HER2 overexpression correlates with survival after curative resection of pancreatic cancer

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HER2 overexpression has been linked to clinical outcomes in several solid tumors, such as breast cancer. However, the correlation between HER2 overexpression and survival in pancreatic carcinoma remains unclear. The impact of HER2 overexpression on survival in pancreatic ductal cancer was examined. Immunohistochemical staining of 129 pancreatic cancers without hematogenous metastases or peritoneal dissemination treated by macroscopically curative resection were analyzed in association with survival data. To determine HER2 overexpression in this pancreatic cancer series, the polyclonal antibody included in HercepTest, which is used worldwide for clinical examination of HER2 overexpression in breast cancer, was used. Immunoreactivity was classified according to the scale presented in the HercepTest Scoring Guidelines. Twenty-two cases (17.1%) had a score of 0, 28 cases (21.7%) had of a score of 1+, 41 cases (31.8%) had a score of 2+, and 38 cases (29.4%) had a score of 3+. Therefore, HER2 overexpression (score 2+ or 3+) was observed in 79 cases (61.2%). Patients with HER2 overexpression tumors had significantly shorter survival times than those with HER2 normal expression (score 0 or 1+) tumors (median survival time, 14.7 *vs* 20.7 months, respectively; $P = 0.0078$ on the log-rank test). On multivariate survival analysis, HER2 overexpression remained an independent prognostic factor (hazard ratio, 1.806; $P = 0.0258$). A significant percentage of pancreatic cancers were demonstrated to have HER2 overexpression, and overexpression of this tyrosine kinase receptor proved to be an independent factor for a worse prognosis. These results should encourage further investigation of treatments using new molecular targeting agents against HER2 protein to improve the survival of pancreatic cancer patients. (*Cancer Sci* 2009; 100: 1243–1247)

The *HER2* gene, known as ErbB-2/neu, is located on the long arm of chromosome 17 (17q12-21.32) and encodes the 185-kDa transmembrane tyrosine kinase receptor.[1] HER2 is recognized as a member of the epidermal growth factor receptor family of transmembrane receptors encoded by erbB1 (HER1 or EGFR), erbB3 (HER3), and erbB4 (HER4).[2] EGFR is the coreceptor for the formation of dimers with HER2. After a ligand binds to a single-chain EGFR, the receptor forms a dimer with HER2 that signals within the cell by activating receptor autophosphorylation through tyrosine kinase activity.[2] These dimers play a role in tumor cell proliferation, survival, adhesion, and migration.[3]

Many studies have demonstrated that HER2 overexpression is correlated with aggressiveness in breast,[4–8] lung,[9] and gastric carcinomas.[10,11] However, investigations correlating HER2 overexpression with survival in pancreatic carcinoma have been very limited.[12–14] The objective of this study was to examine the impact of HER2 overexpression on the survival of patients with pancreatic ductal carcinoma.

Materials and Methods

Patients. Pancreatic cancer tissue samples were obtained from 129 patients who underwent macroscopically curative resection at the Department of Surgical Oncology, Osaka City University Hospital, from January 1983 to December 2007. The age of the pancreatic cancer patients was 66.2 ± 10.1 years (mean ± SD), with a range of 33–85 years. The median follow-up period was 18.3 months, with a range of 3–129 months. The patients' tumor characteristics are shown in Table 1. No patients had hematogenous metastases or peritoneal dissemination. The stages of the tumors classified by the International Union against Cancer[15] were: stage I, 12 cases; stage II, 99 cases; stage III, nine cases; and stage IV, nine cases. All stage IV cases in this study were classified as due to the presence of metastases to paraaortic lymph nodes (not regional lymph nodes), which were clearly dissected at surgery.

Table 1. Tumor characteristics

Characteristics	Number
Tumor location	
Head/whole	88
Body/tail	41
Tumor size	
≤2 cm	20
>2 cm	109
Tumor differentiation	
Grade 1	44
Grade 2	64
Grade 3	17
Grade 4	4
T category	
T1	7
T2	18
T3	93
T4	11
N category	
N0	50
N1	79

Tumor differentiation is according to the Digestive System Tumors Classification of the World Health Organization (2000). Grade 1, well differentiated; grade 2, moderately differentiated; grade 3, poorly differentiated; and grade 4, undifferentiated. TNM classification is according to the International Union against Cancer (UICC, 2002).

