Immunohistochemical expression of receptor-tyrosine kinase c-*kit* protein in invasive ductal carcinoma of the pancreas

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The expression of receptor tyrosine kinase c-*kit* and its biologic significance in pancreatic cancer are unclear. We studied the expression of c-*kit* protein (c-KIT) in resectable invasive ductal carcinomas (IDCs) of the pancreas, in order to assess whether a selective c-*kit* inhibitor, STI571 (Glivec), may be applied for the treatment of pancreatic IDCs. This study included 72 pancreatic IDC patients who received a pancreatectomy between 1982 and 2002. The expression of c-KIT was analyzed retrospectively by immunohistochemistry. c-KIT was expressed in 78% (56/72) of the pancreatic IDCs. c-KIT expression did not correlate with any clinicopathological factor of pancreatic IDC and c-KIT expression had no significant influence on the survival of the patients. The survival rate of the adjuvant chemotherapy (ACT) (+) group was significantly higher than that of the ACT (−) group, but c-KIT expression had no significant effects on the efficacy of the ACT. Multivariate analysis indicated that the pTNM stage, grade and ACT were all significant variables for survival in IDCs overall. As c-KIT was expressed in 78% of the pancreatic IDCs, it suggests that STI571 may be a beneficial agent for chemotherapy against human pancreatic IDCs.
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Introduction

The c-*kit* proto-oncogene has been recently identified as a member of the receptor (Rcp) tyrosine kinase family and, more specifically, as a member of the platelet-derived growth factor Rcp family [1–4]. c-*kit* encodes a transmembrane Rcp with a molecular weight of 145–160 kDa [2,5]. c-*kit* has also been shown to be identical with the product of the W locus in mice, and as such is integral to the development of mast cells and hematopoiesis [6–8]. More recently, the ligand of the c-*kit* has been identified and characterized, and was shown to be encoded at the murine steel locus [9].

The activation of c-*kit* by the c-*kit* ligand, also known as stem cell factor (SCF), is essential to melanocyte and germ cell development and during the early stages of hematopoiesis [10]. The expression of c-*kit* protein (c-KIT) was observed in various malignancies such as neuroblastomas [11] and testicular germ cell tumors [12]. Recently, c-*kit* was shown to play an important role in the progression of gastrointestinal stromal tumors (GISTs) [13,14]. In addition, c-KIT and SCF are co-expressed in some breast and colorectal cancers [15,16], suggesting that c-*kit* may serve an autocrine role in normal or malignant epithelial tissues. Accordingly, c-*kit* may play an important role in the regulation of cell growth in various malignancies. Recently, a selective c-*kit* inhibitor Glivec (STI571, imatinib mesylate) has been introduced into the chemotherapy of GISTs and Glivec showed prominent effects against c-KIT (+) GISTs [17,18].

Invasive ductal carcinoma (IDC) of the pancreas is one of the most common causes of cancer death in developed countries [19] and has been a challenge to clinical oncologists. Although recent progress in surgical treatment and other combination therapies for pancreatic IDC has brought about an improvement in the overall results, it is still an undeniable fact that the prognosis of patients with pancreatic IDC is extremely poor and IDC is highly resistant to various cancer therapies [20]. Although a new anticancer agent gemcitabine (GEM) has been introduced into the chemotherapy of pancreatic IDC, its effect seems still marginal against unresectable or advanced pancreatic IDCs: in single use of GEM, the mean response rate was about 10% (5.4–14.3) and the median survival time (MST) was about 5.7 (3.9–6.3) months, and in combination uses with other agents the response rates ranged between 12.8 and 29% and the MST ranged between 5.7 and 8.2 months [21]. Accordingly, it is urgent to develop chemotherapy more effective than the present standard regimens for pancreatic IDCs. Recently, various targeting therapies such as Herceptin

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for HER-2 over-expressing breast cancer [22], Iressa (ZD1839) for HER-1 over-expressing non-small cell lung cancer [23] and Glivec for c-*kit* over-expressing GISTs have been introduced, and resulted in prominent responses [17,18]. We studied c-KIT expression and its clinicopathological significance in resectable IDCs of the pancreas in order to assess whether Glivec may be applied for the treatment of pancreatic IDC.

Materials and methods

Patients

Informed consent for this study on the genetic and histopathological background of the patients was obtained from the patients or their family according to the recommendation by the ethical committee of our department from 1999.

