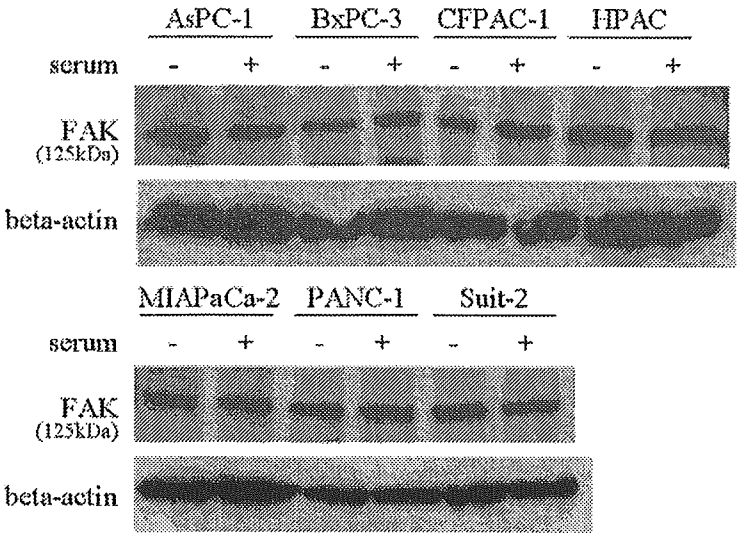


**Figure 3.** FAK phosphorylation at tyrosin 397 in pancreatic cancer cell lines. The level of FAK phosphorylation at tyrosine 397 was not changed by serum in all cell lines.

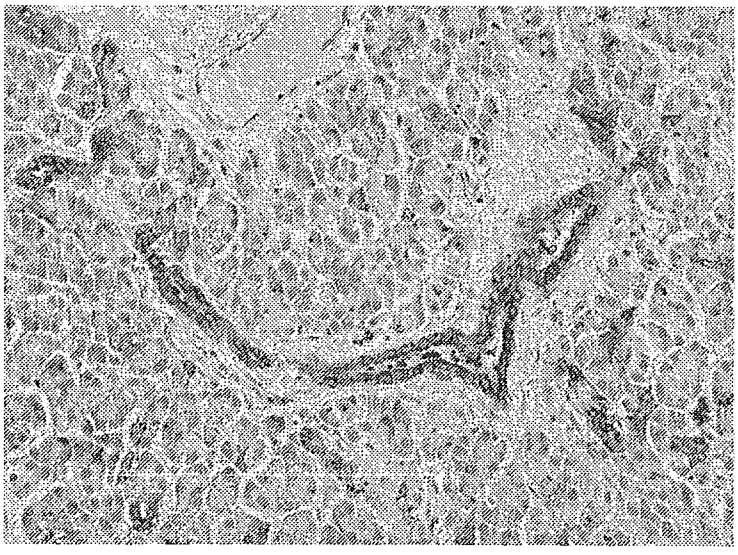

**Figure 4.** Immunohistochemical detection of FAK in normal pancreatic tissue. In normal pancreatic tissue FAK staining was observed in the cytoplasm of ductal cells and faintly in islet cells, but not in acinar cells. The nuclei are counterstained with Mayer's haematoxylin. The scale bar is 200 μm.

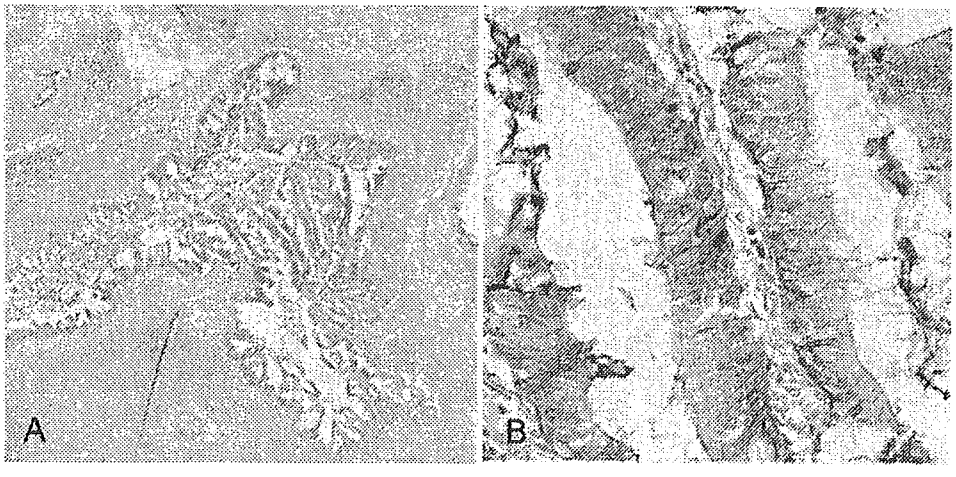


**Figure 5.** Immunohistochemical detection of FAK in pancreatic adenocarcinoma. In pancreatic cancer tissue, FAK was expressed in the cytoplasm and on the plasma membranes of the cancer cells. Original magnification; × 200 (A): x1000 (B).

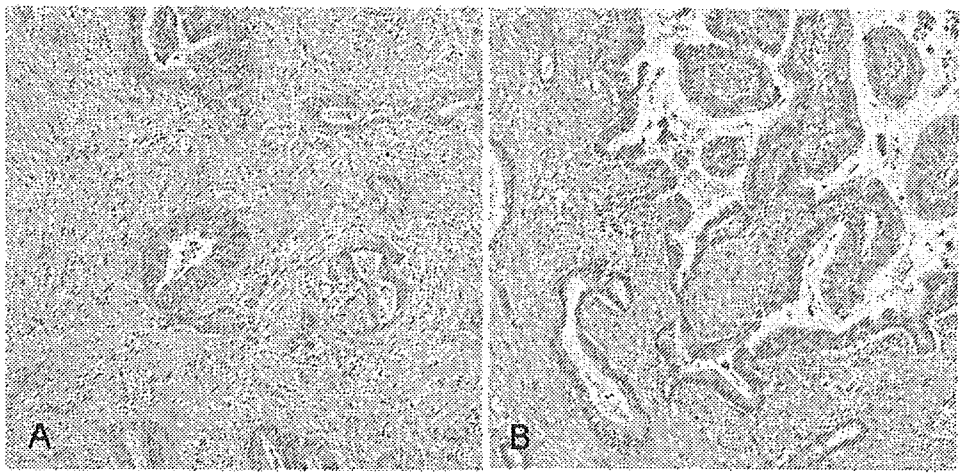


**Figure 6.** Heterogenous expression of FAK in pancreatic cancer tissues. Several sections showed labeling of the majority of cancer cells (A), whereas in others only a part of tumor area was positive for FAK (B). Original magnification; × 200.

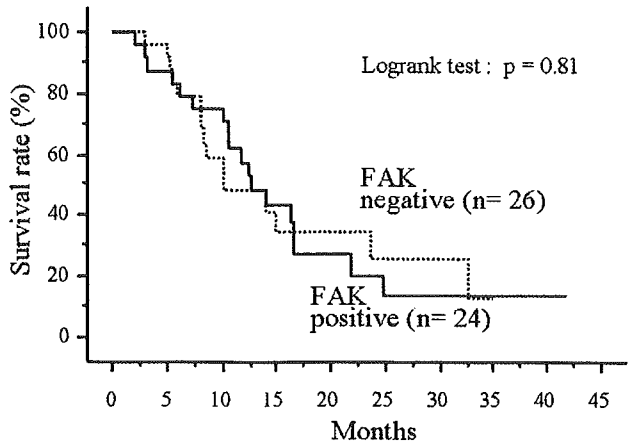


**Figure 7.** Survival curves of the patients with pancreatic cancer. There was no statistically significant difference in the survival between patients with FAK-positive tumors and patients with FAK-negative tumors ($P = 0.81$).

As a result, we found that there is a significant correlation between FAK expression and tumor size in pancreatic cancer patients. It has been reported that astrocytoma cells expressing FAK formed larger tumors in nude mice than tumor cells derived from the parental cell lines.[18] Further, expression of a hyperactive mutant of FAK, SuperFAK,[19] in the breast cancer cell line resulted in an increase in the size of tumors in nude mice.[3] In addition, the FAK dominant negative, FAK-related nonkinase (FRNK) expression inhibited the growth of human carcinoma cells into tumors in nude mice.[20] These reports are consistent with our results and suggest that greater tumor size might be associated with an increased rate of cell proliferation by FAK expression.

Previous reports on different kinds of cancer have shown that FAK expression correlated with survival rate,[9,15] although FAK was not a prognostic factor by itself in those studies. We showed that the survival curve of FAK-positive and FAK-negative patients showed no significant separation. It is well documented that the most important prognostic factor in completely resected patients is nodal status;[21,22] however, other predictors of a favorable outcome include a tumor size < 3 cm, negative margins, well-differentiated tumors, and intraoperative blood loss of less than 750 ml.[23–25] Focal adhesion kinase expression was significantly associated with tumor size in our study, although it was not a prognostic predictor for survival of the pancreatic cancer patients.

Recent studies have demonstrated that suppression of FAK by small interfering RNA (siRNA) enhanced the chemosensitivity of pancreatic adenocarcinoma to gemcitabine,[26] promoted anoikis, and inhibited metastasis of pancreatic cancer cells in vivo.[27] These observations suggest that FAK might be an important determinant of malignant cellular behavior and could be a rational target for therapeutic intervention in pancreatic cancer. We noted, however, FAK was expressed in the normal pancreatic duct. It is not clear why normal pancreatic ductal cells express FAK, although this might be an obstacle when molecular target therapy for FAK would be conducted in vivo.

In conclusion, this is the first report to show the relationship between FAK expression and the clinicopathological factors in pancreatic cancer. We have revealed that FAK expression was related to tumor size in pancreatic cancer, but it was not related to other clinicopathological factors. Focal adhesion kinase expression may not be a prognostic marker for pancreatic cancer

**Table 2.**
Comparison between the expression of FAK and clinicopathological features of pancreatic cancer.

| Category | FAK-positive (n = 24) | FAK-negative (n = 26) | *P* Value |
|---|---|---|---|
| UICC* classification system (6th edition) | | | |
| Histological grade | | | |
| Well–moderate | 21 | 25 | 0.34 |
| Poor | 3 | 1 | |
| pT | | | |
| 1, 2 | 4 | 5 | >0.999 |
| 3, 4 | 20 | 21 | |
| pN | | | |
| 0 | 7 | 6 | 0.751 |
| 1 | 17 | 20 | |
| pM | | | |
| 0 | 18 | 23 | 0.281 |
| 1 | 6 | 3 | |
| Stage | | | |
| 1 | 1 | 3 | 0.375 |
| 2 | 12 | 13 | |
| 3 | 5 | 9 | |
| 4 | 6 | 3 | |
| JPS† classification system (5th edition) | | | |
| Tumor size | | | |
| <4 cm | 15 | 25 | 0.004 |
| ≥4 cm | 9 | 1 | |
| Lymphatic invasion | | | |
| − | 2 | 4 | 0.669 |
| + | 22 | 22 | |
| Portal venous system invasion | | | |
| − | 5 | 5 | >0.999 |
| + | 19 | 21 | |
| Nerve invasion (intrapancreatic) | | | |
| − | 2 | 1 | 0.602 |
| + | 22 | 25 | |
| Nerve invasion (extrapancreatic) | | | |
| − | 11 | 12 | >0.999 |
| + | 13 | 14 | |
| Arterial invasion | | | |
| − | 21 | 23 | >0.999 |
| + | 3 | 3 | |
| Anterior serosal invasion | | | |
| − | 17 | 17 | 0.767 |
| + | 7 | 9 | |
| Retroperitoneal invasion | | | |
| − | 11 | 12 | >0.999 |
| + | 13 | 14 | |

*Union Internationale Contra Cancrum (International Union Against Cancer).
†Japan Pancreas Society.

patients, but it could be a molecular target for therapeutic intervention in these patients.

## ACKNOWLEDGMENTS

This work was supported by grants-in-aid 17390364 and 17659409 from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

## REFERENCES

1. Schaller MD, Borgman CA, Cobb BS, *et al.* pp125FAK a structurally distinctive protein-tyrosine kinase associated with focal adhesions. Proc Natl Acad Sci USA 1992;89:5192–5196.
2. Kornberg L, Earp HS, Parsons JT, *et al.* Cell adhesion or integrin clustering increases phosphorylation of a focal adhesion-associated tyrosine kinase. J Biol Chem 1992;267:23439–23442.
3. Gabarra-Niecko V, Schaller MD, Dunty JM. FAK regulates biological processes important for the pathogenesis of cancer. Cancer Metastasis Rev. 2003;22:359–374.
4. Sieg DJ, Hauck CR, Ilic D, *et al.* FAK integrates growth-factor and integrin signals to promote cell migration. Nat Cell Biol 2000;2:249–256.
5. Cance WG, Harris JE, Iacocca MV, *et al.* Immunohistochemical analyses of focal adhesion kinase expression in benign and malignant human breast and colon tissues: correlation with preinvasive and invasive phenotypes. Clin Cancer Res 2000;6:2417–2423.
6. Owens LV, Xu L, Dent GA, *et al.* Focal adhesion kinase as a marker of invasive potential in differentiated human thyroid cancer. Ann Surg Oncol 1996;3:100–105.
7. Agochiya M, Brunton VG, Owens DW, *et al.* Increased dosage and amplification of the focal adhesion kinase gene in human cancer cells. Oncogene 1999;18:5646–5653.
8. Judson PL, He X, Cance WG, *et al.* Overexpression of focal adhesion kinase, a protein tyrosine kinase, in ovarian carcinoma. Cancer 1999;86:1551–1556.
9. Miyazaki T, Kato H, Nakajima M, *et al.* FAK overexpression is correlated with tumour invasiveness and lymph node metastasis in oesophageal squamous cell carcinoma. Br J Cancer 2003;89:140–145.
10. Mori T, Doi R, Koizumi M, *et al.* CXCR4 antagonist inhibits stromal cell-derived factor 1-induced migration and invasion of human pancreatic cancer. Mol Cancer Ther 2004; 3:29–37.
11. Schaller MD, Hildebrand JD, Shannon JD, *et al.* Autophosphorylation of the focal adhesion kinase, pp125FAK, directs SH2-dependent binding of pp60src. Mol Cell Biol 1994;14:1680–1688.