Immunohistochemistry. Formalin-fixed, paraffin-embedded tissue was prepared in 4-μm-thick sections. Immunohistochemical staining for HER2 was performed using the avidin-biotin-peroxidase complex method. In brief, the deparaffinized and hydrated tissues were heated for 40 min at 95–99°C in Target Retrieval Solution (Dako, Carpinteria, CA, USA). After 40 min of incubation, the slides were allowed to cool for 20 min on the counter in the

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Table 2. Distribution of positive staining of cancer cells stratified by immunohistochemical intensities of HER2 in pancreatic cancer

Positive staining of cancer cells	HER2 staining intensities			
	Strong	Weak/moderate	Faint/barely	No staining
≥75%	25	29	12	0
50–74%	13	12	11	0
25–49%	0	0	4	0
10–24%	0	0	1	0
1–9%	0	0	0	0
0%	0	0	0	22

Numbers express tumors with dominant staining intensity.

Target Retrieval Solution at 25°C. The slides were incubated overnight at 4°C with the recommended dilution of 1:300 of the antihuman c-erbB-2 rabbit polyclonal antibody (Dako, Copenhagen, Denmark), which is the same antibody included in HercepTest (Dako, Carpinteria, CA, USA). All slides were examined by two of the authors (MK and BN) who were blinded to the clinical data. Final evaluations of ambiguous cases were decided after discussion between the two authors. For the determination of HER2 protein immunoreactivity, only the membrane staining intensity and pattern were evaluated according to the scale presented on the HercepTest Scoring Guidelines: score 0, no staining is observed, or membrane staining is observed in less than 10% of the tumor cells; score 1+, faint/barely perceptible membrane staining is detected in more than 10% of tumor cells (the cells exhibit incomplete membrane staining); score 2+, weak or moderate complete membrane staining is observed in more than 10% of tumor cells; and score 3+, strong complete membrane staining is observed in more than 10% of tumor cells.

Scores of 0 and 1+ were considered to be negative for HER2 expression, while 2+ and 3+ were considered to be positive (overexpression).

Statistical analysis. The χ^2-test (Fisher's exact test) was used to compare the prevalence or distributions of two variables. Student's *t*-test was used to compare mean values between two groups. Survival data were estimated by the Kaplan–Meier method, and the log-rank test was used for univariate survival analysis. The Cox proportional hazards model was used for the multivariate survival analysis. A P-value < 0.05 was considered statistically significant.

Results

HER2 expression. Typical immunostaining patterns for HER2 in cancer cells are shown in Fig. 1. Overall, 22 cases (17.1%) had score 0, 28 cases (21.7%) had score 1+, 41 cases (31.8%) had score 2+, and 38 cases (29.4%) had score 3+. Thus, 79 cases (61.2%) were positive (scores of 2+ and 3+) for HER2 expression. The cytoplasms of pancreatic duct cells and acinar cells were faint/barely stained in 35 cases and not stained in 94 cases. The membranes of pancreatic duct cells and acinar cells were not stained in all cases.

The HercepTest Scoring Guidelines are usually adopted for breast cancer. To evaluate the details of immunohistochemical staining for HER2 in pancreatic cancer, the distributions of percentages of HER2 staining stratified by HER2 intensities were examined. Most tumors showed unified staining intensity. All tumors with strong and weak/moderate intensities showed positive staining in at least 50% of cancer cells. With respect to faint/barely staining, 82% (23/28) of tumors had positive staining in at least 50% of cancer cells (Table 2).

Correlation between HER2 expression and clinicopathological factors. Tumor size, tumor location, tumor differentiation, histological findings, and T category were not correlated with HER2 expression. There was a significant difference in HER2-positivity between the N0 and N1 groups; N1 tumors had significantly higher positivity of HER2 expression than N0 tumors (Table 3).

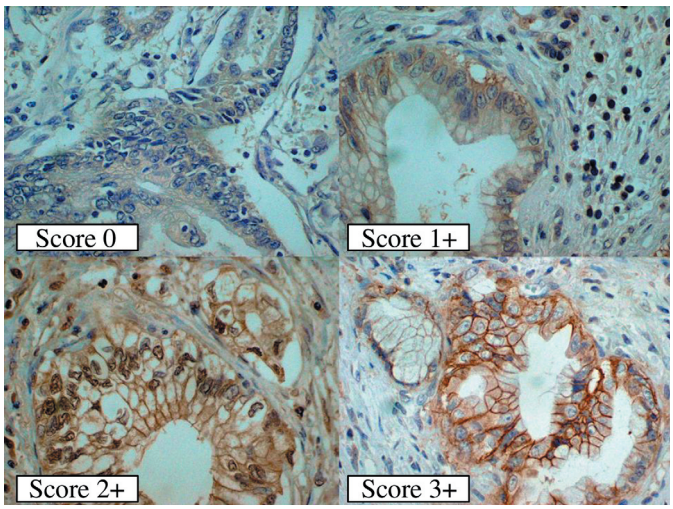

Fig. 1. Representative HER2 staining quantified as scores 0 to 3+ according to the HercepTest Scoring Guidelines.