Seventy-two patients (38 females and 34 males; 35–80 years old; mean 65.4 years) with pancreatic IDC underwent pancreatectomies between 1982 and 2001 at the First Department of Surgery, Shimane Medical University. The present study did not include mucinous cystic adenocarcinomas or intraductal papillary mucinous neoplasms with adenocarcinoma, because they have a better prognosis than IDC. The patients' profiles are summarized in Table 1. A standard or pylorus-preserving pancreatoduodenectomy was performed in 38 patients, a distal pancreatectomy in 23 patients and a total pancreatectomy in 11 patients. The tumors were staged according to the UICC classification (TNM classification) [24]. Histopathologically, all specimens were verified to be IDCs of the pancreas. None of the patients received any type of treatment prior to their surgical procedures. After surgery, some patients were treated with adjuvant chemotherapy (ACT) and/or radiotherapy (RT), and were followed-up. All patients were followed-up in our department and the survival of the patients was surveyed on 1 January 2002. Postoperative survival was defined as the time elapsed from the surgery to a cancer-related death.

Table 1 KIT and transforming growth factor-β1 expression and clinicopathologic characteristics

Feature	No. (%) of patients	No. (%) of c-KIT		Correlation coefficient (p value)
		(+)	(−)	
Overall	72	56 (78)	16 (22)	
Age [(years) mean]	65.3 ± 9.8	66.4 ± 9.1	61.9 ± 11.7	r=0.802 (p=0.1065)
<65	27 (37.5)	20 (74.1)	7 (25.9)	
≥65	45 (62.5)	36 (80)	9 (20)	
Gender				
male	34 (47.2)	26 (76.5)	8 (23.5)	r= −0.030 (p=0.8048)
female	38 (52.8)	30 (78.9)	8 (21.1)	
Grade				
1	34 (47.2)	26 (76.5)	8 (23.5)	r= −0.024 (p=0.8413)
2	33 (45.8)	27 (81.8)	6 (18.2)	
3	5 (6.9)	3 (60)	2 (40)	
4	0	–	–	
pTNM stage				
I	10 (13.8)	6 (60)	4 (40)	r=0.174 (p=0.1431)
II	3 (4.2)	2 (66.7)	1 (33.3)	
III	36 (50)	29 (80.6)	7 (19.4)	
IV	23 (31.9)	19 (82.6)	4 (17.4)	
PT				
1	5 (6.9)	4 (80)	1 (20)	r=0.119 (p=0.3209)
2	25 (34.7)	18 (72)	7 (28)	
3	24 (33.3)	18 (75)	6 (25)	
4	18 (25)	16 (88.9)	2 (11.1)	
PN				
0	5 (6.9)	2 (40)	3 (60)	r=0.131 (p=0.2734)
1,2	67 (93.1)	54 (80.6)	13 (19.4)	
M				
0	68 (94.4)	52 (76.5)	16 (23.5)	r=−0.162 (p=0.1744)
1	4 (5.6)	2 (50)	2 (50)	

ACT

Of the 72 patients who did not experience post-surgical complications, 26 received surgery alone, 42 received ACT, and four received both ACT and RT. In Japan, under the universal health insurance system, the Japanese Ministry of Health, Labor and Welfare strictly regulates the use of anticancer agents. Accordingly, the ACT typically involves only approved agents. In our department, we have no standard regimen for ACT against pancreatic IDC, because there is no evidence supporting the survival benefits of adjuvant therapy for pancreatic IDC at present. Accordingly, the use of ACT was decided upon by the respective doctors with the informed consent of the patients and/or their family. Forty-six patients received ACT after their surgery, Most patients were given 5-fluorouracil (5-FU) or its derivative UFT alone or with cyclophosphamide (CPA), and some received intensive regimens including GEM, adriamycin and cisplatin. Five patients in the ACT group also received adjuvant RT using LINAC (ML-15MDX, 10MVX; Mitsubishi Electric, Tokyo, Japan) at 50 Gy (2 Gy × 25 times) after surgery.

Antibody

The anti-c-KIT rabbit antibody (AB-1; Oncogene Science, Uniondale, NY) was a purified rabbit polyclonal antibody raised against a peptide corresponding to a sequence found at the C-terminal 961–976 amino acids of the human c-*kit* protein. It was diluted at 5 μg/ml for use.