12. Chen HC, Guan JL. Association of focal adhesion kinase with its potential substrate phosphatidylinositol 3-kinase. Proc Natl Acad Sci USA 1994;91:10148–10152.
13. Zhang X, Chattopadhyay A, Ji QS, *et al.* Focal adhesion kinase promotes phospholipase C-gamma1 activity. Proc Natl Acad Sci USA 1999;96:9021–9026.
14. Han DC, Guan JL. Association of focal adhesion kinase with Grb7 and its role in cell migration. J Biol Chem 1999;274:24425–24430.
15. Itoh S, Maeda T, Shimada M, *et al.* Role of expression of focal adhesion kinase in progression of hepatocellular carcinoma. Clin Cancer Res 2004;10:2812–2817.
16. Su JM, Gui L, Zhou YP, *et al.* Expression of focal adhesion kinase and alpha5 and beta1 integrins in carcinomas and its clinical significance. World J Gastroenterol 2002;8:613–618.
17. Komberg LJ. Focal adhesion kinase expression in oral cancers. Head Neck 1998;20:634–639.
18. Wang D, Grammer JR, Cobbs CS, *et al.* p125 Focal adhesion kinase promotes malignant astrocytoma cell proliferation in vivo. J Cell Sci 2000;113(Pt 23):4221–4230.
19. Gabarra-Niecko V, Keely PJ, Schaller MD. Characterization of an activated mutant of focal adhesion kinase: 'Super-FAK'. Biochem J 2002;365(Pt 3):591–603.
20. Aguirre Ghiso JA. Inhibition of FAK signaling activated by urokinase receptor induces dormancy in human carcinoma cells in vivo. Oncogene 2002;21:2513–2524.
21. Trede M, Schwall G, Saeger HD. Survival after pancreatoduodenectomy. 118 consecutive resections without an operative mortality. Ann Surg 1990;211:447–458.
22. Bakkevold KE, Arnesjo B, Dahl O, *et al.* Adjuvant combination chemotherapy (AMF) following radical resection of carcinoma of the pancreas and papilla of Vater—results of a controlled, prospective, randomised multicentre study. Eur J Cancer 1993;29A:698–703.
23. Sohn TA, Campbell KA, Pitt HA, *et al.* Quality of life and long-term survival after surgery for chronic pancreatitis. J Gastrointest Surg 2000;4:355–364discussion 364–355.
24. Millikan KW, Deziel DJ, Silverstein JC, *et al.* Prognostic factors associated with resectable adenocarcinoma of the head of the pancreas. Am Surg 1999;65:618–623; discussion 623–614.
25. Meyer W, Jurowich C, Reichel M, *et al.* Pathomorphological and histological prognostic factors in curatively resected ductal adenocarcinoma of the pancreas. Surg Today 2000;30:582–587.
26. Duxbury MS, Ito H, Benoit E, *et al.* RNA interference targeting focal adhesion kinase enhances pancreatic adenocarcinoma gemcitabine chemosensitivity. Biochem Biophys Res Commun 2003;311:786–792.
27. Duxbury MS, Ito H, Zinner MJ, *et al.* Focal adhesion kinase gene silencing promotes anoikis and suppresses metastasis of human pancreatic adenocarcinoma cells. Surgery 2004;135:555–562.

*Int. J. Cancer:* **118,** 2337–2343 (2006)


# *In vivo* antitumor effect of the mTOR inhibitor CCI-779 and gemcitabine in xenograft models of human pancreatic cancer

**Daisuke Ito, Koji Fujimoto*, Tomohiko Mori, Kazuhiro Kami, Masayuki Koizumi, Eiji Toyoda, Yoshiya Kawaguchi and Ryuichiro Doi**

*Department of Surgery and Surgical Basic Science, Kyoto University, Kyoto, Japan*

**Mammalian target of rapamycin (mTOR) is considered to be a major effector of cell growth and proliferation that controls protein synthesis through a large number of downstream targets. We investigated the expression of the phosphatidylinositol 3′-kinase (PI3K)/mTOR signaling pathway in human pancreatic cancer cells and tissues, and the *in vivo* antitumor effects of the mTOR inhibitor CCI-779 with/without gemcitabine in xenograft models of human pancreatic cancer. We found that the Akt, mTOR and p70 S6 kinase (S6K1) from the PI3K/mTOR signaling pathway were activated in all of the pancreatic cancer cell lines examined. When surgically resected tissue specimens of pancreatic ductal adenocarcinoma were examined, phosphorylation of Akt, mTOR and S6K1 was detected in 50, 55 and 65% of the specimens, respectively. Although CCI-779 had no additive or synergistic antiproliferative effect when combined with gemcitabine *in vitro*, it showed significant antitumor activity in the AsPC-1 subcutaneous xenograft model as both a single agent and in combination with gemictabine. Furthermore, in the Suit-2 peritoneal dissemination xenograft model, the combination of these 2 drugs achieved significantly better survival when compared with CCI-779 or gemcitabine alone. These results demonstrate promising activity of the mTOR inhibitor CCI-779 against human pancreatic cancer, and suggest that the inhibition of mTOR signaling can be exploited as a potentially tumor-selective therapeutic strategy.**


**Key words:** pancreatic cancer; mTOR; CCI-779

Pancreatic cancer is the fifth leading cause of cancer-related death in most western countries.[1] Since it is difficult to detect this disease at an early stage and because pancreatic cancer shows resistance to almost all available chemotherapy and radiation regimens, the prognosis remains dismal with a 5-year survival rate under 10%.[2] Currently, the only active agent for advanced pancreatic ductal adenocarcinoma (PDA) appears to be gemcitabine, a DNA chain terminator. Even with this drug, however, the objective response rate is less than 20%.[3] Until recently, the resistance of this cancer has generally been attributed to increased expression of detoxification mechanisms such as P-glycoprotein or antioxidants or to alterations of drug-metabolizing enzymes. Although these "classical" mechanisms are detectable in pancreatic cancer, there is no compelling evidence that their importance is greater than that in more responsive cancers,[4] and so such mechanisms may not explain the high level of drug resistance seen in pancreatic cancer patients.

The mammalian target of rapamycin (mTOR) is one of the effectors regulated *via* the phosphatidylinositol 3′-kinase (PI3K)/Akt signaling pathway and it plays a central role in cell survival and proliferation.[5] mTOR is a 289 kDa serine-threonine kinase that consists of 2,549 amino acids, and appears to modulate cellular signals in response to mitogenic stimuli and various nutrients, especially amino acids. In mammalian cells, growth factors (such as IGF, EGF and VEGF) regulate mTOR signaling through the PI3K/Akt pathway.[5,6] Intriguingly, *ras* shows mutation in more than 90% of PDAs, and it also positively regulates Akt.[7] This pathway involves phosphatase and tensin homologue deleted from chromosome 10 (PTEN), 3-phosphoinositide-dependent kinase-1 (PDK1) and tuberous sclerosis complexes-1 and -2 (TSC-1 and -2). p70 S6 kinase (S6K1) and 4E-binding protein 1 (4E-BP1) are 2 major downstream targets of mTOR. These substrates bind to regulatory-associated protein of mTOR (Raptor) and are activated by phosphorylation of certain residues.[8] In turn, S6K1 phosphorylates the 40s ribosomal S6 protein, leading to the translation of mRNAs bearing a 5′-TOP tract. Hypophospholyrated 4E-BP1 binds avidly to eIF4E, inhibiting the formation of the eIF4E complex and cap-dependent initiation of translation. After being hyperphosphorylated by mTOR, 4E-BP1 releases eIF4E and promotes cap-dependent protein synthesis.

Rapamycin is a macrocyclic lactone that causes cell cycle arrest in G1 phase and apoptosis of many normal cells and tumor cells.[9–14] It is well known that rapamycin forms a complex with FK506-binding protein (FKBP), and specifically inhibits mTOR activity through the binding of this complex to the FKBP rapamycin-binding domain of mTOR, although the details of the mechanism by which this complex acts are still unclear. CCI-779 is a novel water-soluble synthetic rapamycin ester that is more stable *in vivo* than rapamycin, and is currently being developed for the treatment of patients with cancer.

Dysregulation of mTOR signaling occurs in a wide variety of human tumors, and can lead to increased susceptibility to mTOR inhibitors.[15–18] Since activation of Akt has been reported in pancreatic cancer[19–21] and since the mTOR-S6K1 signaling pathway is constitutively activated and is essential for proliferation of pancreatic cancer cells *in vitro*,[22,23] mTOR (which lies downstream of the PI3K/Akt pathway) might be a promising objective of novel molecular targeting therapy for pancreatic cancer. In addition, recent studies have revealed that CCI-779 showed antitumor activity against advanced refractory renal cell carcinoma in a randomized phase II study[24] and human multiple myeloma cells in a xenograft model.[25] These considerations also provide a rationale for using the mTOR inhibitor CCI-779 to treat pancreatic cancer.

The aims of this study were to investigate whether or not mTOR signaling is activated in PDA tissues and pancreatic cancer cell lines, as well as whether or not CCI-779 (an mTOR inhibitor) has an antiproliferative effect in animal models of human pancreatic cancer as a single agent and in combination with gemcitabine.

## Material and methods

### *Antibodies*

Rabbit anti-Akt (no. 9272), anti-mTOR (no. 2972), anti-S6K1 (no. 9202), anti-4E-BP1 (no. 9271), anti-phospho-Akt (Ser473, no. 9277), anti-phospho-PDK1 (Ser241, no. 3061), anti-phospho-GSK3 (Ser9, no. 9336), anti-phospho-mTOR (Ser2448, no. 2971), anti-phospho-S6K1 (Thr421/Ser424, no. 9204) and anti-phospho-4E-BP1 (Thr70, no. 9455) were purchased from Cell Signaling Technology, Inc. (Beverly, MA). Mouse anti-PTEN (clone 6H2.1, no. ABM2052) was purchased from Cascade Bioscience (Winchester, MA). Mouse anti-β-actin (cat. no. A-5441) was obtained from Sigma (St. Louis, MO).

Grant sponsor: The Ministry of Education, Culture, Sports, Science and Technology of Japan.
***Correspondence to:** Department of Surgery and Surgical Basic Science, Kyoto University, 54 Shogoinkawara-cho, Sakyo, Kyoto 606-8507, Japan. Fax:+81-75-751-4390. E-mail: kofuji@kuhp.kyoto-u.ac.jp
Received 14 March 2005; Accepted after revision 5 August 2005
DOI 10.1002/ijc.21532
Published online 5 December 2005 in Wiley InterScience (www.interscience.wiley.com).

*Tissue samples*

Formalin-fixed, paraffin-embedded tissues were obtained from the Department of Surgery and Basic Surgical Science at Kyoto University (Kyoto, Japan) between January 1994 and December 2000. These specimens were harvested from 20 patients with invasive PDA and were collected after informed consent was given, in accordance with institutional guidelines.

*Immunohistochemical analysis*

Serial sections (4-μm thick) were deparaffinized in 3 changes of xylene, rehydrated in descending concentrations of ethanol, and washed 3 times for 5 min each with double distilled water. After rehydration, the sections were heated in a microwave oven in 10 mM sodium citrate buffer (pH 6.0) for 1 min at full power followed by 9 min at medium power, and then incubated for 10 min at room temperature (RT) in 1% hydrogen peroxide in methanol to block endogenous peroxidase activity. Next, the sections were incubated for 60 min at RT with 10 mM phosphate-buffered saline (PBS) (pH 7.4) containing 5% normal goat serum, followed by overnight incubation at 4°C with the primary antibody diluted 1:50 to 1:100 in 10 mM PBS containing 5% normal goat serum. Then the sections were washed 3 times for 5 min in PBS and incubated for 60 min with horseradish peroxidase-conjugated anti-rabbit immunoglobulin G (Envision Kit, DakoCytomation, Kyoto, Japan) as the secondary antibody. The serial sections were stained for assessment of p-AKT, p-mTOR and p-S6K1 expression. Negative controls were done using nonspecific rabbit IgG as the primary antibody. The expression of each phosphorylated molecule was classified into 3 grades: 0 = undetectable, 1 = weak staining and 2 = strong staining. The proportion of positive cancer cells was also classified into 3 grades: 0 = none, 1 = 1–49% and 2 = 50–100%. Then the staining intensity score was multiplied by the score for positive cells to obtain the overall score. Specimens with a score of $\geq 2$ were regarded as positive, while specimens with a score $\leq 1$ as negative. Evaluation of immunostaining was performed by 2 investigators (D. I. and K. F.).

*Cell lines and culture conditions*

Six human pancreatic cancer cell lines were used. AsPC-1, BxPC-3 and Panc-1 cells were obtained from the ATCC (Rockville, MD). KMP-3 and KMP-4 were cell lines established in our department,[26] while Suit-2 cells were kindly provided by Dr. Tomoda (National Kyushu Cancer Center, Fukuoka, Japan). Cells were maintained in the following media during incubation at 37°C in a humidified atmosphere of 5% $CO_2$. AsPC-1 cells and BxPC-1 cells were cultured in RPMI 1640 medium (Gibco-BRL, Grand Island, NY) with 10% fetal bovine serum (FBS) (Gibco-BRL). KMP-3 cells and KMP-4 cells were cultured in an equal mixture of RPMI 1640 and F-12 nutrient medium (HAM) (Gibco-BRL) with 2% FBS. Panc-1 cells and Suit-2 cells were cultured in DMEM (Gibco-BRL) with 10% FBS. Each medium contained 100 units/ml of penicillin and 0.1 mg/ml of streptomycin.

*Agents*

CCI-779 was kindly provided by Wyeth-Ayerst Reseach Laboratories (Princeton, NJ) and gemcitabine was obtained from Eli Lilly (Bad Homburg, Germany). CCI-779 was stored as a dry powder at −20°C and dissolved in ethanol on the day of use. Gemcitabine was stored as a 50 mg/ml solution in sterile PBS at −20°C.

*Cell proliferation assay*

Cells ($1.5 \times 10^4$/ml) were seeded into 24-well tissue culture dishes. After overnight incubation with complete medium, the cells were cultured under serum-starved conditions for 24 hr. Then the cells were treated with various concentrations of CCI-779 alone or combined with gemcitabine in the presence of 10% FBS. After 48 hr, the number of cells was counted using a cell counter (Coulter Z1; Beckman-Coulter, Fullerton, CA).