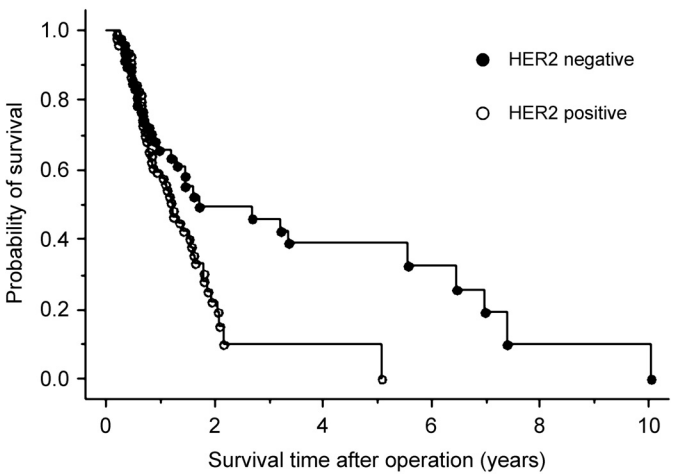

Fig. 2. Probability of survival for patients with resectable pancreatic cancer in relation to HER2 expression in tumor cells. A statistically significant difference in survival was observed between patients with HER2-positive and HER2-negative tumors (P = 0.0078). Closed circle, HER2-negative; open circle, HER2-positive.

Impact of HER2 overexpression and tumor characteristics on survival. Univariate analysis demonstrated that the median survival time (MST) of patients with HER2-positive tumors was 14.7 months, whereas that of the HER2-negative cases was 20.7 months (P = 0.0078) (Fig. 2; Table 4). There were no statistically significant

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Table 3. Association between HER2 expression and clinicopathological factors in resectable pancreas cancer

Variable	HER2		*P*-values
	Negative	Positive	
Tumor location			
Head/whole	51	37	0.2618
Body/tail	28	13	
Tumor size			
≤2 cm	9	11	0.5332
>2 cm	41	68	
Tumor differentiation†			
Grade 1/2	40	68	0.3624
Grade 3/4	10	11	
Cancer-stroma relationship‡			
Scirrhous	21	30	0.6487
Intermediate/medullary	29	49	
Growth patterns of tumor infiltrating surrounding tissue‡			
INFα/β	42	64	0.6658
INFγ	8	15	
Lymphatic invasion‡			
ly0/1	26	37	0.5675
ly2/3	24	42	
Venous invasion‡			
v0/1	40	69	0.2617
v2/3	10	10	
Intrapancreatic nerve invasion‡			
ne0/1	19	38	0.2604
ne2/3	31	41	
T category§			
T1/T2	14	11	0.5492
T3/T4	65	39	
N category§			
N0	25	25	0.0482
N1	52	25	

P-values were examined by χ^2-test.
†Tumor differentiation is according to Digestive System Tumors Classification of the World Health Organization (2000).
Grade 1, well differentiated; grade 2, moderately differentiated; grade 3, poorly differentiated; and grade 4, undifferentiated.
‡Histological findings are according to the classification of pancreatic carcinoma in Japan Pancreatic Carcinoma (2nd ed., 2003).
§TNM classification is according to the International Union against Cancer (UICC, 2002).
INFα/β, infiltration alpha and beta; INFγ, infiltration gamma.

