

Potential crosstalk between insulin-like growth factor receptor type 1 and epidermal growth factor receptor in progression and metastasis of pancreatic cancer

Shigeto Ueda¹, Kazuo Hatsuse¹, Hitoshi Tsuda², Sho Ogata³, Nobuaki Kawarabayashi¹, Toshimichi Takigawa¹, Takahiro Einama¹, Daisaku Morita¹, Kazuhiko Fukatsu^{1,5}, Yoshiaki Sugiura⁴, Osamu Matsubara² and Hidetaka Mochizuki¹

¹Department of Surgery I, National Defense Medical College, Tokorozawa, Saitama, Japan; ²Department of Pathology II, National Defense Medical College, Tokorozawa, Saitama, Japan; ³Department of Laboratory Medicine, National Defense Medical College, Tokorozawa, Saitama, Japan; ⁴Department of Surgery II, National Defense Medical College, Tokorozawa, Saitama, Japan and ⁵Department of Basic Traumatology, National Defense Medical College, Tokorozawa, Saitama, Japan

The insulin-like growth factor receptor type 1 (IGF1R) and epidermal growth factor receptor (EGFR) are reportedly overexpressed in pancreatic cancer. However, the correlation between activated EGFR and IGF1R and their clinicopathological implications still remain unclear. The cellular localization and overexpression of IGF1R and EGFR were investigated immunohistochemically in primary invasive ductal pancreatic carcinomas obtained from 74 patients who underwent radical surgical resection. We also compared the status of IGF1R and EGFR overexpression between primary tumors and hepatic metastatic tumors obtained from 44 autopsied patients. Among the 74 surgically resected primary tumors, cytoplasm- and membrane-dominant EGFR overexpression was detected in 22 (30%) and 7 (9%), respectively, whereas cytoplasm- and membrane-dominant IGF1R overexpression was detected in 8 (11%) and 28 (38%), respectively. Membrane-dominant EGFR and cytoplasm-dominant IGF1R were more frequent in lower-grade tumors and correlated with favorable prognosis, whereas cytoplasm-dominant EGFR and membrane-dominant IGF1R were more frequent in higher-grade tumors and correlated with poor prognosis. In 36 autopsy specimens of pancreatic tumor with concurrent overexpression of IGF1R and EGFR, there was an inverse correlation between the IGF1R and EGFR localization patterns ($P=0.001$). In the hepatic metastatic tumors obtained by autopsy, the incidences of both IGF1R and EGFR overexpression were much higher than in the surgically resected primary tumors. More than half of the autopsy cases consistently showed membrane-dominant EGFR expression in both the primary tumor and hepatic metastases, whereas IGF1R expression showed considerable variation. Crosstalk between differently localized IGF1R and EGFR might play a role in determining the biological aggressiveness of pancreatic cancer, although their cellular localization may often alter during the process of metastasis.

Modern Pathology (2006) 19, 788–796. doi:10.1038/modpathol.3800582; published online 31 March 2006

Keywords: EGFR; IGF1R; immunohistochemistry; pancreatic cancer

Insulin-like growth factor receptor type 1 (IGF1R), a tyrosine kinase receptor belonging to the insulin receptor family, is overexpressed in a variety of human cancers, including pancreatic cancer. IGF1R signaling plays a pivotal role in cellular transforma-

tion, tumorigenesis and tumor vascularization through the mitogen-activated protein (MAP) kinase and phosphatidylinositol 3-kinase (PI3K) pathways.^{1–6} In addition, the ligand-binding activation of IGF1R through the PI3K signaling pathway is known to promote proliferation in some cancer cell lines, and to protect many different cell types from a variety of proapoptotic damage.^{3,4,7,8–10} Among the tyrosine kinase receptor families, the mechanism of action of the epidermal growth factor receptor (EGFR) family, especially EGFR and HER-2 (c-erbB2), is that which has been best characterized.^{11,12} EGFR

Correspondence: Dr H Tsuda, MD, Department of Pathology II, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan.

E-mail: htsuda@ndmc.ac.jp

Received 14 November 2005; revised 9 February 2006; accepted 10 February 2006; published online 31 March 2006

and HER-2 are also thought to be involved in the development of cancers. Many studies have shown that overexpression of EGFR and HER-2 is correlated with advanced disease stage, low patient survival rate, development of tumor metastasis and acquisition of chemoresistance.^{13–15} As is the case for the IGF1R signaling pathway, the EGFR signaling pathway is also involved in cellular transformation, tumorigenesis and tumor vascularization. However, differences between the biological behaviors mediated by these two signaling pathways still remain unclear. Recently, Lu *et al*¹⁶ suggested that the acquisition of resistance to trastuzumab anti-HER-2 therapy by HER-2-overexpressing metastatic breast cancer might be caused by the switching of signaling pathways. In the presence of trastuzumab, the growth signal conveyed through the HER-2-mediated pathway might be repressed, and bypassed via the alternatively upregulated IGF1R pathway. It has been shown that EGFR or HER-2 is overexpressed in 10–30% of pancreatic cancers.¹³ EGFR overexpression was associated with a high risk of death, whereas HER-2 overexpression was correlated with glandular differentiation and early oncogenesis.^{17–19} Previously, we reported that the clinical implication of cytoplasmic EGFR overexpression was distinct from that of membrane EGFR overexpression in pancreatic cancer, the former being correlated with a higher tumor grade and poorer patient prognosis than the latter.²⁰ EGFR overexpression in pancreatic cancer is therefore expected to be a potential therapeutic target, and a multicenter phase II trial of cetuximab, a monoclonal antibody targeting EGFR, in combination with gemcitabine, for advanced pancreatic cancer is underway.²¹ In view of the apparently alternating expression of the IGF1R and EGFR pathways in breast cancer, we speculated that there might be a link between IGF1R and EGFR overexpression, and that both might be correlated with clinical aggressiveness of pancreatic cancer cells.