*Western blot analysis*

Cells were incubated with 20 nM CCI-779 or vehicle for 6 hr after culture with serum starvation for 24 hr. Then the cells were lysed in RIPA buffer containing 50 mM HEPES (pH 7.0), 250 mM NaCl, 0.1% Nonidet P-40, 1 mM phenylmethylsulfonylfluoride, and 20 μg/ml gabexate mesilate, followed by incubation on ice for 10 min. Subsequently, the lysate was sonicated for 10 sec. The extracts were cleaned by centrifugation at 15,000 rpm for 10 min at 4°C and the supernatants were harvested. The protein concentration was measured by a protein assay (cat. no. 23223, 23224; Pierce, Rockford, IL). Next, the lysates were resuspended in 1 vol of gel loading buffer containing 50 mM Tris–HCl (pH 6.7), 4% SDS, 0.02% bromophenol blue, 20% glycerol and 4% 2-mercaptoethanol. After heating at 95°C for 5 min, the extracted protein was subjected to western blotting as described previously.[27] In brief, 30-μg aliquots of protein were size-fractionated to a single dimension by SDS-PAGE (8–15% gels) and transblotted onto a 0.45-μm polyvinylidene difluoride membrane (IPVH304F0; Millipore, Billerica, MA) using a semidry electroblot apparatus (Bio-Rad, Richmond, CA). The blots were then washed 3 times in Tris-buffered saline with 0.1% Tween-20 (TBST) and incubated for 1 hr at room temperature in blocking buffer (Block Ace; Dainipponseiyaku, Osaka, Japan). Subsequently, the blots were immunoblotted with an appropriate primary antibody for 1 hr at room temperature or overnight at 4°C. Unbound antibody was removed by washing the membrane 3 times for 10 min each with TBST, after which the membrane was incubated with a horseradish peroxidase-conjugated secondary antibody for 1 hr at room temperature. Reaction products were detected by the enhanced chemiluminescence system (Amersham, Buckinghamshire, UK) and membranes were treated with enhanced chemiluminescence reagents according to the manufacturer's protocol and were exposed to X-ray film for 5–120 sec.

*Animal study*

The animal study was performed in accordance with the guidelines for animal experiments of Kyoto University. To create the subcutaneous xenograft model, athymic female nude mice (4–6 weeks old) were subcutaneously inoculated with $1 \times 10^6$ AsPC-1 cells in 100-μl of Hank's Balanced Salt Solution (HBSS) (Gibco-BRL) containing 20% matrigel (BD Biosciences, Bedford, MA). Tumor-bearing animals were divided into the following 5 treatment groups: (*i*) CCI-779 (1 mg/kg, 5 times/week for 2 weeks), (*ii*) CCI-779 (10 mg/kg, 5 times/week for 2 weeks), (*iii*) gemcitabine (125 mg/kg, 2 times/week for 2 weeks), (*iv*) CCI-779 + gemcitabine (10 and 125 mg/kg for 2 weeks) and (*v*) vehicle alone (control). Each group consisted of 10 animals. CCI-779 and gemcitabine were administered intraperitoneally and treatment was started when subcutaneous tumors reached 20 $mm^3$ in size. Tumor size was measured weekly with calipers and the volume was calculated by the following formula: tumor volume = (length × width$^2$)/2.

For the peritoneal dissemination xenograft model, $1 \times 10^6$ Suit-2 cells in 250-μl sterile PBS were injected into the intraperitoneal cavity of nude mice. After injection, the mice were divided into the following 5 treatment groups:(*i*) CCI-779 (10 mg/kg, 5 times/week for 1 week), (*ii*) CCI-779 (20 mg/kg, 5 times/week for 1 week), (*iii*) gemcitabine (125 mg/kg, 2 times/week for 2 weeks), (*iv*) CCI-779 + gemcitabine (20 mg/kg, 5 times/week for 1 week and 125 mg/kg, 2 times/week for 2 weeks) and (*v*) vehicle alone (control). Each group consisted of 5 animals. Treatment was initiated 3 days after the injection of Suit-2 cells, and survival was measured from the date of injection.

*Statistical analysis*

Results are presented as the mean ± SEM for quantitative experiments. For statistical analysis of differences between the groups, one-way ANOVA was performed and statistical significance was considered to be present at $p < 0.05$. Each experiment was performed at least 3 times independently. Survival was calcu-

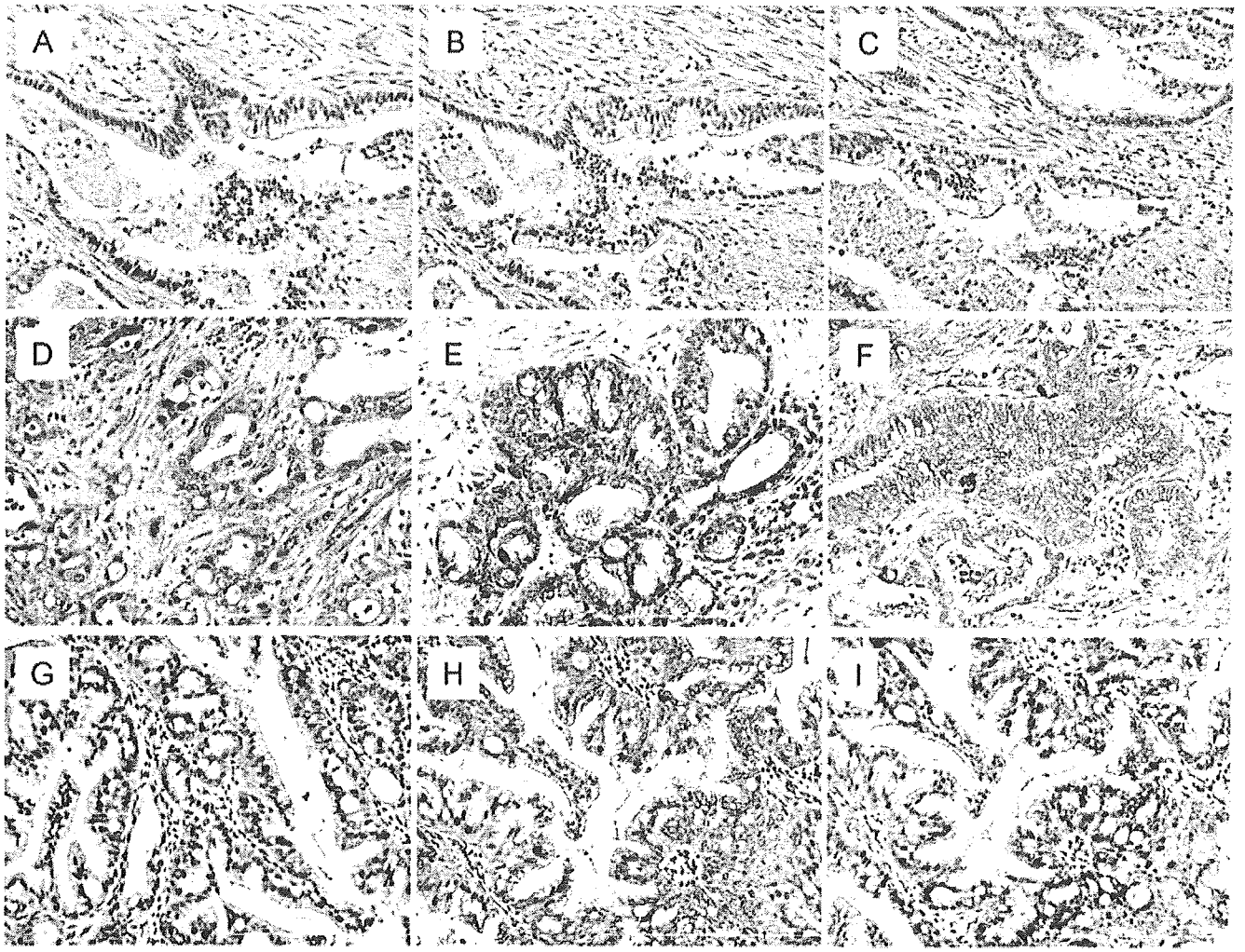


FIGURE 1 – Immunohistochemical staining of phospho-Akt (Ser473, no. 9277) (*a*, *d* and *g*), phospho-mTOR (Ser2448, no. 2971) (*b*, *e* and *h*) and phospho-p70S6K (Thr421/Ser424, no. 9204) (*c*, *f* and *i*) in specimens of pancreatic ductal adenocarcinoma (PDA). The upper panel (*a*, *b* and *c*, serial sections) represents the sections of PDA specimens regarded as negative. The middle panel (*d*, *e* and *f*) shows typical photomicrographs of positive sections of PDA specimens. The lower panel (*g*, *h* and *i*) shows expression of these 3 molecules in serial sections of PDA. Strong cytosolic staining for p-Akt, p-mTOR and p-p70S6K is seen. The nuclei were counterstained with Mayer's hematoxylin (original magnification: ×400).

lated by the Kaplan–Meier method, and differences between each group were evaluated with the log-rank test.

## Results

### *Expression of mTOR pathway-related molecules in PDA tissue specimens*

To examine the role of the mTOR signaling pathway in human pancreatic cancer, we performed immunohistochemical analysis of tissue specimens from PDAs. Expression of p-Akt (Ser473), p-mTOR (Ser2448) and p-S6K1 (Thr421/Ser424) was observed in 50, 55 and 65% of the specimens, respectively (Fig. 1*d*–1*f*). Staining was mainly seen in the cytoplasm. As shown in Figure 1*g*–1*i*, positive expression of p-Akt was associated with positive expression of p-mTOR and p-S6K1 in serial sections of the same specimen. There was a significant correlation among the expression of these 3 molecules (p-Akt vs p-mTOR: $p = 0.0013$, p-mTOR vs p-S6K1: $p = 0.045$ and p-mTOR vs p-S6K1: $p = 0.0004$, respectively). When the expression of these molecules was compared with various clinicopathological features (pT, pN, pM, UICC Stage, tumor size and histological grade), however, there were no significant associations. There was also no statistical correlation between the expression of these molecules and survival.

### *Expression of mTOR pathway-related molecules by pancreatic cancer cell lines*

Next, we performed western blot analysis to examine the expression of mTOR pathway-related molecules by pancreatic cancer cell lines. As previously reported,[28] PTEN (a negative regulator of PI3K) was not expressed by KMP-3 and KMP-4 cells. However, the other molecules, including Akt, mTOR, S6K1 and 4E-BP1, were expressed by all of the cell lines examined (Fig. 2*a*).

We also examined the phosphorylation of these molecules after 24 hr of serum starvation. Phospho-PDK1, which phosphorylates Akt, showed almost equal expression in all of the cell lines. With respect to phospho-Akt, which reflects loss of PTEN expression, its expression was much stronger in KMP-3 and KMP-4 cells than in the other cell lines. Interestingly, mTOR was almost equally phosphorylated in all of the cell lines, a result that was inconsistent with p-Akt expression. Furthermore, phosphorylation of S6K1 and 4E-BP1 was also observed in all of the cell lines. On the other hand, the pattern of phospho-GSK expression, which is another target of Akt, was the same as that of phospho-Akt expression (Fig. 2*b*).

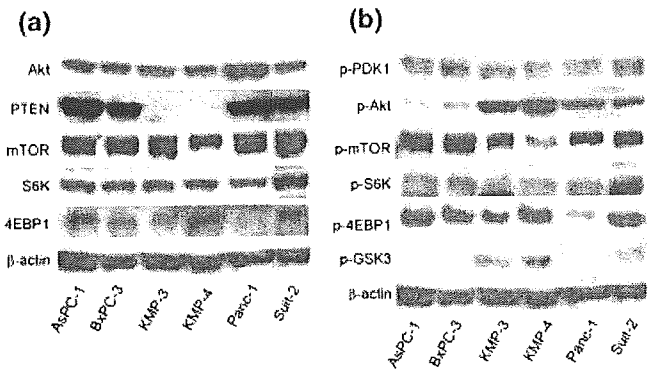


FIGURE 2 – (*a*) Western blot analysis of mTOR signaling pathway-related proteins in pancreatic cancer cell lines. Twenty micrograms of total protein extracted from cells starved of serum for 24 hr was applied to each lane. Akt, mTOR, p70S6K and 4E-BP1 were expressed at almost equivalent levels by all of the cell lines. PTEN expression was not detected in KMP3 and KMP-4 cells. Representative results from 3 independent experiments are shown. (*b*) Western blot analysis of the phosphorylation of mTOR signaling pathway-related proteins in pancreatic cancer cell lines. The phosphorylation site and catalog number of each protein are as follows: phospho-Akt (Ser473, no. 9277), phospho-PDK1 (Ser241, no. 3061), phospho-GSK3 (Ser9, no. 9336), phospho-mTOR (Ser2448, no. 2971), phospho-S6K1 (Thr421/Ser424, no. 9204), and phospho-4E-BP1 (Thr70, no. 9455). Twenty micrograms of total proteins was applied to each lane. All of the proteins examined were phosphorylated in all of the cell lines even after 24 hr of serum starvation. Reflecting the loss of PTEN expression, Akt was strongly phosphorylated in KMP-3 and KMP-4 cells. Representative results from three independent experiments are shown.

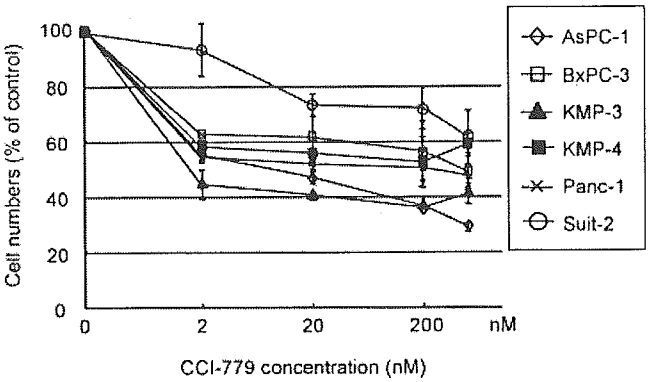


FIGURE 3 – Growth-inhibitory effect of CCI-779 on pancreatic cancer cell lines. Cells were cultured in the serum-free medium for 24 hr, followed by incubation with various concentrations of CCI-779 for 48 hr and the number of viable cells was counted. Data represent the mean ± SEM of triplicate determinations from 1 of 3 representative experiments.