Immunohistochemistry

The specimens were immunostained primarily according to the labeled polymer method using the EnVision + peroxidase, rabbit kit (Dako, Carpenteria, CA), which is a goat anti-rabbit immunoglobulin conjugated to a peroxidase labeled-dextran polymer. A false-positive staining caused by endogenous biotin can be completely overcome by this method [25]. Formalin-fixed, paraffin-embedded

specimens were cut into 4-μm sections. The sections were deparaffinized in xylene for 5 min × 3, hydrated in 100, 95 and 45% ethanol, and finally in phosphate-buffered saline (PBS). The immuno-staining was performed according to instructions from the manufacturer (Dako) as follows. The slides were pretreated in 6 M urea at 95°C for 10 min for antigen retrieval [26]. The slides were then cooled at room temperature in PBS. Endogenous peroxidase activity and non-specific binding were blocked by treatment with 3% hydrogen peroxide for 15 min. The specimens were incubated with the primary antibody for 2 h at room temperature and then rinsed twice in PBS. The specimens were incubated with EnVision + system HRP rabbit at room temperature for 30 min and then rinsed twice in PBS. Finally, the specimens were treated with a 0.05% 3,3′-diaminobenzidine solution for 5 min at room temperature. After washing in distilled water, the specimens were counterstained with hematoxylin and then mounted in Entellan-new with a cover slip.

Evaluation of immunostaining

The immunostaining was considered positive for c-KIT only when strong cytoplasmic immunoreactivity in tumor cells was diffusely observed. Those cases with only faint immunostaining were regarded as negative.

Statistical analysis

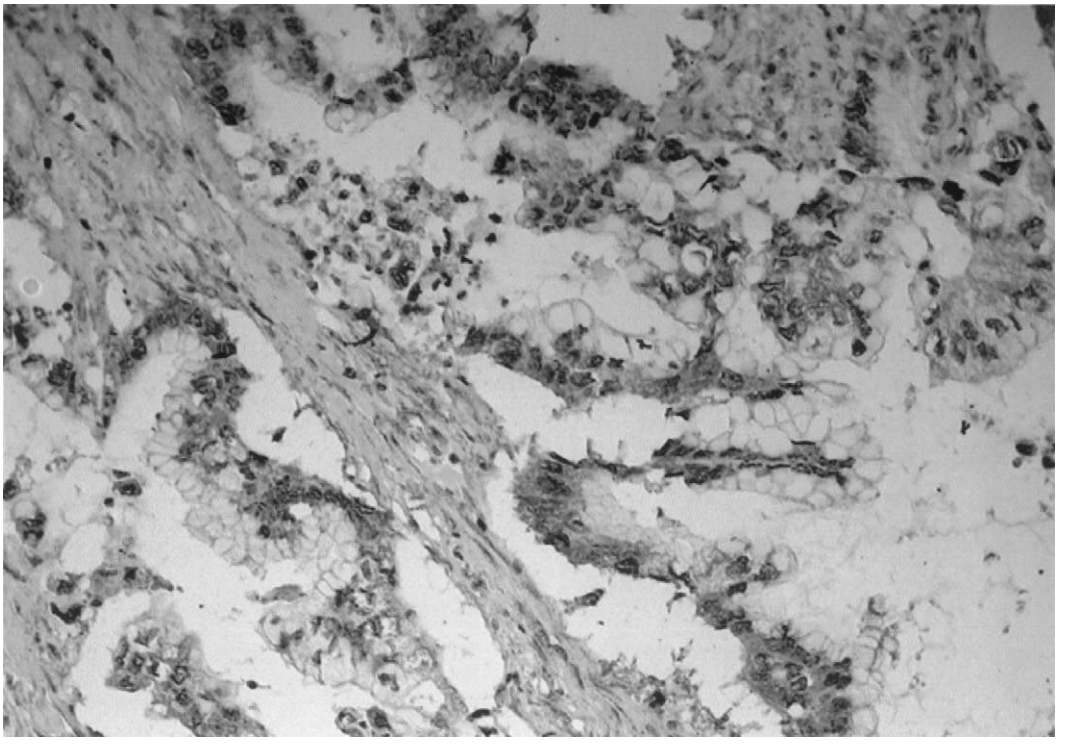
The correlations between the clinicopathological factors and the expression of c-KIT were examined using Pearson's correlation analysis. The post-surgical status of all patients was surveyed on 1 December 2002. The cumulative survival rates were calculated according to the Kaplan–Meier method and were compared by the Cox–Mantel test. A multivariate analysis of Cox's proportional hazard risk model was used to obtain the conditional risk of death due to IDC of the pancreas. Statistically significant differences were defined at $p < 0.05$.