In the present study using immunohistochemistry, we studied the incidence of membrane and cytoplasm overexpression of IGF1R, and its correlation with that of EGFR, in relation to clinicopathological parameters in surgically resected archival specimens of 74 primary pancreatic cancers. Furthermore, we compared the incidence and localization of IGF1R and EGFR overexpression between the primary pancreatic tumors and corresponding hepatic metastases in 44 individual autopsy cases of pancreatic cancer.

Materials and methods

Patients and Tumor Specimens

The subjects of this study were 74 patients who underwent radical surgical resection of primary pancreatic cancer between 1988 and 2001 at the National Defense Medical College Hospital,

Tokorozawa, Japan. The clinicopathological characteristics of these cases are summarized in Table 1a. Pancreatoduodenectomy was performed on 61 patients with pancreatic head cancer, and distal or total pancreatectomy was performed on another 13 patients in whom the cancer was located in the pancreatic body or tail. The clinical stages of the disease were I, II, III, and IV in 4, 13, 24, and 33 patients, respectively. In addition, primary tumors

Table 1 Clinicopathological characteristics of cases

Parameter	Number (n = 74) (%)
<i>(a) Surgical specimens</i>	
Age (mean ± s.d., year)	62.3 ± 10.5
≥ 65	34 (46)
< 65	40 (54)
Gender	
Male	56 (76)
Female	18 (24)
Site	
Head	61 (82)
Body or/and tail	13 (18)
Stage	
I	4 (5)
II	13 (18)
III	24 (32)
IV	33 (45)
Grade	
1	12 (16)
2	35 (47)
3	27 (36)
Median survival (months)	19.3
Parameter	Number (n = 44) (%)
<i>(b) Autopsy specimens</i>	
Age (mean ± s.d., year)	62.1 ± 32.1
< 65	24 (55)
≥ 65	20 (45)
Gender	
Male	30 (68)
Female	14 (32)
Primary pancreatic tumor	
Grade	
1	6 (14)
2	9 (20)
3	29 (66)
Hepatic metastatic tumor	
Grade	
1	3 (7)
2	18 (41)
3	23 (52)
Chemotherapy before death	
Yes	11 (25)
No	15 (34)
Unknown	18 (41)
Median survival (months)	4.9

s.d., standard deviation.

and hepatic metastatic tumors were obtained at autopsy from 44 other patients who had died of inoperable pancreatic cancer between 1975 and 2002 at the same hospital (Table 1b). To ensure accuracy in determining that tumors were true hepatic metastases from primary pancreatic cancer, we excluded patients whose pancreatic tumors showed direct invasion to the liver and patients with double primary cancers that had developed simultaneously in the pancreas and another organ.

Using these tumor specimens from a total of 118 patients, formalin-fixed paraffin-embedded tissue blocks were prepared and sections were cut and stained with hematoxylin and eosin (HE) for routine histopathological examination. All of the cases were diagnosed histologically as ductal adenocarcinomas of the pancreas. After a histological review of the sections by two observers (SU, HT), one representative tissue block of the primary tumor and, if present, a hepatic metastatic tumor, were selected per case.

Histological Classification

The two observers graded the degree of tumor differentiation according to the criteria stipulated in the TNM classification of the International Union Against Cancer (UICC). Tumor differentiation was subclassified into Grade 1 (well differentiated type), Grade 2 (moderately differentiated type), and Grade 3 (poorly differentiated type), according to the degree of tubule formation. The grade of each resected primary tumor specimen was decided according to the grade evident in the widest cross-sectional area.

Immunohistochemical Detection of IGF1R and EGFR

Antibodies used for the analysis were a mouse monoclonal anti-EGFR antibody (Clone 31G7, diluted 1:200; Zymed Lab. Inc., San Francisco, CA, USA) and a rabbit polyclonal anti-IGF1R antibody (ready to use; NeoMarkers Inc., Fremont, CA, USA). Briefly, 5- μ m-thick tissue sections were deparaffinized and subjected to antigen retrieval, which involved pretreatment with 0.1% type XXIV protease (Sigma, St Louis, MO, USA) for 20 min at 37°C for anti-EGFR, and microwave treatment in 10 mM sodium citrate (pH 6.0) for 15 min at 95°C for anti-IGF1R. The sections were treated with 5% hydrogen peroxide for 5 min to quench endogenous peroxidase activity, followed by incubation with 3.0% skim milk for 10 min at room temperature to suppress nonspecific staining. Subsequently, the sections were incubated with the primary antibody at 4°C overnight, and then with dextran polymer reagent conjugated with the secondary antibody and preoxidase (Envision Plus; Dakocytomation, Glostrup, Denmark) for 2 h at room temperature. The immunoreaction was visualized by immersing