TABLE I – SUMMARY OF EXPRESSION PATTERNS OF mTOR RELATED MOLECULES AND THE EFFECTS OF CCI-779 IN PANCREATIC CANCER CELL LINES

| | AsPC-1 | BxPC-3 | KMP-3 | KMP-4 | Panc-1 | Suit-2 |
|---|---|---|---|---|---|---|
| PTEN | + | + | − | − | + | + |
| p-Akt | + | + | ++++ | ++++ | ++ | +++ |
| p-mTOR | +++ | +++ | ++ | + | ++ | ++ |
| p-p70S6K | + | + | + | + | + | ++ |
| p-4EBP1 | ++ | ++ | ++ | ++ | + | ++ |
| $IC_{50}$ (nM) | 20 | >200 | 2 | 100 | 100 | >500 |

*Effect of CCI-779 alone and combined with gemcitabine on cultured pancreatic cancer cells*

These results suggested that the mTOR signaling pathway might be constitutively activated in PDAs. Therefore, we next examined the growth-inhibitory effect of a specific mTOR inhibitor, CCI-779, on cultured pancreatic cancer cells. After serum starvation for 24 hr, 6 pancreatic cancer cell lines were cultured for 48 hr with complete growth medium containing increasing concentrations of CCI-779. KMP-3 (a PTEN-deficient cell line) and AsPC-1 showed a high sensitivity to CCI-779 ($IC_{50}$ = 2 and 20 nM, respectively), while Suit-2 cells had the highest resistance ($IC_{50}$ > 500 nM) (Fig. 3, Table I).

We also examined suppression of the phophorylation of S6K1 by CCI-779 treatment. As shown in Figure 4, phosphorylation of S6K1 was inhibited in all of the cell lines by treatment with 20 nM CCI-779 for 6 hr. In AsPC-1 cells, the phospho-specific band of S6K1 was decreased by 88% after CCI-779 treatment when compared with that after vehicle treatment. In Suit-2 cells that were resistant to this drug, however, p70S6K phosphorylation was only decreased by 39% (Fig. 4). From these data, AsPC-1 and Suit-2 cells were chosen as representative sensitive and resistant cells to CCI-779 for further experiments.

Before we performed *in vivo* experiments, the growth inhibitory effect of CCI-779 combined with gemcitabine was examined *in vitro* using AsPC-1 and Suit-2 cells. Our preliminary study demonstrated that AsPC-1 cells were resistant to gemcitabine, while Suit-2 cells were sensitive (data not shown). As shown in Figure 5, combination therapy had neither a synergistic nor an additive effect on both cell lines.

*Antitumor activity of CCI-779 in xenograft models*

The antitumor effect of CCI-779 was also examined using AsPC-1 and Suit-2 xenograft models. After inoculation of $1 \times 10^6$ cells into the right flank (AsPC-1) or the peritoneal cavity (Suit-2) of nude mice, CCI-779 and/or gemcitabine was administered by intraperitoneal injection. In the AsPC-1 subcutaneous xenograft model, CCI-779 was able to delay tumor growth as single agent (Fig. 6). Treatment with a low dose of CCI-779 significantly decreased tumor volume by 74% after 35 days ($p$ = 0.037) and treatment with a high dose of CCI-779 also significantly decreased tumor volume by 68% when compared with the control ($p$ = 0.009). Consistent with the *in vitro* results, no growth inhibition was observed in the mice treated with gemcitabine alone. Interestingly, treatment with a combination of CCI-779 and gemcitabine caused a synergistic decrease of tumor volume by 41% when compared with the control ($p$ = 0.0002). In addition, the combination therapy resulted in a significant decrease of tumor volume even when compared with the CCI-779 alone as well as the gemcitabine alone ($p$ = 0.0042).

In the Suit-2 intraperitoneal xenograft model, our preliminary study revealed that untreated mice died of peritoneal dissemination after approximately 7 weeks with bloody ascites. All of the mice in the control and CCI-779 monotherapy group died within 45 days, and there were no significant differences among the groups (Fig. 7). Consistent with the high *in vitro* sensitivity to gemcitabine, the mice treated with gemcitabine alone showed significantly longer survival time than untreated mice or CCI-779-treated mice ($p$ = 0.0198 and 0.0026, respectively). Interestingly, just as in the AsPC-1 model, mice treated with a combination of CCI-779 and gemcitabine showed the best survival. In fact, 2 of the 5 mice given combination therapy survived for more than 100 days, and no tumor was seen in the peritoneal cavity when the mice were killed for autopsy on day 107.

## Discussion

The present study demonstrated that the signaling pathways regulating mTOR are frequently activated in pancreatic cancer, and that a rapamycin derivative (CCI-779) could inhibit tumor growth

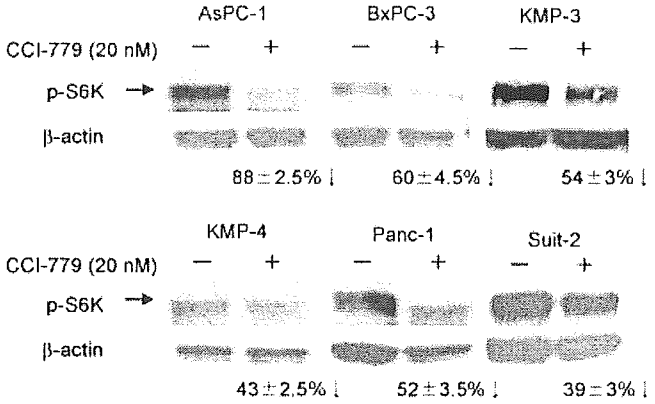


FIGURE 4 – Western blot analysis of the phosphorylation of S6K in pancreatic cancer cells after treatment with CCI-779. Twenty micrograms of total protein extracted from untreated cells or cells treated with 20 nM CCI-779 was applied to each lane. The inhibitory rate was calculated by densitometry (ATTO, Osaka, Japan). A representative result from 3 independent experiments is shown.

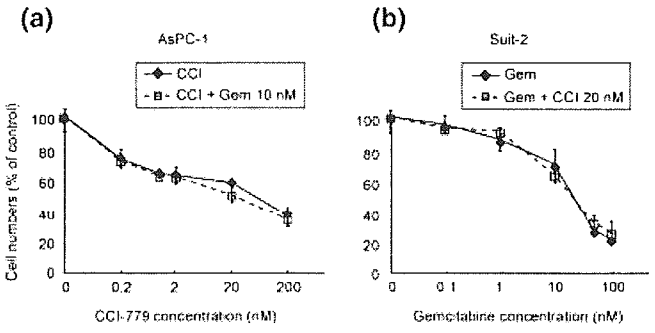


FIGURE 5 – Growth-inhibitory effect of combined treatment with CCI-779 and gemcitabine in (*a*) AsPC-1 and (*b*) Suit-2 cells. Since AsPC-1 cells were sensitive to CCI-779 and resistant to gemcitabine, they were treated with increasing concentrations of CCI-779 and a fixed concentration of gemcitabine (10 nM) for 48 hr. In contrast to AsPC-1 cells, Suit-2 cells were incubated with a fixed concentration of CCI-779 (20 nM) and increasing concentrations of gemcitabine for 48 hr, since these cells were resistant to CCI-779 and sensitive to gemcitabine. As shown in both graphs for AsPC-1 and Suit-2 cells, no additive or synergistic effects were found for both cell lines. Data represent the mean ± SEM of 9 determinations from 3 representative experiments.

in xenograft models as a single agent as well as combined with gemcitabine.

It is well known that *ras* mutation can be detected in more than 90% of pancreatic cancers and that ras protein binds and activates the catalytic subunit of PI3K.[29,30] Recent studies have revealed that the genes encoding the catalytic subunit of PI3K and Akt are amplified in various human cancers, including pancreatic cancer.[19–21,31] Interestingly, Hu *et al.* have demonstrated that transfection of mutant K- or N-*ras* genes into multiple myeloma cells confers a high sensitivity to CCI-779.[15] Furthermore, it has been reported that Akt regulates the sensitivity of tumors to mTOR inhibitors.[32] These studies lead to the hypothesis that mTOR and its downstream targets, which are considered to be essential for cell survival and proliferation through regulation of protein synthesis, might be activated in pancreatic cancer, and suggest that the inhibition of mTOR signaling could block tumor growth.

First, we examined the expression and constitutive phosphorylation of molecules from the mTOR signaling pathway in pancreatic cancer tissues and cell lines. Using specific antibodies, immunohistochemical analysis revealed that pospho-Akt, phospho-mTOR,

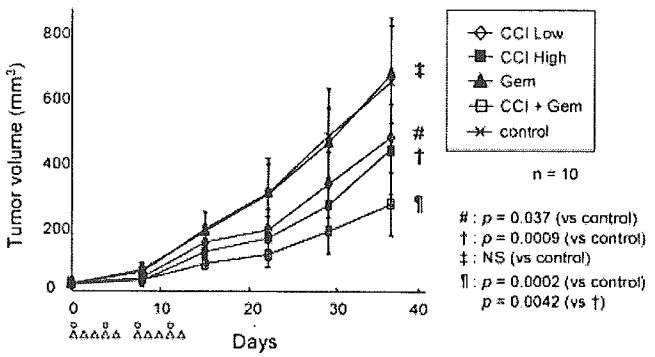


FIGURE 6 – Suppression of the growth of subcutaneous xenografts of AsPC-1 cells by CCI-779, gemcitabine, or a combination of both agents. Athymic mice bearing AsPC-1 xenografts were divided into 5 treatment groups. Open triangles indicate the day of CCI-779 administration and open circles indicate gemcitabine administration.

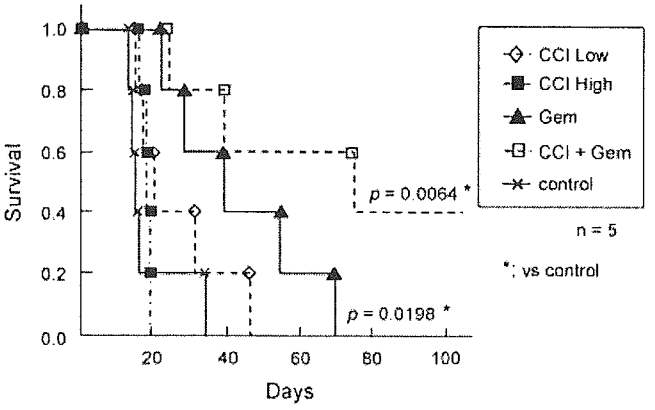


FIGURE 7 – Kaplan–Meier plot showing the survival of Suit-2-bearing mice after treatment with CCI-779, gemcitabine, or a combination of both agents. Suit-2 cells were injected intraperitoneally into athymic mice. Three days after injection, the mice were divided into 5 treatment groups and drug administration was initiated.

and phospho-S6K1 were expressed in at least half of the PDA specimens examined (50, 55 and 65%, respectively). Western blot analysis also demonstrated that the Akt/mTOR signaling pathway, including the downstream effectors S6K1 and 4E-BP1, was constitutively activated in all of the pancreatic cancer cell lines examined. These data suggested that the mTOR signaling pathway might play a role in the development of pancreatic cancer and that mTOR inhibitors might be active against pancreatic cancer.

Next, we used a cell proliferation assay to examine the *in vitro* growth inhibitory effect of CCI 779 against 6 pancreatic cancer cell lines, including 2 PTEN-deficient cell lines (KMP-3 and KMP-4 cells[26,28]). Although PTEN protein has been suggested to have multiple important functions pertaining to cell cycle regulation and survival, its best characterized role is related to regulation of the PI3K/Akt signaling pathway.[31] Recently, Neshat *et al.* have shown that CCI-779 inhibits the hyperproliferation of PTEN-deficient cells.[33] Also, the administration of CCI-779 to PTEN heterozygous mice, which develop multiple neoplasms, attenuates tumor development.[34] PTEN is a well-known negative regulator of PI3K, and acquired mutations of the PTEN gene have been reported in several tumors[35]; however, PTEN mutation occurs rarely in patients with pancreatic cancer.[36] In this study, we demonstrated that KMP-3 and 4 cells were PTEN-deficient cells and were sensitive to CCI-779 ($IC_{50}$: 2 and 100 nM, respectively) in spite of the relatively minimal suppression of S6K1 phosphorylation in both cells. We consider that PTEN status is also associated with the sensitiv-

ity of CCI-779 in KMP-3 and 4 cells besides S6K1 dephosphorylation.

Recent work has suggested that S6K1 activity may be an appropriate marker for mTOR-interacting agents.[37,38] It has been reported that rapamycin dephosphorylates S6K1 and inhibits the proliferation of pancreatic cancer cell lines such as BxPC-3, Panc-1 and MiaPaCa-2.[22,23] Consistent with these data, the suppression of S6K1 phosphorylation was maximum (88%) in AsPC-1 cells and was lowest (39%) in Suit-2 cells, which were highly sensitive and resistant to CCI-779, respectively, suggesting that suppression of S6K1 phosphorylation was correlated with the inhibitory effect of CCI-779 on pancreatic cancer cells (Fig. 4). With respect to 4E-BP1, another major downstream target of mTOR, CCI-779 had no effect on its phosphorylation in this study (data not shown). Interestingly, it has been shown that the treatment of nude mice bearing a CCI-779-sensitive breast cancer cell line (MDA-468) with CCI-779 results in marked inhibition of tumor tissue S6K1 activity, which is identical to the inhibition seen in peripheral blood mononuclear cells (PBMCs).[37] This suggests that PBMCs may be an adequate surrogate tissue for measuring S6K1 activity *in vivo*. In the clinical setting, it may be very useful to assess the pharmacodynamic effects of CCI-779 by using an S6K1 assay of PBMCs. From these data, we considered that the S6K1 activity should be regarded as a suitable surrogate marker of biological effect for an mTOR inhibitor, CCI-779.

We hypothesized that constitutively elevated levels of phosphorylated mTOR in pancreatic cancer cells could protect against apoptosis induced by chemotherapy agents and contribute to drug resistance. Therefore, the inhibition of mTOR phosphorylation by CCI-779 may enhance the sensitivity of pancreatic cancer cells to gemcitabine. To clarify this hypothesis, the effects of combined therapy with CCI-779 and gemcitabine were investigated *in vitro* and *in vivo*. From the results shown in Figure 3, we chose AsPC-1 and Suit-2 cells as CCI-779-sensitive and -resistant cells, respectively. In the *in vitro* proliferation assay, AsPC-1 was resistant and Suit-2 was sensitive to gemcitabine treatment as a single agent, unlike their susceptibility to CCI-779. As shown in Figure 5, combined therapy with CCI-779 and gemcitabine did not have a synergistic effect on both cell lines. Although numerous studies have demonstrated that rapamycin and its derivatives could induce apoptosis in various cancer cell lines,[39] the mechanisms of antiproliferative effects of CCI-779 *in vitro* in this study remain unclear. To elucidate the mechanisms, further investigation is now on-going in our laboratory. Next, the *in vivo* antitumor activity of CCI-779 as a single agent or combined with gemcitabine was examined. Since it is well known that many patients with PDA die from peritoneal dissemination, we studied an *in vivo* Suit-2 peritoneal dissemination model as well as an AsPC-1 subcutaneous model. Consistent with the data from our *in vitro* proliferation assay, CCI-779 showed an antitumor effect in the AsPC-1 subcutaneous model but not the Suit-2 peritoneal dissemination model, whereas gemcitabine showed the reverse activity as a single agent. Combined therapy with CCI-779 and gemcitabine led to delayed tumor growth in the AsPC-1 subcutaneous model and longer survival in the Suit-2 peritoneal dissemination model when compared with single agent therapy, suggesting that a synergistic effect might be obtained in the clinical setting.