Results

Representative immunostaining for c-KIT is shown in Figure 1. The c-KIT was expressed in 77.8% (56/72) of the patients, but the c-KIT expression did not correlate with any clinicopathological factor (Table 1).

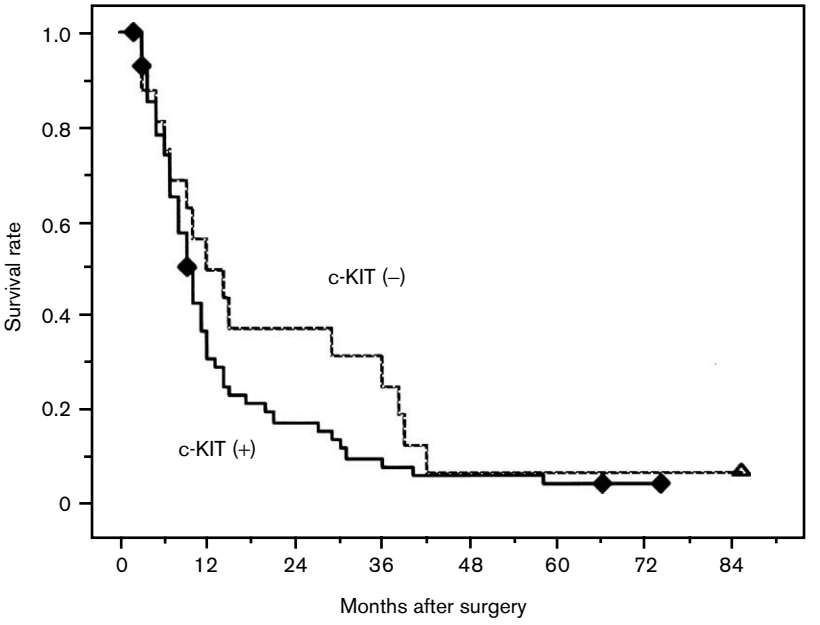
The MST was 14.8 months for all patients. The cumulative survival rate of the c-KIT (−) group ($n = 16$) was higher than that of the c-KIT (+) group ($n = 56$), and the MST was 14.4 months for c-KIT (+) IDCs and 19.4 months for c-KIT (−) IDCs, but there were no significant differences between them ($p = 0.1237$), which suggests that c-KIT expression was not significantly associated with survival (Fig. 2).

Fig. 1



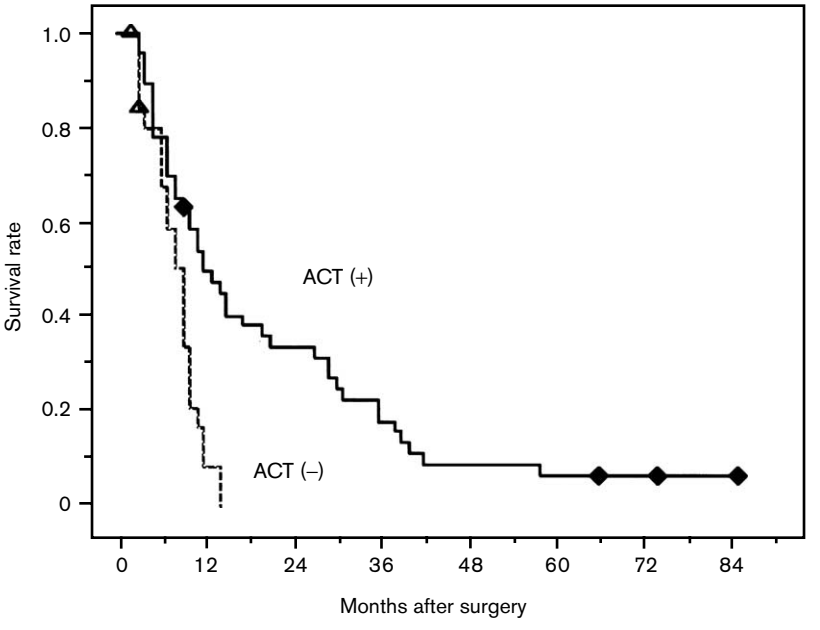
Representative immunostaining for c-KIT (66-year-old woman, moderately differentiated adenocarcinoma).