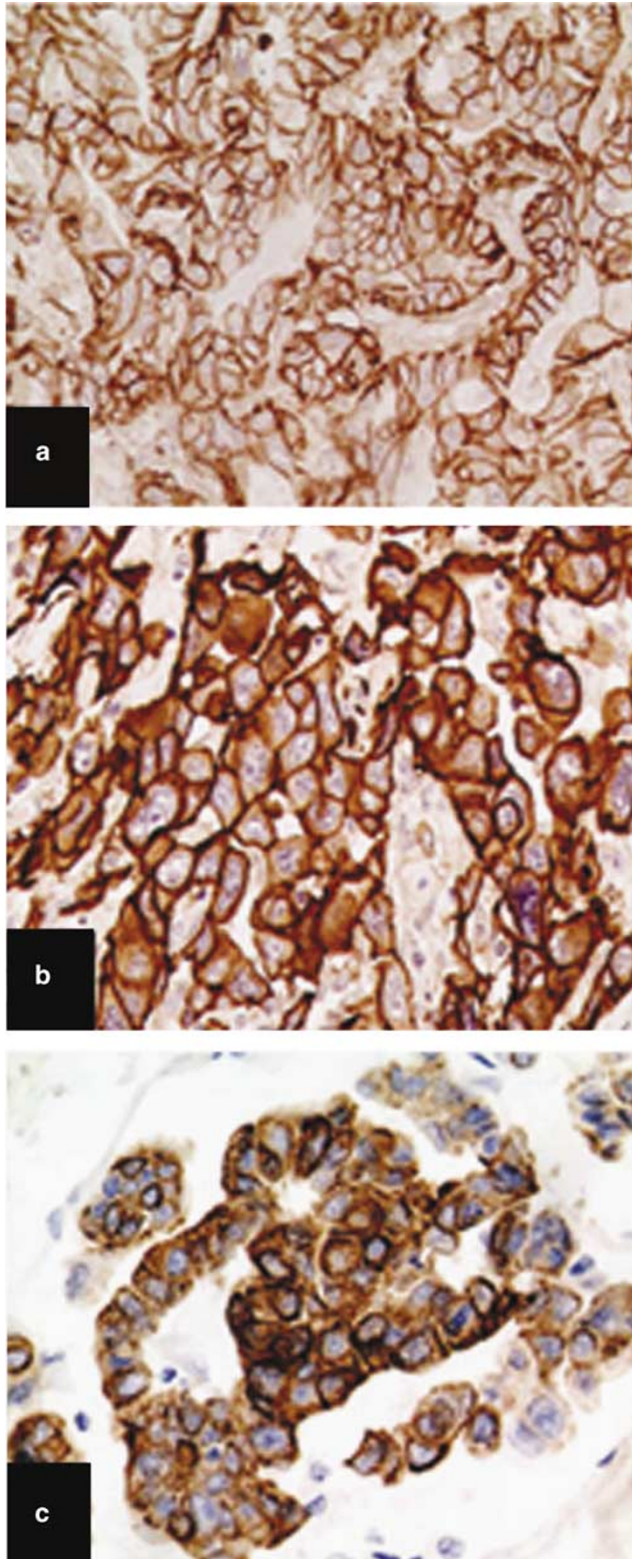
the sections in 0.06 mM 3,3'-diaminobenzidine tetrahydrochloride containing 0.01% hydrogen peroxide. Counterstaining was performed using Mayer's hematoxylin. A case of stomach cancer with *EGFR* gene amplification and a case of breast cancer with IGF1R overexpression were used as positive controls. Tissue sections incubated with normal swine serum were included in each assay as a negative control.

Evaluation of IGF1R and EGFR Protein Expression

The intensity of IGF1R and EGFR immunostaining was evaluated for both the cell membrane and cytoplasm of invasive cancer components. Cell membrane staining was divided into 4 score levels (0, 1+, 2+, 3+), and 2+ and 3+ were judged to show overexpression according to the scoring system used for HER-2 evaluation by Herceptest²² as follows: IGF1R and EGFR expression was scored as 2+ and 3+ if the entire circumference of the cell membrane was weakly or moderately stained and strongly stained, respectively, in 10% or more of the constituent cancer cells. A score of 1+ was assigned if incomplete membrane staining was observed in 10% or more of the cancer cells, and a score of 0 was given if there was membrane staining in less than 10% of constituent cells or no membrane staining. Score 2+ and 3+ were divided only from the intensity of membrane staining. It has proved that HER-2 expression of score 3+ is more correlated with Her2 gene amplification and response to trastuzumab (Herceptin) than that of score 2+ in breast cancer. Membrane-dominant pattern appears to represent the activation of these proteins through a normal signal transduction pathway. Therefore, we divided overexpression of membranous patterns into 2+ and 3+.

Cytoplasmic staining was divided into 3 score levels (0, 1+, 2+) as follows: EGFR and IGF1R expression was scored as 1+ and 2+ if the cytoplasmic staining was similar to, or weaker than, that of normal islet and ductular cells and was stronger than that of these cells, respectively, in 10% or more of the constituent cancer cells. Cytoplasmic granular staining was also scored as 2+. A score of 0 was assigned if there was cytoplasmic staining in less than 10% of constituent cells or no cytoplasmic staining. Cases with a score of 2+ were judged to show overexpression. In both the membrane and cytoplasm of normal hepatocytes, EGFR was strongly positive, whereas in both the membrane and cytoplasm of normal biliary epithelium, IGF1R was intensely stained. These cells were used as an internal positive control for scoring of the metastatic cancer cells. We defined tumors showing membrane overexpression, with or without cytoplasmic staining, as 'membrane-dominant type', and tumors showing cytoplasmic overexpression without membrane overexpression as 'cytoplasm-dominant type',

for both IGF1R and EGFR (Figure 1). Although EGFR overexpression had been examined in the 74 primary tumors in our previous study,²⁰ we reclassified their tumor grade and EGFR overexpression type according to the present definition.