This discrepancy between the susceptibility to combination therapy *in vitro* and *in vivo* is interesting. It was recently reported that rapamycin has a potent antiangiogenic effect by decreasing the production of vascular endothelial growth factor (VEGF).[40] El-Hashemite *et al.* have also reported that the loss of TSC-1 and TSC-2, which are upstream negative regulators of mTOR, induces VEGF production *via* activation of mTOR.[41] With respect to the relationship between VEGF and pancreatic cancer, we have previously shown that VEGF may play an important role in tumor angiogenesis.[42] Recently, it has been reported that treatment of rapamycin suppressed primary and metastatic liver tumor growth by the inhibition of tumor angiogenesis in an orthotopic model of AsPC-1, one of pancreatic cancer cell lines.[39] Intriguingly, in this model, combination treatment of rapamycin and anti-VEGF antibody 2C3 further improved the antitumor effects compared with single treatment of rapamycin or 2C3. Moreover, other investigators have demonstrated synergistic growth-inhibitory effects by combined treatment of gemcitabine and rapamycin in another orthotopic model of L3.6pl pancreatic cancer cells.[43] They have revealed that rapamycin induced apoptosis of endothelial cells and tumor vessel thrombosis in this model and suggested that combining rapamycin with other cytotoxic drugs could be a feasible therapy of pancreatic cancer. From these data, we speculate that an antiangiogenic effect of CCI-779 might be involved in the mechanism of its synergistic antitumor activity in this study.

In conclusion, mTOR and related molecules are frequently activated in pancreatic cancer, and an mTOR inhibitor (CCI-779) inhibits the proliferation of certain pancreatic cancer cells *in vitro* and shows a synergistic effect with gemcitabine *in vivo*. These data suggest that mTOR might be a promising objective of novel molecular targeting therapy for pancreatic cancer.

## References


1. Greenlee RT, Hill-Harmon MB, Murray T, Thun M. Cancer statistics, 2001. CA Cancer J Clin 2001;51:15–36.
2. Rosewicz S, Wiedenmann B. Pancreatic carcinoma. Lancet 1997;349: 485–9.
3. Burris HA, III, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, Tarassoff P, Nelson R, Dorr FA, et al. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. J Clin Oncol 1997;15:2403–13.
4. Goldstein LJ, Galski H, Fojo A, Willingham M, Lai SL, Gazdar A, Pirker R, Green A, Crist W, Brodeur GM, Lieber M, Cossman J, et al. Expression of a multidrug resistance gene in human cancers. J Natl Cancer Inst 1989;81:116–24.
5. Schmelzle T, Hall MN. TOR, a central controller of cell growth. Cell 2000;103:253–62.
6. Riesterer O, Zingg D, Hummerjohann J, Bodis S, Pruschy M. Degradation of PKB/Akt protein by inhibition of the VEGF receptor/mTOR pathway in endothelial cells. Oncogene 2004;23:4624–35.
7. Krasilnikov MA. Phosphatidylinositol-3 kinase dependent pathways: the role in control of cell growth, survival, and malignant transformation. Biochemistry (Moscow) 2000;65:59–67.
8. Bondar VM, Sweeney-Gotsch B, Andreeff M, Mills GB, McConkey DJ. Inhibition of the phosphatidylinositol 3′-kinase-AKT pathway induces apoptosis in pancreatic carcinoma cells in vitro and in vivo. Mol Cancer Ther 2002;1:989–97.
9. Akselband Y, Harding MW, Nelson PA. Rapamycin inhibits spontaneous and fibroblast growth factor β-stimulated proliferation of endothelial cells and fibroblasts. Transplant Proc 1991;23:2833–6.
10. Francavilla A, Starzl TE, Carr B, Azzarone A, Carrieri G, Zeng QH, Porter KA. The effects of FK 506, cyclosporine, and rapamycin on liver growth in vitro and in vivo. Transplant Proc 1991;23:2817–20.
11. Albers MW, Williams RT, Brown EJ, Tanaka A, Hall FL, Schreiber SL. FKBP-rapamycin inhibits a cyclin-dependent kinase activity and a cyclin D1-Cdk association in early G1 of an osteosarcoma cell line. J Biol Chem 1993;268:22825–9.
12. Jayaraman T, Marks AR. Rapamycin-FKBP12 blocks proliferation, induces differentiation, and inhibits cdc2 kinase activity in a myogenic cell line. J Biol Chem 1993;268:25385–8.
13. Dilling MB, Dias P, Shapiro DN, Germain GS, Johnson RK, Houghton PJ. Rapamycin selectively inhibits the growth of childhood rhabdomyosarcoma cells through inhibition of signaling via the type I insulin-like growth factor receptor. Cancer Res 1994;54:903–7.
14. Marx SO, Jayaraman T, Go LO, Marks AR. Rapamycin-FKBP inhibits cell cycle regulators of proliferation in vascular smooth muscle cells. Circ Res 1995;76:412–7.
15. Hu L, Shi Y, Hsu JH, Gera J, Van Ness B, Lichtenstein A. Downstream effectors of oncogenic ras in multiple myeloma cells. Blood 2003;101:3126–35.
16. Huang S, Houghton PJ. Targeting mTOR signaling for cancer therapy. Curr Opin Pharmacol 2003;3:371–7.

17. Mita MM, Mita A, Rowinsky EK. Mammalian target of rapamycin: a new molecular target for breast cancer. Clin Breast Cancer 2003;4: 126–37.
18. Panwalkar A, Verstovsek S, Giles FJ. Mammalian target of rapamycin inhibition as therapy for hematologic malignancies. Cancer 2004;100: 657–66.
19. Cheng JQ, Ruggeri B, Klein WM, Sonoda G, Altomare DA, Watson DK, Testa JR. Amplification of AKT2 in human pancreatic cells and inhibition of AKT2 expression and tumorigenicity by antisense RNA. Proc Natl Acad Sci USA 1996;93:3636–41.
20. Ruggeri BA, Huang L, Wood M, Cheng JQ, Testa JR. Amplification and overexpression of the AKT2 oncogene in a subset of human pancreatic ductal adenocarcinomas. Mol Carcinog 1998;21:81–6.
21. Altomare DA, Tanno S, De Rienzo A, Klein-Szanto AJ, Skele KL, Hoffman JP, Testa JR. Frequent activation of AKT2 kinase in human pancreatic carcinomas. J Cell Biochem 2003;88:470–6.
22. Grewe M, Gansauge F, Schmid RM, Adler G, Seufferlein T. Regulation of cell growth and cyclin D1 expression by the constitutively active FRAP-p70s6K pathway in human pancreatic cancer cells. Cancer Res 1999;59:3581–7.
23. Shah SA, Potter MW, Ricciardi R, Perugini RA, Callery MP. FRAP-p70s6K signaling is required for pancreatic cancer cell proliferation. J Surg Res 2001;97:123–30.
24. Atkins MB, Hidalgo M, Stadler WM, Logan TF, Dutcher JP, Hudes GR, Park Y, Liou SH, Marshall B, Boni JP, Dukart G, Sherman ML. Randomized phase II study of multiple dose levels of CCI-779, a novel mammalian target of rapamycin kinase inhibitor, in patients with advanced refractory renal cell carcinoma. J Clin Oncol 2004;22: 909–18.
25. Frost P, Moatamed F, Hoang B, Shi Y, Gera J, Yan H, Gibbons J, Lichtenstein A. In vivo antitumor effects of the mTOR inhibitor CCI-779 against human multiple myeloma cells in a xenograft model. Blood 2004;104:4181–7.
26. Kato M, Shimada Y, Tanaka H, Hosotani R, Ohshio G, Ishizaki K, Imamura M. Characterization of six cell lines established from human pancreatic adenocarcinomas. Cancer 1999;85:832–40.
27. Fujimoto K, Sheng H, Shao J, Beauchamp RD. Transforming growth factor-β1 promotes invasiveness after cellular transformation with activated Ras in intestinal epithelial cells. Exp Cell Res 2001;266: 239–49.
28. Matsumoto J, Kaneda M, Tada M, Hamada J, Okushiba S, Kondo S, Katoh H, Moriuchi T. Differential mechanisms of constitutive Akt/PKB activation and its influence on gene expression in pancreatic cancer cells. Jpn J Cancer Res 2002;93:1317–26.
29. Li D, Xie K, Wolff R, Abbruzzese JL. Pancreatic cancer. Lancet 2004;363:1049–57.
30. Downward J. Targeting RAS signalling pathways in cancer therapy. Nat Rev Cancer 2003;3:11–22.
31. Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nat Rev Cancer 2002;2:489–501.
32. Gera JF, Mellinghoff IK, Shi Y, Rettig MB, Tran C, Hsu JH, Sawyers CL, Lichtenstein AK. AKT activity determines sensitivity to mammalian target of rapamycin (mTOR) inhibitors by regulating cyclin D1 and c-myc expression. J Biol Chem 2004;279:2737–46.
33. Neshat MS, Mellinghoff IK, Tran C, Stiles B, Thomas G, Petersen R, Frost P, Gibbons JJ, Wu H, Sawyers CL. Enhanced sensitivity of PTEN-deficient tumors to inhibition of FRAP/mTOR. Proc Natl Acad Sci USA 2001;98:10314–9.
34. Podsypanina K, Lee RT, Politis C, Hennessy I, Crane A, Puc J, Neshat M, Wang H, Yang L, Gibbons J, Frost P, Dreisbach V, et al. An inhibitor of mTOR reduces neoplasia and normalizes p70/S6 kinase activity in Pten+/− mice. Proc Natl Acad Sci USA 2001;98:10320–5.
35. McMenamin ME, Soung P, Perera S, Kaplan I, Loda M, Sellers WR. Loss of PTEN expression in paraffin-embedded primary prostate cancer correlates with high Gleason score and advanced stage. Cancer Res 1999;59:4291–6.
36. Sakurada A, Suzuki A, Sato M, Yamakawa H, Orikasa K, Uyeno S, Ono T, Ohuchi N, Fujimura S, Horii A. Infrequent genetic alterations of the PTEN/MMAC1 gene in Japanese patients with primary cancers of the breast, lung, pancreas, kidney, and ovary. Jpn J Cancer Res 1997;88:1025–8.
37. Peralba JM, DeGraffenried L, Friedrichs W, Fulcher L, Grunwald V, Weiss G, Hidalgo M. Pharmacodynamic Evaluation of CCI-779, an Inhibitor of mTOR, in Cancer Patients. Clin Cancer Res 2003;9: 2887–92.
38. Noh WC, Mondesire WH, Peng J, Jian W, Zhang H, Dong J, Mills GB, Hung MC, Meric-Bernstam F. Determinants of rapamycin sensitivity in breast cancer cells. Clin Cancer Res 2004;10:1013–23.
39. Stephan S, Datta K, Wang E, Li J, Brekken RA, Parangi S, Thorpe PE, Mukhopadhyay D. Effect of rapamycin alone and in combination with antiangiogenesis therapy in an orthotopic model of human pancreatic cancer. Clin Cancer Res 2004;10:6993–7000.
40. Guba M, von Breitenbuch P, Steinbauer M, Koehl G, Flegel S, Hornung M, Bruns CJ, Zuelke C, Farkas S, Anthuber M, Jauch KW, Geissler EK. Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. Nat Med 2002;8:128–35.
41. El-Hashemite N, Walker V, Zhang H, Kwiatkowski DJ. Loss of Tsc1 or Tsc2 induces vascular endothelial growth factor production through mammalian target of rapamycin. Cancer Res 2003;63:5173–7.
42. Fujimoto K, Hosotani R, Wada M, Lee JU, Koshiba T, Miyamoto Y, Tsuji S, Nakajima S, Doi R, Imamura M. Expression of two angiogenic factors, vascular endothelial growth factor and platelet-derived endothelial cell growth factor in human pancreatic cancer, and its relationship to angiogenesis. Eur J Cancer 1998;34:1439–47.
43. Bruns CJ, Koehl GE, Guba M, Yezhelyev M, Steinbauer M, Seeliger H, Schwend A, Hoehn A, Jauch KW, Geissler EK. Rapamycin-induced endothelial cell death and tumor vessel thrombosis potentiate cytotoxic therapy against pancreatic cancer. Clin Cancer Res 2004;10: 2109–19.

Original Paper

# Radiation Therapy, Bypass Operation and Celiac Plexus Block in Patients with Unresectable Locally Advanced Pancreatic Cancer

*Koji Yamaguchi[1], Kiichiro Kobayashi[1], Yasuhiro Ogura[1], Katsumasa Nakamura[2]*
*Kenji Nakano[1], Kazuhiro Mizumoto[1], Masao Tanaka[1]*
Departments of [1]Surgery and Oncology and [2]Radiation Therapy
Graduate School of Medical Sciences Kyushu University, Fukuoka, Japan
Corresponding Author: Koji Yamaguchi, MD, Department of Surgery and Oncology
Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan
Tel: +81 92 642 5438, Fax: +81 92 642 5457, E-mail: yamaguch@med.kyushu-u.ac.jp

## ABSTRACT

**Background/Aims:** The great majority of pancreatic cancers are unresectable due to local invasion and/or distant metastasis. The treatment options for such patients include bypass operation, celiac plexus block, radiation therapy (RT), chemotherapy and immunotherapy. RT is divided into intraoperative radiation therapy (IORT) and external radiation therapy (ERT). Appropriate palliative treatment remains controversial.

**Methodology:** Our experience with palliative treatments including bypass operation, celiac plexus block and RT (IORT and ERT) was retrospectively reviewed in 31 Japanese patients with unresectable locally advanced pancreatic cancer.

The 31 patients consisted of seven with no RT, six with ERT alone, seven with IORT alone and 11 with both IORT and ERT. Gastrojejunostomy was performed in 25 patients and biliary bypass was done in 29 patients for the therapeutic or prophylactic purpose.