Fig. 2



c-KIT expression and survival curves. KIT (+), $n=56$; KIT (−), $n=16$. KIT (+) versus KIT (−), $p=0.2047$.

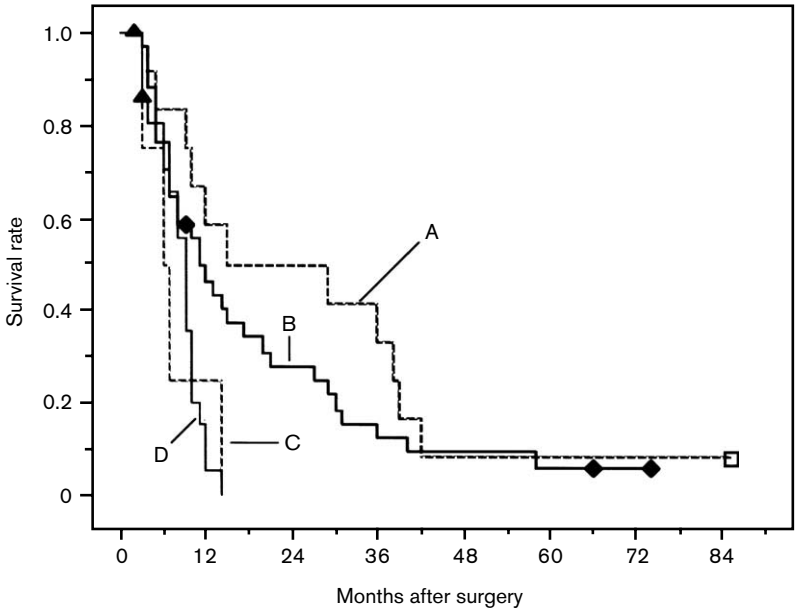
Fig. 3



Effect of ACT on patient survival. ACT (+), $n=46$; ACT (−), $n=26$. ACT (+) versus ACT (−), $p=0.0002$.

The survival rate of the ACT group ($n=46$) was significantly higher than that of the surgery alone group ($n=26$), and the MST was 8.1 for the ACT (−) group and 19.9 for the ACT (+) group ($p=0.0002$) (Fig. 3). When the survival rate was analyzed in combination of c-KIT expression and ACT, the MST was 23.3 months for the c-KIT (−)/ACT (+) group, 18.1 months for the c-KIT (+)/ACT (+) group, 8.2 months for the c-KIT (+)/ACT (−) group and 7.5 months for the c-KIT (−)/ACT (−) group ($p=0.0003$) (Fig. 4).

Fig. 4



Effect of c-KIT expression on the efficacy of ACT. (A) c-KIT (−)/ACT (+) group ($n=12$), (B) c-KIT (+)/ACT (+) group ($n=34$), (C) c-KIT (−)/ACT (−) group ($n=4$) and (D) c-KIT (+)/ACT (−) group ($n=22$). $p=0.0003$ among the four groups.

Table 2 Univariate analysis by Cox's proportional hazard risk model[a]

Variables	Conditional risk ratio (95% confidence limit)	p value (χ^2)
Clinical stage (pTNM)	1.749 (1.297–2.360)	0.0003
Adjuvant chemotherapy	0.360 (0.200–0.651)	0.0007
Histological grade	1.717 (1.081–2.726)	0.0220
c-KIT expression	1.593 (0.859–2.954)	0.1393
Gender	1.342 (0.810–2.222)	0.2533
Age	1.008 (0.979–1.038)	0.5799

[a]Dependent variable = month, censoring variable = death due to pancreatic cancer.