Statistical Analysis

We used the χ^2 or Fisher's exact test to examine the correlation between IGF1R and EGFR overexpression and their correlation with histological grade. Survival curves of patients were drawn by the Kaplan–Meier method. Differences in survival curves were analyzed by the log-rank test. All statistical analyses were performed using Statview 5.0 software (SAS Institute Inc., Cary, NC, USA).

Results

IGF1R and EGFR Overexpression in Surgically Resected Pancreatic Cancer

Membrane- and cytoplasm-dominant EGFR overexpressions were observed in 7 (9%) and 22 (30%) of the 74 surgically resected pancreatic cancers, respectively. Thus, EGFR was overexpressed in a total of 29 (39%) of the 74 cases. Membrane- and cytoplasm-dominant IGF1R overexpressions were observed in 28 (38%) and 8 (11%) of the 74 cases, respectively, and thus IGF1R was overexpressed in a total of 36 cases (49%) (Table 2). Overexpressions of IGF1R and EGFR were not correlated with patient gender or age, T-factor, N-factor, or clinical stage. We found a significant correlation between the type of IGF1R and EGFR overexpression and histological grade. Membrane-dominant EGFR overexpression was more frequent in lower-grade tumors; 2 (29%) and 4 (57%) tumors were Grade 1 and Grade 2, respectively, but only one (14%) was Grade 3. On the other hand, cytoplasm-dominant EGFR overexpression was more frequent in higher-grade tumors: 4 (18%) were Grade 1 and 4 (18%) were Grade 2, but 14 (64%) were Grade 3. There was significant correlation between membrane- or cytoplasm-dominant EGFR overexpression and histological grade (Grade 1, 2 vs Grade 3; $P=0.001$ and 0.0004 , respectively). Conversely, membrane-dominant IGF1R overexpression was more frequent in higher-grade tumors; 4 (14%) and 9 (32%) tumors were Grade 1 and Grade 2 respectively, but 15 (54%) were Grade 3. In contrast, cytoplasm-dominant IGF1R was detected in one (13%) and 7 (87%) Grade 1 and Grade 2 tumors, respectively, but in none (0%) of the Grade 3 tumors. There was a significant correlation between IGF1R cytoplasm-dominant overexpression and histological grade

←
Figure 1 Overexpression of IGF1R and its cellular localization in pancreatic adenocarcinoma. (a and b) Membrane-dominant IGF1R overexpression. (a) Tumor with membrane IGF1R overexpression (3+) and faint diffuse cytoplasmic IGF1R staining (1+). (b) Another tumor with membrane IGF1R overexpression (3+) and strong granular IGF1R staining in the cytoplasm (2+). (c) Cytoplasm-dominant IGF1R overexpression. A tumor with a granular pattern of cytoplasmic overexpression (2+) and weak membrane staining (1+). (a)–(c) Immunoperoxidase stain ($\times 200$).

Table 2 Relationship of histological grade with EGFR and IGF1R immunophenotype in surgical specimens of pancreas

Parameters	Total		Number of cases (%)										
			EGFR overexpression			IGF1R overexpression							
			Membrane-dominant type	Cytoplasm-dominant type	Negative	Membrane-dominant type	Cytoplasm-dominant type	Negative					
Histological grade													
1	12	2 (29)] $P=0.001$	4 (18)] $P=0.0004$	6 (13)] NS	4 (14)] NS	1 (13)] $P=0.0001$	7 (18)] NS
2	35	4 (57)		4 (18)		27 (60)		9 (32)		7 (87)		19 (50)	
3	27	1 (14)		14 (64)		12 (27)		15 (54)		0 (0)		12 (32)	
Total	74	7 (100)		22 (100)		45 (100)		28 (100)		8 (100)		38 (100)	

Correlation of the EGFR or IGF1R overexpression type with histological grade (Grade 1, 2 vs 3). NS, nonsignificant.

(Grade 1, 2 vs 3; $P=0.0001$). Overall survival curves were significantly different among the patient group with tumor showing cytoplasm-dominant type EGFR, those with tumors showing membrane-dominant type EGFR, and those with tumors showing no EGFR overexpression. In comparison with the first group, the later two groups showed better prognosis (Figure 2a) ($P=0.003$). Overall survival curves also differed among the group of patients with tumors showing membrane-dominant IGF1R, those with tumors showing cytoplasm-dominant IGF1R, and those with tumors showing no IGF1R overexpression. In comparison with the groups showing cytoplasm-dominant IGF1R or no IGF1R overexpression, the group showing membrane-dominant IGF1R overexpression had a poorer prognosis (Figure 2b) ($P=0.002$). There was a significant correlation between higher tumor grade and poorer prognosis (Figure 2c) ($P=0.02$). Thus, tumors with cytoplasm-dominant EGFR and membrane-dominant IGF1R overexpression were related with high histological grade and a poor prognosis.