Table 4. Univariate survival analyses in resectable pancreatic cancer

Variable	Comparison	Patients	Median survival (months)	*P*-values
HER2	Positive:Negative	79:50	14.7:20.7	0.0078
Tumor location	Head/whole:body/tail	88:41	17.9:9.7	0.5334
Tumor size	>2 cm:≤2 cm	109:20	14.5:20.3	0.1055
Tumor differentiation	Grade 3/4:Grade 1/2	21:108	11.5:17.7	0.8162
T category	T3/4:T1/2	104:25	14.9:60.9	0.0092
N category	N1:N0	79:50	14.5:19.4	0.2437

P-values were examined by the log-rank test.
Tumor differentiation is according to the Digestive System Tumors Classification of the World Health Organization (2000).
Grade 1, well differentiated; grade 2, moderately differentiated; grade 3, poorly differentiated; and grade 4, undifferentiated.
TNM classification is according to the International Union against Cancer (UICC 2002).

differences in treatments between the HER2-positive and -negative cases (Table 5). The MST of patients with T3-T4 was 14.9 months, whereas that of patients with T1-T2 was 60.9 months ($P = 0.0092$) (Table 4).

On multivariate survival analysis, HER2-positive and T3-T4 were independent prognostic factors (Table 6).

Discussion

A wide range, from 16% to 69%, of pancreatic cancers, has been reported to demonstrate HER2 overexpression.[12–14,16,17] In the present series, HER2 overexpression was observed in 61.2% of the cases. The substantially different percentages of HER2 overexpression among the reports may be attributed to the differences in the procedures used for immunohistochemistry, antibodies, and determination criteria of HER2 overexpression, as well as the patients' racial differences.

In the present study, antihuman c-erbB-2 rabbit polyclonal antibody, which is included in the HercepTest kit, was used for immunohistochemistry, and the HercepTest Scoring Guidelines were used to determine HER2 overexpression. HER2 is known as a transmembrane receptor; therefore, only membrane staining

Table 5. Comparison of treatment background by HER2 expression

Treatment	HER2		*P*-values
	Negative	Positive	
Surgery			0.2454
Pancreaticoduodenectomy	35	46	
Distal pancreatectomy	14	27	
Total pancreatectomy	1	6	
Chemotherapy			0.1351
None	29	32	
Adjuvant oral 5-FU derivative	17	30	
Adjuvant intravenous gemcitabine	2	9	
Neoadjuvant + adjuvant intravenous 5-FU	2	8	
Intraoperative radiotherapy			0.8021
Not done	44	67	
Done	6	12	
External radiotherapy			0.6989
Not done	45	68	
Done	5	11	

P-values were examined by χ^2-test.

Table 6. Multivariate survival analyses in resectable pancreatic cancer

Variable	Comparison	Hazard ratio	*P*-values	95% confidence interval
HER2 expression	2–3:0-1	1.806	0.0258	1.074–3.036
Tumor location	head:body + tail	1.232	0.4516	0.715–2.123
Tumor size	>2 cm:≤2 cm	1.297	0.4464	0.664–2.536
Tumor differentiation	Grade 3/4:Grade 1/2	1.009	0.9781	0.540–1.885
T category	T3-T4:T1-T2	2.493	0.0188	1.163–5.341
N category	N1:N0	1.04	0.8714	0.650–1.663

P-values were examined by the Cox proportional hazard model for multivariate survival analysis.
Tumor differentiation is according to Digestive System Tumors Classification of the World Health Organization (2000).
Grade 1, well differentiated; grade 2, moderately differentiated; grade 3, poorly differentiated; and grade 4, undifferentiated.
TNM classification is according to the International Union against Cancer (UICC, 2002).

should be evaluated as positive for HER2 overexpression, as described in the HercepTest Scoring Guidelines. In the present series, most pancreatic cancer cells showed unified intensities of HER2 staining (Table 2). Therefore, the criterion of the HercepTest Scoring Guidelines, which requires at least 10% positive cancer cells, was easily met in this investigation of pancreatic cancer.