**Results:** No patients developed gastroduodenal obstruction or jaundice until death. Imaging findings after the treatment showed a decrease in tumor size in 11 of the 18 patients examined, an increase in four and no change in the other three. Of 19 patients complaining of back pain before the operation, the pain had disappeared in 12 but persisted in the other seven after the operation. No patients developed back pain after the treatment. Of the 12 patients with pain relief, nine had both RT and celiac plexus block, two RT alone and the other neither RT nor celiac block. Cumulative 0.5-year and 1.0-year survival rates in the group with RT(-), ERT alone, IORT alone IORT and ERT and IORT were 42.9%, 100%, 100%, 100% and 0%, 33.3%, 57.1% and 45.5%, respectively. The survival curve of the RT(-) group was significantly worse than that of the ERT alone group ($P$=0.0029), IORT alone group ($P$=0.0101) and IORT and ERT group ($P$=0.0109). The survival curves of the three RT groups were similar.

**Conclusions:** RT significantly prolonged survival of patients with unresectable locally advanced pancreatic cancer and combined palliative treatments including bypass operation, celiac plexus block and RT (ERT or IORT) are recommended for such patients.

**KEY WORDS:** Intraoperative radiation therapy; External radiation therapy; Pancreatic cancer

**ABBREVIATIONS:** Radiation Therapy (RT); Intraoperative Radiation Therapy (IORT); External Radiation Therapy (ERT); 5-Fluorouracil (FU); Carcinoembryonic Antigen (CEA); Carbohydrate Antigen (CA), Pancreatic Function Diagnostant test (PFD); American Society of Anesthesiologists Score (ASA score); Ultrasonography (US); Computed Tomography (CT); Magnetic Resonance Imaging (MRI)

## INTRODUCTION

Despite the recent progress in diagnostic and therapeutic modalities, pancreatic carcinomas are usually detected at advanced stages, with the resectability rate being about 30% (1-4). The great majority of pancreatic cancers are unresectable due to local aggressiveness, distant metastases and/or peritoneal dissemination. Patients with advanced pancreatic carcinoma develop obstructive jaundice, nausea and vomiting due to biliary and duodenal obstruction. Back pain is usually a sign of neural plexus invasion. Palliative treatment for locally advanced pancreatic carcinoma includes chemotherapy, radiation therapy, biliary and gastrointestinal bypass operation and celiac plexus block. The Gastrointestinal Tumor Study Group (GITSG) studies showed that for locally unresectable disease, both 5-fluorouracil chemotherapy and radiotherapy prolonged the survival and relieved the back pain (5,6). Combined therapy of radiation and 5-fluorouracil (FU) has been the standard treatment in locally unresectable pancreatic cancer. Intraoperative radiation therapy (IORT) permits high dose delivery to tumors while reducing radiation exposure to normal tissues by direct shielding or operative mobilization from the treatment field. However, the survival benefit by the presence or absence of radiation therapy (RT) and the methods of radiation (external radiation therapy (ERT), IORT, or ERT and IORT) and the usefulness of bypass operation and celiac plexus block remain controversial. In this communication, we ret-

Hepato-Gastroenterology 2005; 52:1605-1612

**FIGURE 1**
Percutaneous cholangiography shows hepatico-cholecysto-jejunostomy and stenosis of the distal common bile duct caused by pancreatic head cancer.

rospectively reviewed our experiences with palliative treatment of unresectable locally advanced pancreatic carcinoma.

## METHODOLOGY

This series is composed of 31 Japanese patients with unresectable locally advanced pancreatic cancer which were judged as unresectable only because of local invasion by laparotomy. Hepatic metastasis or peritoneal dissemination was absent. The diagnosis of adenocarcinoma was verified by needle biopsy during laparotomy and/or cytology of the pancreatic juice during preoperative ERCP. All of them underwent bypass operation with or without RT at the Department of Surgery I, Kyushu University Hospital, Fukuoka, Japan, from April, 1991 through March, 2002. The clinical charts were reviewed concerning the following factors; age, sex, preoperative red and white blood cell counts, lymphocyte count, platelet count, hemoglobin, preoperative serum levels of total protein, albumin, total cholesterol, fasting blood sugar, carcinoembryonic antigen (CEA) and carbohydrate antigen (CA19-9), pancreatic function diagnostant test (PFD) value, presence or absence of diabetes mellitus based on the criteria set by the 1985 World Health Organization study group on diabetes mellitus (7), American Society of Anesthesiologists Score (ASA score) (8), operative procedures, location of pancreatic cancer, size of tumor, degree of histological differentiation, stage following the General Rules of Pancreatic Cancer published by the Japan Pancreas Society (9), the use of IORT, ERT, and celiac plexus block (20mL of 99.9% ethanol injection into the celiac plexus), operation time, intraoperative blood loss, postoperative complications, postoperative peripheral red and white blood cell and lymphocyte counts, hemoglobin, platelets, postoperative serum levels of total protein, albumin, total cholesterol, fasting blood sugar, CEA, and CA19-9, total hospital stay and postoperative hospital stay.

They were divided into four groups; seven patients without RT, six with ERT alone, seven with IORT alone and 11 with both IORT and ERT. Gastrojejunostomy was performed in 25 of the 31 patients but not in the other six. Biliary drainage was performed in 29 of the 31 patients; choledochoduodenostomy in one, choledochojejunostomy in three, hepaticocholecystojejunostomy **(Figure 1)** in 14, hepaticojejunostomy in 10, and hepaticojejunostomy and cholecystojejunostomy in one. Obstructive jaundice was evident in 19 of the 29 patients and not in the other 10. Celiac plexus

**TABLE 1. Preoperative clinicopathological features of patients with unresectable locally advanced pancreatic cancer**

| | RT(-) | RT(+) | | | Statistical significance |
|---|---|---|---|---|---|
| | | ERT alone | IORT alone | IORT+ERT | |
| No. of patients | 7 | 6 | 7 | 11 | |
| Age (yrs) | 69.3±3.5[1] | 62.7±4.3 | 59.7±3.1 | 57.1±2.6[1] | [1]: $P$=0.0120 |
| Sex (M/F) | 5/2 | 3/3 | 6/1 | 8/3 | NS |
| **Preoperative** | | | | | |
| Total protein (g/dL) | 5.9±0.3[1] | 6.6±0.3 | 6.5±0.3 | 6.6±0.1[1] | [1]: $P$=0.0158 |
| Albumin (g/dL) | 3.3±0.2[1] | 3.7±0.2 | 3.7±0.2 | 3.6±0.1[1] | [1]: $P$=0.0085 |
| Red blood cells ($10^4$/μL) | 342.7±27.6 | 384.5±29.6 | 387.4±18.8 | 390.0±27.6 | NS |
| Hemoglobin (g/dL) | 10.5±8.8[1] | 11.8±0.8 | 11.9±0.6 | 12.4±0.3[1] | [1]: $P$=0.0228 |
| White blood cells (μL) | 7074.3±849.5 | 5203.3±412.8 | 5087.1±574.6 | 6382.7±607.4 | NS |
| Lymphocytes (μL) | 959.8±176.7 | 1118.0±127.5 | 1257.8±98.1 | 1480.0±176.7 | NS |
| Platelets ($10^4$/μL) | 26.8±2.7[1] | 17.5±1.8[1] | 22.1±4.2 | 47.6±23.2 | [1]: $P$=0.0180 |
| FBS (mg/dL) | 155.4±27.8 | 117.2±15.0 | 146.7±25.7 | 118.2±9.4 | NS |
| Cholesterol (mg/dL) | 146.1±12.9 | 164.8±22.3 | 120.0±8.0 | 140.8±12.9 | NS |
| CEA (ng/mL) | 4.3±2.0 | 1.2±0.4 | 1.4±0.2 | 2.3±0.7 | NS |
| CA19-9 (IU/mL) | 4849.4±3215.3 | 523.7±186.4[1] | 1088.5±73.9 | 122.0±39.5[1] | [1]: $P$=0.0139 |
| PFD (%) | 59.3±4.0 | 54.1±13.2 | 57.4±10.0 | 61.9±4.0 | NS |
| DM (No/Bor/Yes) | 2/0/5 | 3/0/3 | 2/1/4 | 3/6/2 | NS |
| ASA score | | | | | NS |
| II | 4 | 3 | 6 | 9 | |
| III | 3 | 3 | 1 | 2 | |

RT: radiation therapy; ERT: external radiation therapy; IORT: intraoperative radiation therapy; M: male; F: female; FBS: fasting blood sugar; CEA: carcinoembryonic antigen; CA: carbohydrate antigen; PFD: pancreatic function diagnostant test; DM: diabetes mellitus; Bor: borderline; ASA: American Society of Anesthesiologists; NS: not significant.

block was done in 16 of the 31 patients and not in the other 15. Of the 16, 12 complained of back pain related to pancreatic carcinoma before operation and the other four did not.

IORT was done in 18 patients with 6 to 15-MeV electron beams. The IORT field set by the use of treat-

TABLE 2. Operative Findings of Patients with Unresectable Locally Advanced Pancreatic Cancer

| | RT(-) | RT(+) | | | Statistical significance |
|---|---|---|---|---|---|
| | | ERT alone | IORT alone | IORT+ERT | |
| No. of patients | 7 | 6 | 7 | 11 | |
| Site of origin | | | | | NS |
| Head | 7 | 5 | 7 | 9 | |
| Body | 0 | 1 | 0 | 2 | |
| Size of tumor (cm) | 4.3±0.4 | 3.6±0.7 | 4.0±0.2 | 4.7±0.5 | NS |
| Degree of differentiation | | | | | NS |
| Well | 0 | 0 | 1 | 7 | |
| Mod | 3 | 3 | 4 | 2 | |
| Poor | 0 | 1 | 0 | 1 | |
| NA | 4 | 2 | 2 | 1 | |
| Gastrojejunostomy | | | | | NS |
| GJ | 3 | 2 | 6 | 8 | |
| GJ (PT) | 3 | 1 | 0 | 2 | |
| None | 1 | 3 | 1 | 1 | |
| Biliary bypass | | | | | NS |
| CD | 0 | 0 | 1 | 0 | |
| CJ | 2 | 0 | 1 | 0 | |
| HCJ | 1 | 5 | 2 | 6 | |
| HJ | 4 | 0 | 3 | 3 | |
| HJ,CJ | 0 | 1 | 0 | 0 | |
| None | 0 | 0 | 0 | 2 | |
| Operation time (min) | 249±26 | 319±24 | 595±181 | 393±29 | NS |
| Intraoperative bleeding (g) | 471±137 | 866±236 | 844±125 | 691±83 | NS |
| Stage | | | | | NS |
| III | 1 | 0 | 0 | 0 | |
| IVa | 6 | 6 | 7 | 11 | |

Well: well differentiated; mod: moderately differentiated; poor: poorly differentiated; NA: not available; GJ: loop gastrojejunostomy without partial transection of the stomach; GJ (PT): loop gastrojejunostomy with partial transection of the stomach; CD: choledochoduodenostomy; CJ: choledochojejunostomy; HCJ: hepaticocholecystojejunostomy; HJ: hepaticojejunostomy; Stage: following the General Rules of Pancreatic Cancer published by the Japan Pancreas Society, Kanehara publisher, Tokyo, Japan, 1996; NS: not significant.

TABLE 3. Postoperative Findings of Patients with Unresectable Locally Advanced Pancreatic Cancer

| | RT(-) | RT(+) | | | Statistical significance |
|---|---|---|---|---|---|
| | | ERT alone | IORT alone | IORT+ERT | |
| No. of patients | 7 | 6 | 7 | 11 | |
| At discharge | | | | | |
| Total protein (g/dL) | 6.3±0.3 | 6.2±0.1 | 6.2±0.1 | 6.0±0.2 | NS |
| Albumin (g/dL) | 3.2±0.2 | 3.2±0.2 | 3.2±0.1 | 3.4±0.1 | NS |
| Red blood cells ($10^4$/μL) | 393.7±45.6 | 338.3±27.5 | 349.0±13.4 | 332.7±11.7 | NS |
| Hemoglobin (g/dL) | 11.2±0.4 | 10.5±0.7 | 10.8±0.4 | 10.8±0.4 | NS |
| White blood cells (μL) | 8852.9±2294.0 | 6818.3±1438.5 | 5764.3±732.3 | 4962.7±703.4 | NS |
| Lymphocytes (μL) | 1818.3±287.2[1,2] | 972.3±131.6[1,3] | 1142.0±195.4[4] | 587.8±103.7[2,3,4] | [1]: $P$=0.0317, [2]: $P$=0.0014 [3]: $P$=0.0494, [4]:$P$=0.0268 |
| Platelets ($10^4$/μL) | 32.8±4.9 | 23.6±3.2 | 31.1±5.0 | 24.4±2.1 | NS |
| FBS (mg/dL) | 161.6±34.4 | 131.2±16.8 | 103.4±8.5 | 111.1±11.2 | NS |
| Total cholesterol (mg/dL) | 137.7±10.6 | 124.0±9.7 | 110.9±6.5 | 129.8±11.4 | NS |
| CEA (ng/mL) | 3.5±1.5 | 1.3±0.3 | 1.2±0.4 | 1.2±0.3 | NS |
| Decline of CEA (ng/mL) | -1.1±1.7 | 0.3±0.3 | 0.5±0.4 | 1.2±0.6 | NS |
| CA19-9 (IU/mL) | 6063.7±5265.2 | 279.4±114.4[1] | 313.2±153.3[2] | 54.3±21.3[1,2] | [1]: $P$=0.0095, [2]: $P$=0.0426 |
| Decline of CA19-9 (IU/mL) | -68.1±589.6 | 482.6±249.7[1] | 952.8±857.3 | 25.0±16.6[1] | [1]: $P$=0.0061 |
| Total hospital stay (days) | 52.1±7.1 | 67.8±22.8 | 66.6±6.1 | 75.3±7.4 | NS |
| Postoperative hospital stay (days) | 32.0±3.3 | 32.3±13.2 | 33.0±5.1 | 49.9±6.7 | NS |

RT: radiation therapy; ERT: external radiation therapy; IORT: intraoperative radiation therapy; FBS: fasting blood sugar; CEA: carcinoembryonic antigen; CA: carbohydrate antigen; NS: not significant.