Concordance of EGFR and IGF1R Overexpression Patterns between Primary and Metastatic Tumors

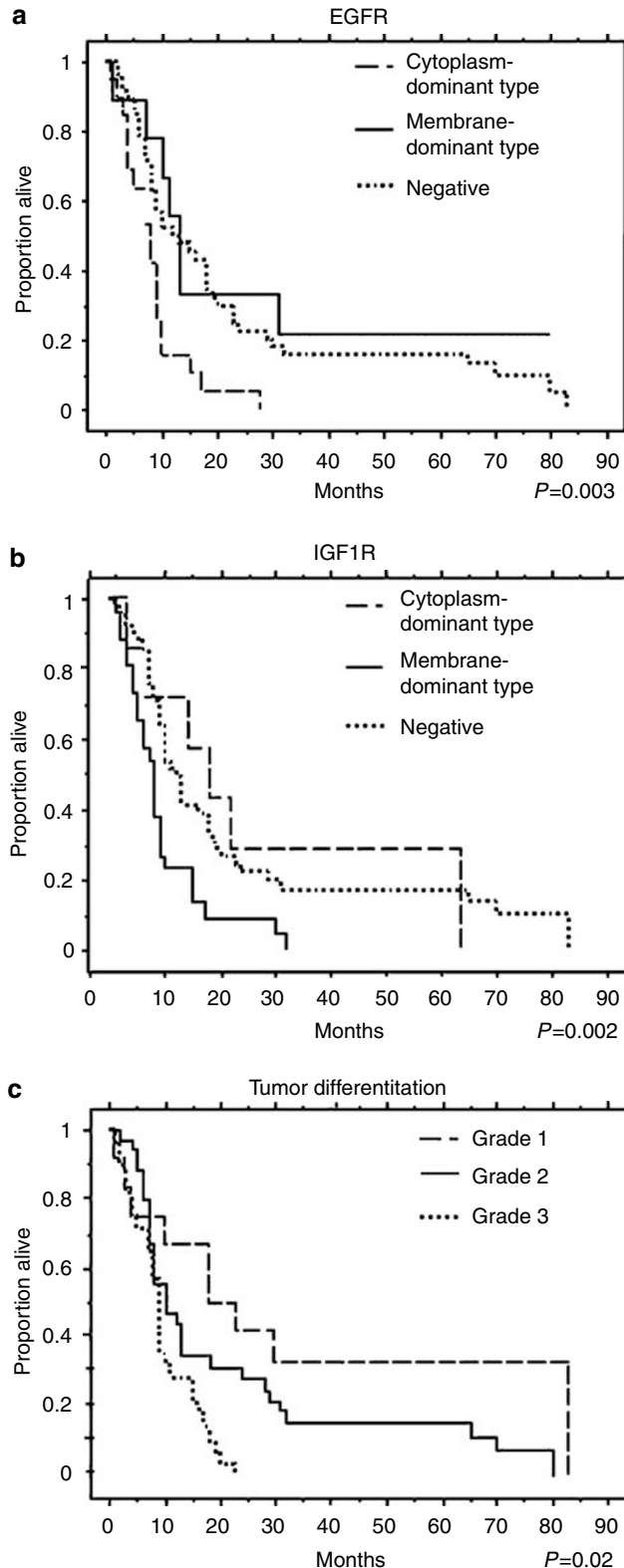
We compared the localization of EGFR and IGF1R overexpression between primary tumors and hepatic metastatic tumors in 44 autopsy cases. In autopsy specimens of primary pancreatic tumors, EGFR and IGF1R overexpressions were not correlated with patient gender or age, or with other clinicopathological factors: The primary tumors and hepatic metastatic tumors were histological grade 1 in six and three cases, grade 2 in nine and 18 cases, and grade 3 in 29 and 23 cases, respectively (Table 1b). There was a significantly higher incidence of EGFR and/or IGF1R overexpression in autopsy specimens than in surgical specimens (Table 3). As most of the tumors were positive for EGFR and IGF1R overexpressions, the difference between EGFR or IGF1R

overexpression and histological grade was not significant (Table 3). Membrane-dominant EGFR overexpression was detected in 26 (59%) of the 44 primary pancreatic tumors and in 32 (73%) of the 44 hepatic metastatic tumors. Cytoplasm-dominant EGFR overexpression was detected in 15 (34%) of the 44 primary tumors and in 9 (20%) of the 44 hepatic metastases. Only three cases were negative for EGFR overexpression in either the primary or the metastatic tumors. Thus, 23 (72%) of 32 hepatic metastases showing membrane-dominant EGFR also showed membrane-dominant EGFR in the primary tumor. However, 9 (28%) of the 32 primary tumors with cytoplasm-dominant EGFR showed a shift in staining localization to the membrane-dominant type in the hepatic metastases (Table 4a). Membrane-dominant IGF1R overexpression was detected in 23 (52%) of the 44 primary tumors and 14 (32%) of the 44 hepatic metastases, while cytoplasm-dominant IGF1R overexpression was detected in 15 (34%) of the primary tumors and 25 (57%) of the hepatic metastases. Membrane- or cytoplasm-dominant IGF1R was absent in 6 (14%) and 5 (11%) of the primary tumors and hepatic metastases, respectively. In contrast to EGFR, 16 (64%) of 25 primary tumors with membrane-dominant IGF1R showed a shift in staining localization to the cytoplasm-dominant type in the hepatic metastases. In 9 (36%) of the 25 primary tumors with cytoplasm-dominant IGF1R staining, this type of IGF1R expression was retained in the hepatic metastases (Table 4b). Thus, shifts in localization of EGFR and IGF1R overexpression frequently occurred in half or more of cases between tumor at the primary site and hepatic metastases.