Few studies have attempted to examine the impact of HER2 overexpression on survival in pancreatic cancer patients, and they resulted in inconsistent conclusions. Lei *et al*. demonstrated that 10 of 21 (47.6%) pancreatic cancers, including 10 unresectable tumors, showed HER2 overexpression. In their series, patients with HER2 overexpression had shortened survival (7.3 ± 3.8 months for the overexpression group *vs* 19.1 ± 11.7 months for the no overexpression group).[12] Yamanaka *et al*. showed that HER2 overexpression was observed in 45% of 76 pancreatic cancer patients who underwent surgery, though they did not describe how many tumors underwent curative surgery. They examined the survival period of part of their series (53 patients); patients with HER2 overexpression cancers had shorter survival (10.8 ± 1.5 months) than patients with HER2 non-overexpression cancers (12.4 ± 0.8 months), but the difference between the groups was not statistically significant.[13] Koka *et al*. immunohistochemically investigated the HER2 status of 308 pancreatic adenocarcinomas and found that only 48 tumors (16%) had HER2 overexpression. Their survival results were contrary to those previously reported; the mean survival was 11 months in the HER2 overexpression group and 7 months in the HER2 non-overexpression group.[14] In the paper by Koka *et al.* the number of patients who underwent operation was not clear, and the details of other treatments, such as chemotherapy and radiation therapy, were not reported. The present investigation focused on the prognostic power of HER2 overexpression in pancreatic cancer that was resected in a macroscopically curative manner. HER2 overexpression may prompt cancer-cell proliferation, blocking apoptosis, activating invasion and metastasis, and stimulating tumor-induced neovascularization.[2] In resectable pancreatic cancer, microscopic residual tumor cells with HER2 overexpression may have worse biological behavior than those that are HER2-negative, resulting in shorter patient survival. However, patients with unresectable pancreatic cancer usually have such a poor prognosis that the survival difference between HER2-positive and -negative cases may be very small.

HER2 overexpression was marginally correlated with N category. To the best of our knowledge, the correlation between these two features has not been reported in pancreatic cancer or in breast cancer. In any case, the univariate analysis demonstrated that N category had no impact on survival (Table 4). These results suggested that the impact of HER2 overexpression on survival might be independent of N category. In fact, the multivariate analysis including N category indicated that HER2 overexpression was an independent indicator of short survival (Table 6).

The importance of coevaluation of *HER2* gene amplification using fluorescence *in situ* hybridization (FISH) analysis with immunohistochemistry has been emphasized to predict disease progression and poor clinical outcome in breast cancer.[18] However, to date, it has been reported that there was no association between *HER2* gene amplification and survival in pancreatic cancer.[19,20] Therefore, *HER2* gene amplification was not examined as a survival

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indicator in the present study. HER2 overexpression might occur at the transcriptional and/or translational levels in pancreatic cancer.

In breast cancer, HER2 is used to forecast a patient's therapeutic response to and select patients for anti-HER2 monoclonal antibody (trastuzumab) immunotherapy. Treatment with trastuzumab has been shown to reduce tumor volume, augment chemotherapeutic effects, and enhance survival rates in both primary and metastatic breast cancer patients.[21] Recently, Kimura *et al*. reported the effect of trastuzumab on pancreatic cancer with a high level of HER2 expression *in vitro* and in an *in vivo* experimental setting. They demonstrated that trastuzumab had no antiproliferative effect in any of 16 pancreatic cancer cell lines in an *in vitro* experimental setting. However, xenografts in nude mice made by inoculation of a HER2 overexpression cell line (Capan-1) were suppressed by trastuzumab. They suggested that the mechanism might be antibody-dependent, cell-mediated cytotoxicity (ADCC).[22] As ADCC is dependent on the patient's immunocompetence, the efficacy of trastuzumab monotherapy may be insufficient for some patients. In fact, a phase II clinical trial of trastuzumab for pancreatic cancer showed only a 6% response rate, a median survival time of 7 months, and a 1-year survival of 19% by the combined therapy with trastuzumab and gemcitabine in patients with metastatic pancreatic cancer. This trial enrolled 34 tumors with 2+/3+ HER2 expression on immunohistochemistry.[23] The efficacy of the combined therapy was similar to that of the gemcitabine monotherapy.

This clinical result may not rule out the potential clinical efficacy for patients with HER2 overexpression pancreatic cancer of other HER2 targeted therapeutic agents, such as lapatinib, which is a small molecule agent targeted for HER2 and EGFR. Lapatinib blocks downstream signaling pathways of its target receptors through inhibition of the autophosphorylation sites on these receptors.[24] Consequently, this agent is expected to have direct antiproliferative effects on cancer cells. A clinical trial demonstrated that trastuzumab-failure metastatic breast cancer with HER2 overexpression showed a significantly better clinical outcome with lapatinib plus capecitabine treatment than capecitabine alone.[25] In pancreatic cancer, a phase II study for unresectable pancreatic cancer using lapatinib has been carried out in the USA.

In conclusion, the present results demonstrated that HER2 overexpression was an indicator of a poor prognosis in curatively resected pancreatic cancer patients. Further investigation is recommended to examine the prognostic impact of HER2 expression on unresectable pancreatic cancer using tissue obtained from endoscopic ultrasound-guided, fine-needle aspiration biopsy. Developing new molecular target therapy against HER2 may be one possible strategy for the treatment of such patients.

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