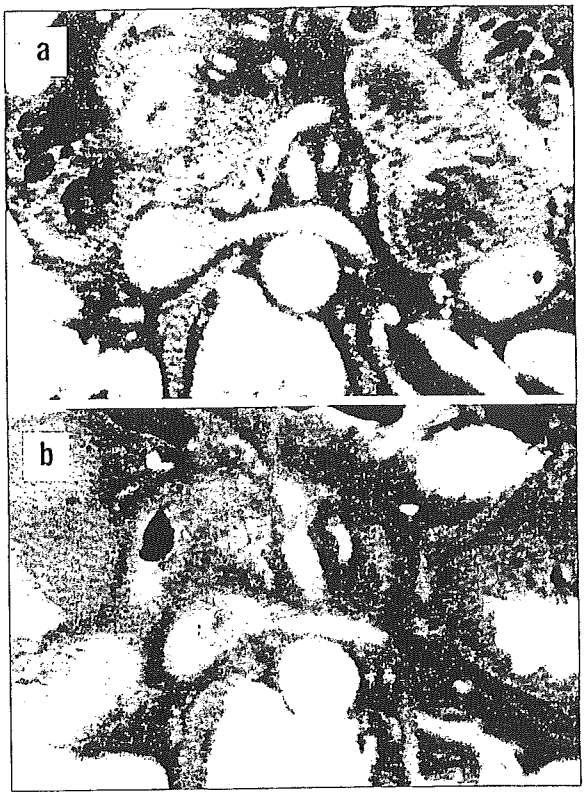


FIGURE 2
Computed tomography shows a pancreatic head carcinoma (a) and a decrease in size of the tumor after IORT and ERT (b).

ment applicators of 7-10cm in diameter covered the tumor plus a more than 1-cm margin. The IORT dose was 15-30 (22.3±5.2) Gy. Postoperatively, ERT (6-50, 38.2±3.4) Gy at 1.5-2.0 Gy/fraction was given by 6-MV X-rays in 17 patients. At the time of ERT, drip infusion of 5-FU (250mg) was done immediately prior to ERT. Presence or absence of IORT was decided by the referred physician's opinion of IORT and the patient's physical condition and ERT was given as far as the patient's postoperative conditions permitted.

Imaging [ultrasonography (US), computed tomography (CT) and/or magnetic resonance imaging (MRI)] after the treatment was obtained in 18 patients. The clinical outcome was updated as of June 30, 2002. The follow-up period ranged from 32 days to 685 days with a mean of 324±163 days. Thirty patients died of pancreatic cancer and another patient was alive for three months at the time of this writing. Half-year, 1-year and 1.5-year cumulative survival rates were calculated and the differences were examined by the log-rank test. Survival curves were measured by the Kaplan-Meier method and the difference of the curves was examined by the generalized Wilcoxon test.

The values were expressed as mean ±standard deviation. The mean value was examined by the chi-square test and the distribution of patients was examined by the Student's $t$ test. A $p$-value less than 0.05 was considered statistically significant.

## RESULTS

### Clinicopathologic Features

The 31 patients consisted of seven with no RT, six with ERT alone, seven with IORT alone and 11 with both IORT and ERT (**Table 1**). The mean age was 69.3, 62.7, 59.6, and 57.1 years, respectively. The Male/female ratio was 5/2, 3/3, 6/1, and 8/3. Preopera-

TABLE 1. Complications and Clinical Course of Patients with Unresectable Locally Advanced Pancreatic Cancer

| | RT(-) | RT(+) ERT alone | RT(+) IORT alone | RT(+) IORT+ERT | Statistical significance |
|---|---|---|---|---|---|
| No. of patients | 7 | 6 | 7 | 11 | |
| Complication | | | | | NS |
| GI bleeding | 1 | 0 | 0 | 2 | |
| Anastomosis obstruction | 0 | 0 | 0 | 1 | |
| Liver abscess | 0 | 0 | 1 | 0 | |
| Change of size by imaging | | | | | NS |
| Decrease in size | 0 | 3 | 4 | 4 | NS |
| Increase in size | 0 | 1 | 1 | 2 | |
| No change in size | 0 | 1 | 0 | 2 | |
| Celiac plexus block | | | | | NS |
| Yes | 1 | 1 | 7 | 0 | |
| No | 6 | 5 | 0 | 4 | |
| Effect of celiac plexus block | | | | | NS |
| Yes | 1 | 1 | 7 | 5 | |
| No | 0 | 0 | 0 | 2 | |
| Mean survival (days) | 178.6±30.7[1,2,3] | 390.5±52.8[1] | 369.3±63.8[2] | 350.4±30.7[3] | [1]: $P$=0.0042, [2]: $P$=0.0195, [3]: $P$=0.0281 |
| Cumulative survival rates | | | | | |
| 0.5 years | 42.9% | 100% | 100% | 81.8% | |
| 1.0 year | 0% | 33.3% | 57.1% | 45.5% | |
| 1.5 years | 0% | 16.7% | 14.3% | 9.1% | |
| 2.0 years | 0% | 0% | 0% | 0% | |

RT: radiation therapy; ERT: external radiation therapy; IORT: intraoperative radiation therapy; GI: gastrointestinal; NS: not significant; Cumulative survival rates: RT(-) *vs.* ERT alone: $P$=0.0029; RT(-) *vs.* IORT alone: $P$=0.0101; RT(-) *vs.* IORT+ERT: $P$=0.0109.

tive complete blood cell counts and serum chemistry were not different among the four groups, excluding total protein, albumin, hemoglobin, platelets and CA19-9.

### Operative Features

Of the 31 pancreatic carcinomas, 28 were located in the head of the pancreas and the other three in the body (**Table 2**). The mean diameter of the tumor in each group was 4.3cm, 3.6cm, 4.0cm, and 4.7cm. The majority of pancreatic carcinomas were well to moderately differentiated adenocarcinoma. Of the 31 patients, 30 were in stage IVa and the other in stage III. Gastrojejunostomy without partial transection of the stomach was done in 19 patients and with partial transection in the other six. Biliary drainage was performed in 29 patients; hepaticocholecystojejunostomy in 14, choledochojejunostomy in three, choledochoduodenostomy in one, hepaticojejunostomy in 10, hepaticojejunostomy and choledochojejunostomy in the other. The ASA score, operation time and intraoperative blood loss were not different among the four groups.

### Postoperative Features

Complete blood cell counts and serum chemistry were not different among the four groups, excluding the white blood cell and lymphocyte counts (**Table 3**). The decline of CEA and CA19-9 were -1.1ng/mL and -68.1 IU/mL (RT (-) group), 0.3ng/mL and 483 IU/mL[a] (ERT alone group), 0.5ng/mL and 953 IU/mL (IORT alone group), and 1.2ng/mL and 25 IU/mL[a] (IORT and ERT group), respectively ([a]: $P=0.0061$). Postoperative hospital stay in each group was 32 days, 32, 33 and 50.

### Complications and Effects of Treatment

During treatment, one patient without RT and two with IORT and ERT developed gastrointestinal bleeding (**Table 4**). Transient anastomotic obstruction was evident in one patient with IORT and ERT and liver abscess in one with IORT alone. One of the 25 patients underwent gastrojejunostomy for the treatment of vomiting due to duodenal obstruction by pancreatic cancer but the other 24 patients did prophylactically. None of the 31 patients developed vomiting due to cancerous invasion after the treatment. Nineteen patients underwent biliary bypass operation for the therapeutic purpose and the other 10 for the prophylactic purpose. After the treatment, none of these patients developed jaundice due to tumor invasion to the biliary tree. No patients underwent reoperation for obstruction of gastrojejunostomy or biliary bypass.

Imaging findings immediately after the treatment showed a decrease in size (**Figure 2**) in 11 of the 18 patients examined, an increase in four and no change in the other three. Of 19 patients with back pain before the operation, the pain disappeared in 12 but persisted in the other seven (**Table 5 and Figure 3**). No patients developed back pain after the treatment. Of the 12 patients in whom back pain disappeared,

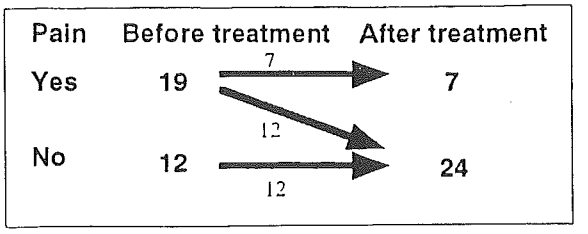


FIGURE 3 Alteration of back pain related to pancreatic carcinoma before and after the treatment.

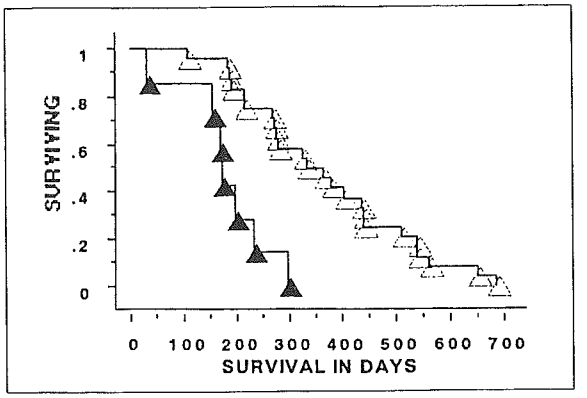


FIGURE 4 Survival curves of 24 patients with RT and of seven without RT. △: 24 patients with RT, ▲: seven without RT.

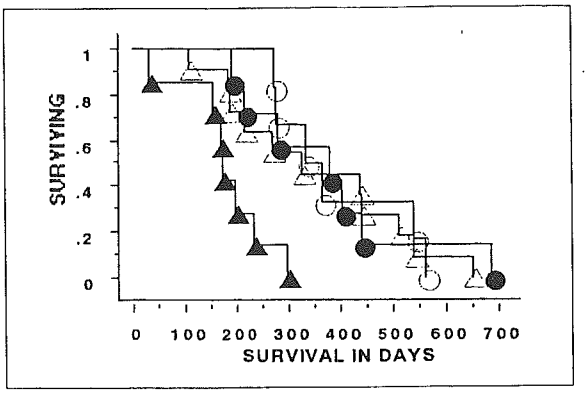


FIGURE 5 Survival curves of six with ERT alone, seven with IORT alone, 11 with IORT and ERT and seven without RT. ○: six with ERT alone, ●: seven with IORT alone, △: 11 patients with IORT and ERT, ▲: seven without RT.

nine had both RT and celiac plexus block, two had RT alone and the other had no RT or celiac block. Mean survival of the RT(-) group was 178.6±30.7 days[a,b,c] ERT alone 390.5±52.8 days[a] IORT alone 369.3±63.8 daysb and IORT and ERT group 350.4±30.7 days[c] ([a]: $P=0.0042$, [b]: $P=0.0195$, [c]: $P=0.0281$). Cumulative 0.5-year survival rates were 42.9% in the RT(-) group, 100% in ERT alone group, 100% in IORT alone group, 100% in IORT and ERT group, respectively (**Figure 4**). The 1.0-year survival rate in each of these groups was 0%, 33.3%, 57.1%, and 45.5%, respectively. The survival curve of the RT(-) group was significantly

TABLE 5 Change in Back Pain

| | Alterations of back pain related to pancreatic cancer | | |
|---|---|---|---|
| | No to No | Yes to No | Yes to Yes |
| RT(-), Block(-) | 2 | 1 | 2 |
| RT(-), Block(+) | 1 | 0 | 1 |
| RT(+), Block(-) | 6 | 2 | 1 |
| RT(+), Block(+) | 3 | 9 | 3 |

RT: radiation therapy; Block: celiac plexus block.

worse than that in the ERT alone group ($P$=0.0029), IORT alone group ($P$=0.0101), and IORT and ERT group ($P$=0.0109). The survival curves of the three RT groups were similar not different **(Figure 5)**.

## DISCUSSION

Experience with palliative treatments including bypass operation, celiac plexus block and RT (IORT and ERT) was retrospectively reviewed in 31 with unresectable locally advanced pancreatic cancer. The results obtained are as follows;

1. Therapeutic or prophylactic biliary and gastrointestinal bypass yielded satisfactory results.

2. Satisfactory pain relief was obtained by IORT and/or celiac plexus block.

3. RT produced survival benefits, whichever the type of radiation (IORT alone, ERT alone, or IORT and ERT) may be.

Initial experience with radiation therapy for pancreatic cancer showed that the disease is relatively unresponsive to radiotherapy. However, the GITSG studies showed that radiotherapy and 5-FU chemotherapy in a relatively non-aggressive regimen prolonged the survival following apparently curative resection (10,11). Recently, the European Organization for Research and Treatment of Cancer (EORTC) (12) showed that the benefit of the combination therapy was small and routine use of adjuvant chemoradiotherapy was not warranted as a standard treatment for cancer of the head of the pancreas. In this series, the survival curve of the RT (+) group was more favorable than that of the RT (-) group, although the number of patients was small and this was not a prospective randomized study.

IORT permits delivery of high dose irradiation to tumors while reducing the exposure of normal tissues by direct shielding or operative mobilization from the treatment volume (13). Techniques of IORT for pancreatic cancer were initially developed in Japan during the early 1970s. Experiences at the Massachusetts General Hospital in early 1980s (14) suggested that IORT enhanced survival in selected patients with locally advanced but non-metastatic disease. However, subsequent investigations by a variety of institutions including the Mayo Clinic failed to establish any conclusive evidence that IORT significantly prolonged the survival of unresectable pancreatic cancer patients (15,16). A prospective multi-institutional study carried out by the Radiation Therapy Oncology Group (RTOG) (17) showed an 8-month median survival, similar to conventional therapy. In our series, the effects of radiation therapy were compared by the methods of radiation (ERT alone, IORT alone and ERT and IORT). RT had survival benefits in patients with unresectable locally advanced pancreatic cancer and there was no difference of survival by the way of radiation.

Major adverse effects of RT are occurrence of pseudoaneurysm, upper gastrointestinal bleeding and liver abscess at the early phase and bone fracture at the late phase probably due to organic stenosis or occlusion of the artery and proliferation of fibrous tissue around the artery (18,19). In this series, these early complications were evident in five patients, four of whom had had RT. No late complications occurred because of short survival. Some reported increased occurrence of liver metastasis (20) and peritoneal dissemination after IORT and others claimed that IORT was not effective to control liver metastasis (21,22). In this series, the survival rate was better in patients with RT than in patients without RT.

Jaundice is the dominant symptom in patients with biliary obstruction by carcinoma of the pancreatic head. The symptoms associated with prolonged biliary stasis (malnutrition, coagulopathy, pruritis, hepatic failure, renal dysfunction, angiocolitis) are commonly resolved or relieved by biliary drainage. Palliation is frequently the only feasible treatment in these patients due to the biological aggressiveness of the tumor characterized by the early infiltration to adjacent tissues. Biliary-enteric bypass is associated with less morbidity or mortality than celiotomy alone (23). Hugueir *et al.* (24) reviewed retrospective multicenter experiences of 2,493 patients with pancreatic cancer. They mentioned that cholecystoenteric bypass (n=237) was associated with higher postoperative mortality (20% *vs.* 14%), lower long-term morbidity (26% *vs.* 35%) and shorter survival time (means: 3.2 *vs.* 5.2 months) in comparison with choledochoenteric bypass (n=1,770). Choledochoduodenostomy (n=1,159) and choledochojejunostomy (n=611) had similar rates of postoperative morality (14% *vs.* 13%), morbidity (26% *vs.* 27%), recurrent jaundice (8% and 7%) and mean survival time (5.4 *vs.* 5.0 months). They consequently recommended choledochoenterostomy. To ensure a safe, effective and long-lasting biliary-drainage, it is imperative that the proximal biliary limb or channel for the enteric anastomosis is functional, accessible, and secreted bile can enter the gut freely. In this series, we usually utilized hepaticocholecystojejunostomy proposed by Koga *et al.* (25) and obtained favorable patency of the anastomosis.