Inverse Correlation between Localization Patterns of EGFR and IGF1R Overexpression

We evaluated the correlation between localization patterns of IGF1R and EGFR overexpression in

specimens of primary pancreatic cancer. Table 5 shows the correlation of IGF1R and EGFR localization patterns in surgical specimens and autopsy specimens. In autopsy specimens shown in Table



5b, membrane-dominant EGFR overexpression with cytoplasm-dominant IGF1R overexpression was detected in 14 (32%) of 44 cases, while cytoplasm-dominant EGFR with membrane-dominant IGF1R overexpression was detected in 13 (30%). Membrane-dominant overexpression of both IGF1R and EGFR was detected in 8 (18%) of 44 cases while cytoplasm-dominant overexpression of both IGF1R and EGFR was detected in 1 (2%) of 44 cases. Among the 36 cases (82%) that showed both IGF1R and EGFR overexpression, the localization patterns of these two types differed in 27 (75%). Among the 23 primary tumors with membrane-dominant IGF1R, 13 (56%) showed cytoplasm-dominant EGFR overexpression, whereas among 15 primary tumors with cytoplasm-dominant IGF1R, 14 (93%) showed membrane-dominant EGFR overexpression. There was a significant inverse correlation between the IGF1R and EGFR localization patterns ($P=0.001$). Among the surgical specimens shown in Table 5a, only 16 (22%) of 74 cases showed both IGF1R and EGFR overexpression, so although there was a tendency for an inverse correlation between the IGF1R and EGFR localization patterns, this was not significant ($P=0.068$).

Thus, there was a tendency for inverse correlation between IGF1R and EGFR localization in both surgical specimens and autopsied specimens of primary tumors.

Discussion

IGF1R and EGFR function biologically via multiple signaling pathways including those mediated by PI3K/Akt and by Ras/MAPK. Recent studies have proved that IGF1R activation is mediated mainly via the PI3K/Akt signaling pathway, whereas EGFR activation is mediated mainly via the Ras/MAPK signaling pathway. Most *in vitro* studies have shown that cancer cells modulated by EGFR activation have increased propensity for mitosis, proapoptosis, chemoresistance and angiogenesis.^{4,5,13-16,23} In addition, some reports have indicated that membranous EGFR activation is responsible for the regulation of intercellular adhesion such as cell-cell and cell-matrix adhesion. IGF1R activation has also been

Figure 2 Survival curves for patients with pancreatic adenocarcinoma after surgical therapy stratified by the states of EGFR (a), IGF1R (b), and tumor grade (c). (a) Curve for patients with tumors showing cytoplasm-dominant EGFR overexpression (—) was significantly worse than that of patients with tumors showing membrane-dominant EGFR overexpression (---) or patients with tumors lacking EGFR overexpression (.....) ($P=0.003$). (b) Curve for patients with tumors showing membrane-dominant IGF1R overexpression (—) was significantly worse than that of patients with tumors showing cytoplasm-dominant IGF1R overexpression (---) or patients with tumors lacking IGF1R overexpression (.....) ($P=0.002$). (c) There were significant differences among the curves for patients with grade 1 (---), grade 2 (—), and grade 3 (.....) tumors ($P=0.02$).

Table 3 Relationship of histological grade with EGFR or IGF1R immunophenotype in autopsied specimens of pancreas

Parameters	Total	Number of cases (%)					
		EGFR overexpression			IGF1R overexpression		
		Membrane-dominant type	Cytoplasm-dominant type	Negative	Membrane-dominant type	Cytoplasm-dominant type	Negative
<i>Histological grade</i>							
1	6	3 (12)	2 (13)	1 (33)	5 (22)	1 (7)	0 (0)
2	9	5 (19)	4 (27)	0 (0)	5 (22)	4 (27)	0 (0)
3	29	18 (69)	9 (60)	2 (67)	13 (56)	10 (66)	6 (100)
Total	44	26 (100)	15 (100)	3 (100)	23 (100)	15 (100)	6 (100)

No significant difference of the EGFR and IGF1R overexpression types with histological grade (grade 1, 2 vs 3). NS, nonsignificant.

Table 4 Comparison of EGFR and IGF1R localization patterns between primary pancreatic cancer and hepatic metastatic tumor in individual patients

Primary tumor	Total	Membrane-dominant type	Cytoplasm-dominant type	Negative
<i>(a) EGFR</i>				
	<i>EGFR overexpression (%)</i>			
	<i>Hepatic metastatic tumor</i>			
Membrane-dominant type	26	23 (72)	3 (33)	0 (0)
Cytoplasm-dominant type	15	9 (28)	5 (56)	1 (33)
Negative	3	0 (0)	1 (11)	2 (67)
Total	44	32 (100)	9 (100)	3 (100)
<i>(b) IGF1R</i>				
	<i>IGF1R overexpression (%)</i>			
	<i>Hepatic metastatic tumor</i>			
Membrane-dominant type	23	7 (50)	16 (64)	0 (0)
Cytoplasm-dominant type	15	5 (36)	9 (36)	1 (20)
Negative	6	2 (14)	0 (0)	4 (80)
Total	44	14 (100)	25 (100)	5 (100)

shown to play roles similar to EGFR activation *in vivo*. Furthermore, IGF1R can upregulate several aspects of the malignant phenotype and metastasis *in vitro*,^{12,13} although the specific roles played by IGF1R in this process remain unclear. We consider that normal membrane-dominant-type IGF1R and EGFR overexpression would play a major role in the proliferation, migration and differentiation of carcinoma cells. However, their cytoplasmic overexpression might also be of positive biological significance.