Table 3 Multivariate analysis by Cox's proportional hazard risk model[a]

Variables	Conditional risk ratio (95% confidence limit)	p value (χ^2)
Overall patients		
clinical stage	1.707 (1.242–2.346)	0.0010
adjuvant chemotherapy	0.431 (0.239–0.778)	0.0052
histological grade	1.566 (1.008–2.432)	0.0460
gender	1.280 (0.745–2.200)	0.3711
age	1.002 (0.973–1.031)	0.9085
KIT expression	1.044 (0.569–1.951)	0.8904
ACT (+)		
clinical stage	1.672 (1.171–2.388)	0.0047
histological grade	1.871 (1.041–3.364)	0.0364
gender	1.273 (0.637–2.541)	0.4945
age	1.000 (0.964–1.036)	0.9882
c-KIT expression	1.058 (0.501–2.233)	0.8823
ACT (−)		
clinical stage	1.846 (0.814–4.183)	0.1421
histological grade	1.267 (0.613–2.617)	0.5232
gender	1.408 (0.564–3.514)	0.4637
age	1.012 (0.955–1.073)	0.6775
c-KIT expression	1.102 (0.341–3.562)	0.8717

[a]Dependent variable = month, censoring variable = death due to pancreatic cancer.

In the univariate analysis (Table 2), crude relative hazards for clinical stage, ACT and histological grade were significant prognostic factors. Furthermore, in the multivariate analysis (Table 3), the clinical stage, histological grade and ACT were all significant variables for survival in the IDCs overall, but c-KIT expression was not a significant variable. In ACT group clinical stage and histological grade were significant variables and in ACT (–) group there were no significant variables.

Discussion

It has been reported that the protein or mRNA for c-*kit* was expressed in various human solid cancers and their cell lines, such as breast cancer [27,28], lung cancer [29] and colon cancer [30]. To our knowledge, only one report studied KIT expression in human pancreatic cancer, in which three cases of human pancreatic cancer did not express c-KIT in an immunohistochemical study [29]. However, in the present study, c-KIT was expressed in about 80% of human pancreatic IDCs. These different results may be due to the different methods for antigen retrieval, because we pretreated the paraffin sections with 6 M urea at 95°C for 10 min according to the manufacturer's instructions, whereas the previous authors did not use an antigen retrieval method. We studied

c-KIT expression in GISTs by applying STI571 for the treatment of c-KIT (+) GISTs. In our experience, when antigen retrieval was not performed, the immunostaining of c-KIT was very weak. One GIST case, which was known to express c-*kit* mRNA by RT-PCR and responded to STI571 treatment, was evaluated as c-KIT (–) in an immunohistochemical study. Furthermore, in the present study, a new method, a labeled polymer method, was used for the immunohistochemical staining in order to avoid false-positive staining, which is caused by a non-specific staining of the endogenous biotin in the tissue [25]. Accordingly, we are confident to present these results; however, the expression of c-KIT in pancreatic IDC should be further replicated.

In the present study, c-KIT expression did not correlate with any clinicopathological factors and c-KIT expression had no significant influence on the survival of these patients. c-KIT was expressed in 78.9% (60/76) of pancreatic IDCs, which indicates that c-*kit* may play an important role in the progression of pancreatic IDCs. Furthermore, c-KIT expression had no significant effects on the efficacy of the ACT. Although there are no reports on the involvement of c-KIT expression in the response to chemotherapy, it has been reported that the leukemia cell line MO7e, which was transduced by a mutant c-*kit* cDNA, acquired a growth advantage and resistance to apoptosis in response to chemotherapeutic agents and ionizing radiation [31]. This report supports a possible implication for c-KIT expression in the response of these cells to the chemotherapy. In the present study, most patients from the ACT group received 5-FU or its derivative, UFT and CPA. In the patients received ACT, the MST of the c-KIT (+) group was 18.1 months and that of the c-KIT (–) group was 23.3, months. Although there was no significant difference between them, this result suggests a resistance of c-KIT (+) IDCs against chemotherapy. To our knowledge, there have been no reports on the relationship between c-KIT expression and the efficacy of these chemotherapeutic agents.

Recently, various molecular targeting therapies, such as Herceptin for HER-2, Iressa for HER-1 and Glivec for c-KIT, have been introduced into chemotherapy for malignancies. Now their applications are restricted for only a few types of malignancies and they will be applied more widely for other malignancies expressing these target molecules. The present study demonstrated that c-KIT was expressed in about 80% of human IDCs, which suggests that Glivec may be a beneficial agent for chemotherapy against human pancreatic IDCs.

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