptive nature, no in vitro experiments were performed and no models were used in this study. Accordingly, additional studies are needed to elucidate the molecular functions of CRH–CRHR1 signaling that are associated with poor clinical outcomes in pancreatic cancer patients. Second, the determinants of CRH, CRHR1, and CRHR2 expression in pancreatic cancer cells are not known, and thus the mechanism by which pancreatic cancer cells mediate CRH signaling remains to be clarified. Third, because of the retrospective nature of this study, a prospective cohort study is needed to investigate the clinical importance of CRH–CRHR1 signaling.

We should determine whether the proposed correlation between CRH signaling and proliferation is indeed present, but we were unable to examine the proliferation rate of the pancreatic cancer cells in these tissues. Therefore, this study was unable to confirm the function associated with cancer growth, such as an association of CRH with the CRHR1-mediated proliferation of pancreatic cancer cells. Further examinations are required to clarify the biological functions of CRH in pancreatic cancer.

In multivariate analysis, CRHR1 did not reach the level of statistical significance, but the trend was maintained. This does not appear to be subordinate to other factors, so it may be that our study lacked the power to detect a truly significant difference. Future studies will need to be conducted with a larger sample size and a longer follow-up period.

Furthermore, there are other ligands beyond CRH for CRHR1, such as urocortin,[22] that were not investigated in this study.

Conclusion

The differences in survival observed in this study suggest that CRH plays a role in CRHR1-mediated cancer cell proliferation, the effects of chemotherapy in pancreatic

cancer patients, and the risk of poor survival. This study revealed that survival in patients with pancreatic cancer was significantly associated with expression of CRHR1 by assessing biological progression according to CRH and the expression of its receptors. However, CRHR1 expression was correlated with survival in univariate analysis but not in multivariate analysis, suggesting the need for further studies to elucidate the relationship.

Supplementary Material

Material associated with this article can be found in the online version at https://doi.org/10.1016/j.gastha.2022.09.003.

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The corresponding author, on behalf of all authors, jointly and severally, certifies that their institution has approved the protocol for any investigation involving humans or animals and that all experimentation was conducted in conformity with ethical and humane principles of research.

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