In the present study, we were able to show that IGF1R as well as EGFR was frequently overexpressed in pancreatic adenocarcinomas, and that their overexpression was classified into two patterns: membrane-dominant and cytoplasm-dominant. The former type is the conventional localization pattern of cell membrane according to the scoring system of Herceptest for Her2 overexpressing breast cancer. 3+ membranous patterns of Her2 staining are more correlated with high frequency of Her2 gene amplification than 2+ mem-

branous patterns of that. Membrane-dominant pattern appears to represent the activation of these proteins through a normal signal transduction pathway. Among autopsy specimens of primary pancreatic tumors, those with membrane-dominant IGF1R, usually accompanied by cytoplasm-dominant EGFR, showed higher histological grade and poorer patient prognosis than tumors with cytoplasm-dominant IGF1R usually accompanied by membrane-dominant EGFR.

These findings suggest that in advanced pancreatic cancer, EGFR expression tends to increase while IGF1R expression decreases. Crosstalk between the IGF1R-mediated signaling pathway and the EGFR-mediated signaling pathway might play an important role in determining the biological aggressiveness of pancreatic adenocarcinoma. In hepatic metastases as well as primary tumors obtained at autopsy, a tendency for an inverse correlation between the localization patterns of IGF1R and EGFR overexpression was retained (data not presented). The overexpression pattern of IGF1R and/or EGFR

Table 5 Localization pattern of EGFR and IGF1R overexpressions in (a) surgical specimens of primary pancreatic tumors and (b) autopsied specimens of primary pancreatic tumors

EGFR overexpression	Number of cases (%)			
	Total	IGF1R overexpression		
		Membrane-dominant type	Cytoplasm-dominant type	Negative
<i>(a) Surgical specimens</i>				
Membrane-dominant type	7	0 (0) ^a	2 (25) ^a	5 (13)
Cytoplasm-dominant type	22	9 (32) ^a	5 (63) ^a	8 (21)
Negative	45	19 (68)	1 (12)	25 (66)
Total	74	28 (100)	8 (100)	38 (100)
<i>(b) Autopsied specimens</i>				
Membrane-dominant type	26	8 (35) ^b	14 (93) ^b	4 (66)
Cytoplasm-dominant type	15	13 (56) ^b	1 (7) ^b	1 (17)
Negative	3	2 (9)	0 (0)	1 (17)
Total	44	23 (100)	15 (100)	6 (100)

^aOf 74 cases which showed both IGF1R and EGFR overexpressions, 16 (22%) present no significance, but tendency of inverse correlation between IGF1R and EGFR localization patterns ($P=0.068$).

^bIn total, 36 cases (82%) which showed both IGF1R and EGFR overexpressions present significant change between IGF1R and EGFR localization patterns ($P=0.001$).

was concordant between primary and metastatic tumors in identical patients in nearly half of all cases, although the pattern was discordant in the other half. In some cases, membrane-dominant EGFR overexpression in the primary tumor showed switching to membrane-dominant IGF1R or to coexpression of membrane-dominant EGFR and IGF1R in the hepatic metastasis. These findings suggest that crosstalk between the IGF1R-mediated pathway and the EGFR-mediated pathway might also occur during the process of metastasis of pancreatic adenocarcinoma. In autopsy specimens of primary pancreatic tumors and corresponding hepatic metastases, there was no significant relationship between EGFR or IGF1R overexpression and tumor differentiation, probably because the majority of these tumors were positive for EGFR or IGF1R overexpression. More than the half of pancreatic cancers at far advanced stages consistently showed membrane-dominant EGFR overexpression in both the primary and the metastatic tumors, while IGF1R variously exhibited membrane- or cytoplasm-dominant overexpression in both types of tumors (Table 4). These findings imply that the normal signaling pathway mediated by IGF1R and that mediated by EGFR might generally function in a compensatory manner in primary pancreatic cancer. For the formation of metastatic tumors, these signaling pathways appear to show complex cooperation in which their compensatory relationship is modified. The activities of receptor tyrosine kinases are attenuated by receptor internalization, intracellular trafficking through endosomes, and degradation in lysosomes, resulting in decreased receptor expression. However, there is now considerable evidence that the

EGFR continues signaling in endosomes, which forces us to reevaluate the outcomes of receptor trafficking. Internalized receptors might extend some signaling activities and mediate certain responses, such as cell motility.²⁴ If the signaling pathway mediated by IGF1R can substitute for that mediated by EGFR, we may be able to utilize another therapeutic approach to target IGF1R in pancreatic cancer. As interruption of the EGFR family members has been shown to exert therapeutic effects against human cancers, a strategy for interruption of IGF1R signaling could also be of likely clinical benefit.

In summary, this immunohistochemical study has shown that both IGF1R and EGFR are frequently overexpressed in pancreatic adenocarcinoma at both primary and metastatic sites. The cellular localization patterns of IGF1R and EGFR in the primary tumor are correlated with histological tumor grade and patient prognosis. Throughout the process of progression of pancreatic adenocarcinoma, the tendency toward reciprocal localization of IGF1R and EGFR, and the inverse shift in localization in metastases implies a relationship between two related receptors that may have therapeutic implications.

References

- 1 Wu Y, Yakar S, Zhao L, *et al*. Circulating insulin-like growth factor-I levels regulate colon cancer growth and metastasis. *Cancer Res* 2002;62:1030–1035.
- 2 Werner H, Re GG, Drummond IA, *et al*. Increased expression of the insulin-like growth factor I receptor gene, IGF1R, in Wilms tumor is correlated with

- modulation of IGF1R promoter activity by the WT1 Wilms tumor gene product. *Proc Natl Acad Sci USA* 1993;90:5828–5832.
- 3 Reinmuth N, Fan F, Liu W, *et al*. Impact of insulin-like growth factor receptor-I function on angiogenesis, growth, and metastasis of colon cancer. *Lab Invest* 2002;82:1377–1389.
 - 4 Ellis MJ, Jenkins S, Hanfelt J, *et al*. Insulin-like growth factors in human breast cancer. *Breast Cancer Res Treat* 1998;52:175–184.
 - 5 Hellowell GO, Ferguson DJ, Brewster SF, *et al*. Chemosensitization of human prostate cancer using antisense agents targeting the type 1 insulin-like growth factor receptor. *BJU Int* 2003;91:271–277.
 - 6 Hellowell GO, Turner GD, Davies DR, *et al*. Expression of the type 1 insulin-like growth factor receptor is up-regulated in primary prostate cancer and commonly persists in metastatic disease. *Cancer Res* 2002;62:2942–2950.
 - 7 Fukuda R, Hirota K, Fan F, *et al*. Insulin-like growth factor 1 induces hypoxia-inducible factor 1-mediated vascular endothelial growth factor expression, which is dependent on MAP kinase and phosphatidylinositol 3-kinase signaling in colon cancer cells. *J Biol Chem* 2002;277:38205–38211.
 - 8 Werner H, Roberts Jr CT. The IGFI receptor gene: a molecular target for disrupted transcription factors. *Genes Chromosomes Cancer* 2003;36:113–120.
 - 9 Mauro L, Salerno M, Morelli C, *et al*. Role of the IGF-I receptor in the regulation of cell–cell adhesion: implications in cancer development and progression. *J Cell Physiol* 2003;194:108–116.
 - 10 Ibrahim YH, Yee D. Insulin-like growth factor-I and breast cancer therapy. *Clin Cancer Res* 2005;11:944s–950s.
 - 11 Fantl WJ, Johnson DE, Williams LT. Signalling by receptor tyrosine kinases. *Annu Rev Biochem* 1993;62:453–481.
 - 12 Salomon DS, Brandt R, Ciardiello F, *et al*. Epidermal growth factor-related peptides and their receptors in human malignancies. *Crit Rev Oncol Hematol* 1995;19:183–232.
 - 13 Tobita K, Kijima H, Dowaki S, *et al*. Epidermal growth factor receptor expression in human pancreatic cancer: Significance for liver metastasis. *Int J Mol Med* 2003;11:305–309.
 - 14 Slichenmyer WJ, Fry DW. Anticancer therapy targeting the erbB family of receptor tyrosine kinases. *Semin Oncol* 2001;28:67–79.
 - 15 Yano S, Nishioka Y, Goto H, *et al*. Molecular mechanisms of angiogenesis in non-small cell lung cancer, and therapeutics targeting related molecules. *Cancer Sci* 2003;94:479–485.
 - 16 Lu Y, Zi X, Zhao Y, *et al*. Insulin-like growth factor-I receptor signaling and resistance to trastuzumab (Herceptin). *J Natl Cancer Inst* 2001;93:1852–1857.
 - 17 Tsuda H, Hirohashi S. Multiple developmental pathways of highly aggressive breast cancers disclosed by comparison of histological grades and c-erbB-2 expression patterns in both the non-invasive and invasive portions. *Pathol Int* 1998;48:518–525.
 - 18 Dugan MC, Dergham ST, Kucway R, *et al*. HER-2/neu expression in pancreatic adenocarcinoma: relation to tumor differentiation and survival. *Pancreas* 1997;14:229–236.
 - 19 Hruban RH, Wilentz RE, Kern SE. Genetic progression in the pancreatic ducts. *Am J Pathol* 2000;156:1821–1825.
 - 20 Ueda S, Ogata S, Tsuda H, *et al*. The correlation between cytoplasmic overexpression of epidermal growth factor receptor and tumor aggressiveness: poor prognosis in patients with pancreatic ductal adenocarcinoma. *Pancreas* 2004;29:e1–e8.
 - 21 Xiong HQ, Rosenberg A, LoBuglio A. Cetuximab, a monoclonal antibody targeting the epidermal growth factor receptor, in combination with gemcitabine for advanced pancreatic cancer: a multicenter phase II Trial. *J Clin Oncol* 2004;22:2610–2616.
 - 22 Lebeau A, Deimling D, Kaltz C, *et al*. Her-2/neu analysis in archival tissue samples of human breast cancer: comparison of immunohistochemistry and fluorescence *in situ* hybridization. *J Clin Oncol* 2001;19:354–363.
 - 23 Albanell J, Baselga J. Unraveling resistance to trastuzumab (Herceptin): insulin-like growth factor-I receptor, a new suspect. *J Natl Cancer Inst* 2001;93:1830–1832.
 - 24 Haugh JM. Localization of receptor-mediated signal transduction pathways: the inside story. *Mol Intervent* 2002;2:292–307.