Less than 5% of patients with carcinoma of the pancreas present with duodenal obstruction. However, many patients develop duodenal obstruction as the tumor grows up, reaching 50% in some series (26). The probability of developing duodenal obstruction after biliary bypass alone has been reported to be 15% to 34% (23,24,27). In contrast to bilioenteric anastomosis, there are some negative arguments that potential morbidity of prophylactic gastrojejunostomy outweighs the need, gastrojejunostomy fails to relieve functional gastric-outlet obstruction, and prophylactic gastrojejunostomy is frequently associated with delayed gastric emptying, delaying discharge and/or causing significant other complications (28). Recently, Lillemoe *et al.* (29) performed a prospective randomized trial and demonstrated that prophylactic gastrojejunostomy significantly decreased the incidence of late gastric outlet obstruction. The conduct of prophylactic gastrojejunostomy at the initial surgical procedure does not increase the incidence of postoperative complications or extend the length of hospital stay

(29). In this series, only one patient had gastric outlet obstruction and the other 24 patients underwent prophylactic gastroenterostomy. Loop gastrojejunostomy was performed using two different methods. In one method, the proximal jejunal loop was anastomosed to the posterior aspect of the stomach in an antecolic fashion. In the other, the stomach was partially transected in the body portion and the jejunal loop was anastomosed to the transected site to prevent bleeding by passage of the meal through the infiltrated duodenum. In both procedures, jejunojejunostomy was performed immediately below the gastrojejunostomy. No patients necessitated additional operation to relieve gastric outlet obstruction, but three developed upper gastrointestinal bleeding and one anastomotic obstruction, which might be related to radiation. They were satisfactorily managed by medical treatment. There was no difference between the two methods of gastrojejunostomy in terms of postoperative bleeding and development of obstruction.

The pain in advanced pancreatic carcinoma is persistent, overwhelming and incapacitating. Potentially useful modalities to control pain include antitumor therapy, pharmacotherapy, celiac plexus block, splanchnic nerve block (intraoperatively or percutaneously), intercostal nerve block and psychological intervention (30). The multicenteric analysis of palliative surgery in 2,493 patients with unresectable pancreatic cancer by Hugueir *et al.* (24) showed that radiotherapy was more effective than other symptomatic treatments. Splanchnic nerve transection via thoracoscopic, minimally invasive approach, has been recently introduced by Worsely *et al.* (31). In this series, radiation and celiac plexus block was done in most patients, and the pain was satisfactorily controlled. The number of patients was so small that no conclusion was obtainable whether radiation or celiac plexus block was effective for the pain relief.

In conclusion, RT significantly prolonged survival of patients with unresectable locally advanced pancreatic cancer. Effects of procedures of RT (ERT alone, IORT alone or IORT and ERT) are not different. Bypass operation, celiac plexus block and RT (ERT or IORT) are recommended in patients with unresectable locally advanced pancreatic cancer.

## REFERENCES


1 **Yamaguchi K, Enjoji M:** Carcinoma of the pancreas: A clinicopathologic study of 96 cases with immunohistochemical observations. Jpn J Clin Oncol 1989; 19:14-22.

2 **Yamaguchi K, Shimizu S, Yokohata K, Noshiro H, Chijiiwa K, Tanaka M:** Pancreatic carcinoma: reappraisal of surgical experiences in one Japanese University Hospital. Hepatogastroenterology 1999; 46:3257-3262.

3 **Sunada S, Miyata M, Tanaka Y, Okumura K, Nakamuro M, Kitagawa T, Shirakura R, Kawashima Y:** Aggressive resection for advanced pancreatic carcinoma. Surg Today 1992; 22:74-77.

4 **Matsuno S, Egawa S, Arai K:** Trends in treatment for pancreatic cancer. J Hepato-Biliary-Panc Surg 2001; 8:544-548.

5 **The Gastrointestinal Tumor Study Group:** Treatment of locally unresectable carcinoma of the pancreas: Comparison of combined-modality therapy (chemotherapy plus radiotherapy) to chemotherapy alone. J Nat Cancer Instit 1988; 80:751-755.

6 **The Gastrointestinal Tumor Study Group:** Therapy of locally unresectable pancreatic carcinoma; A randomized comparison of high dose (6000 rads) radiation alone, moderate dose radiation (4000 rads+5-fluorouracil), and high dose radiation+5-fluorouracil. Cancer 1981; 48:1705-1710.

7 **Alberti KGMM, Hochaday TAR:** Diabetes mellitus. In: Weatherall DJ, Ledingham YGG, Warrell DA (Eds.). Oxford Textbook of Medicine. New York: Oxford Medical Publisher, 1987.

8 **Vacanti CJ, VanHouten RJ, Hill RC:** A statistical analysis of the relationship of physical status to postoperative mortality in 68,388 cases. Anesth Analg 1970; 49:564-566.

9 **Japan Pancreas Society:** Classification of Pancreatic Carcinoma. Tokyo: Kanehara & Co., Ltd., 1996.

10 **Kalser MH, Ellenberg SS:** Pancreatic cancer. Adjuvant combined radiation and chemotherapy following curative resection. Arch Surg 1985; 120:899-903.

11 **The Gastrointestinal Tumor Study Group:** Further evidence of effective adjuvant combined radiation and chemotherapy following curative resection of pancreatic cancer. Cancer 1987; 59:2006-2010.

12 **Klinkenbijl JH, Jeekel J, Sahmoud T, van Pel R, Couvreur ML, Veenhof CH, Arnaud JP, Gonzalez DG, de Wit LT, Hennipman A, Wils J:** Adjuvant radiotherapy and 5-fluorouracil after curative resection of cancer of the pancreas and periampullary region: phase III trial of the EORTC gastrointestinal tract cancer cooperative group. Ann Surg 1999; 230:776-782.

13 **Sunamura M, Kobari M, Lozonschi L, Egawa S, Matsuno S:** Intraoperative radiotherapy for pancreatic adenocarcinoma. J Hep Bil Pancr Surg 1998; 5:151-156.

14 **Shipley WU, Wood WC, Tepper JE, Warshaw AL, Orlow EL, Kaufman S, D, Battit GE, Nardi GL:** Intraoperative electron beam irradiation for patients with unresectable pancreatic carcinoma. Ann Surg 1984; 200:289-296.

15 **Moertel CG, Frytak S, Hahn RG, et al:** Therapy of locally unresectable pancreatic carcinoma: a randomized comparison of high dose (6000 rads) radiation alone, moderate dose radiation (4000 rads + 5-fluorouracil), and high dose radiation + 5-fluorouracil: The Gastrointestinal Tumor Study Group. Cancer 1981; 48:1705-1710.

16 **Roldan GE, Gunderson LL, Nagorney DM, Martin JK, Ilstrup DM, Holbrook MA, Kvols LK, McIlrath DC:** External beam versus intraoperative and external beam irradiation for locally advanced pancreatic cancer. Cancer 1988; 61:1110-1116.

17 **Tepper JE, Noyes D, Krall JM, Sause WT, Wolkov HB, Dobelbower RR, Thomson J, Owens J, Hanks GE:** Intraoperative radiation therapy of pancreatic carcinoma: a report of RTOG-8505. Radiation Therapy Oncology Group. Int J Rad Oncol Biol Phys 1991; 21:1145-1149.

18 **Kawamura M, Kataoka M, Fujii T, Itoh H, Ishine M, Hamamoto K, Yokoyama S, Takashima S., Satoh M., Inoue K:** Electron beam intraoperative radiation therapy (EBIORT) for localized pancreatic carcinoma. Int J Rad Oncol Biol Phys 1992; 23:751-757.

19 **Gotoh M, Monden M, Sakon M, Kanai T, Umeshita K, Ikeda H, Mori T:** Intraoperative irradiation in resected carcinoma of the pancreas and portal vein. Arch Surg 1992; 127:1213-1215.

20 **Ishikawa O, Ohigashi H, Sasaki Y, Masao K, Kabuto T, Furukawa H, Imaoka S:** Adjuvant therapies in extended pancreatectomy for ductal adenocarcinoma of the pancreas. Hepatogastroenterology 1998; 45:644-650.

21 **Zerbi A, Fossati V, Parolini D, Carlucci M, Balzano G, Bordogna G, Staudacher C, Di Carlo V:** Intraoperative radiation therapy adjuvant to resection in the treatment of pancreatic cancer. Cancer 1994; 73:2930-2935.

22 **Johnstone PA, Sindelar WF:** Patterns of disease recur-

rence following definitive therapy of adenocarcinoma of the pancreas using surgery and adjuvant radiotherapy: correlations of a clinical trial. Int J Rad Oncol Biol Phys 1993; 27:831-834.

23 **Sarr MG, Cameron JL:** Surgical management of unresectable carcinoma of the pancreas. Surgery 1982; 91:123-133.

24 **Huguier M, Baumel H, Manderscheid JC, Houry S, Fabre JM:** Surgical palliation for unresected cancer of the exocrine pancreas. Eur J Surg Oncol 1993; 19:342-347.

25 **Koga A, Nakayama F:** One stage hepaticocholecystojejunostomy as an easy and long term effective bilioenteric bypass for unresectable carcinoma of the pancreas. Surg Gynecol Obstet 1987; 165:177-179.

26 **Gudjonsson B:** Cancer of the pancreas. Cancer 1987; 60:2284-2303.

27 **Watanapa P, Williamson RC:** Surgical palliation for pancreatic cancer: developments during the past two decades. Br J Surg 1992; 79:8-20.

28 **Konishi M, Ryu M, Kinoshita T, Kawano N, Tanizaki H, Cho A:** Stomach-preserving gastric bypass for unresectable pancreatic cancer. Surg Today 1997; 27:429-433.

29 **Lillemoe KD, Cameron JL, Hardacre JM, Sohn TA, Sauter PK, Coleman J, Pitt HA, Yeo CJ:** Is prophylactic gastrojejunostomy indicated for unresectable periampullary cancer? A prospective randomized trial. Ann Surg 1999; 230:322-328.

30 **Saltzburg D, Foley KM:** Management of pain in pancreatic cancer. Surg Clin N Am 1989; 69:629-649.

31 **Worsey J, Ferson RF, Keenan RJ, Julian TB, Landreneau RJ:** Thoracoscopic pancreatic denervation for pain in irresectable pancreatic cancer. Br J Surg 1993; 80:1051-1052.

## Short Communication

# New Infusion Device for Trans-tissue, Sustained Local Delivery of Anticancer Agent to Surgically Resected Tissue: Potential Use for Suppression of Local Recurrence of Pancreatic Cancer

**Tatsuya Manabe,[1,2] Hidenobu Okino,[2] Ryo Maeyama,[1,2] Kazuhiro Mizumoto,[2] Masao Tanaka,[2] Takehisa Matsuda[1]**

[1] Division of Biomedical Engineering, Graduate School of Medicine, Kyushu University, 3-1-1 Maidashi, Fukuoka 812-8582, Japan

[2] Division of Surgery and Oncology, Graduate School of Medicine, Kyushu University, 3-1-1 Maidashi, Fukuoka 812-8582, Japan

*Received 7 July 2004; revised 30 August 2004; accepted 31 August 2004*
*Published online 30 December 2004 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.b.30186*

**Abstract: Local recurrence is a major cause of death of patients who have undergone resection for pancreatic cancer. To reduce the incidence of local recurrence, a new drug-infusion device, which is fixed on resected tissues immediately after surgery, was devised for trans-tissue, sustained local delivery. The drug-infusion device proposed here has the following functions: tight adhesion of the device on resected tissues, sustained drug infusion to the tissue, drug reloading, and easy removal from the body after use. The fabricated prototype experimental device, described here, was a thin infusion pouch made of a thin elastomer film (segmented polyurethane), which is connected to an elastomeric tube for drug reloading. The suppressive effect of the device delivering the anticancer agent, gemcitabine (GEM), was examined using subcutaneous tumor-bearing athymic mouse. A low-dose, local sustained GEM delivery using the device significantly suppressed the tumor growth and regrowth after surgery. The preliminary model experimental result appears to provide a promising therapeutic procedure for increased survival rate of the patients undergoing surgery for pancreatic cancer.** © 2004 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 73B: 203–207, 2005

**Keywords: local recurrence; cancer; local drug delivery; infusion device; trans-tissue**

## INTRODUCTION

The survival rate of patients who have undergone surgery for pancreatic cancer is still very poor (8–25%),[1–4] because most patients who undergo resection have a high incidence of relapse of cancer.[5–8] Local recurrence caused by residual cancer cells after surgery is one of the most frequent types of relapse. The optimum timing of treating for a local recurrence must be immediately after surgery, and the therapy should continue for an extended period so that residual cancer cells do not establish a solid mass. To this end, we proposed and developed trans-tissue, local delivery systems of anticancer drugs, proteins, or gene-encoding adenoviral vectors using an *in situ* photocured, tissue adhesive gelatin gel or gene-transduced cell sheet, all of which were applicable to resected surfaces immediately after surgery.[9–13] One shortcoming of the systems mentioned above is that, irrespective of systems developed, the delivery period is limited and they do not possess a drug-reloading function.

To overcome this shortcoming, a drug-reloadable and detachable infusion device was developed. Figure 1 shows the schematic of trans-tissue drug delivery from the newly developed, tissue-adhered infusion device that was fixed on the resected tissue with biodegradable sutures. The device is composed of an elastomeric pouch having a laser-abladed microporous film on the tissue-facing side, nonporous elastameric film on the peritoneal cavity-facing side, and an elastomeric tube connected to the pouch, which is guided to an extracorporeal site [Figure

*Correspondence to:* T. Matsuda (e-mail: Matsuda@med.kyushu-u.ac.jp)
Contact grant sponsor: Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan, Grant-in-Aid for Scientific Research; contract grant numbers: A2-12358017, B2-12470277
Contact grant sponsor: Organization for Pharmaceutical Safety and Research (OPSR), Promotion Fundamental Studies in Health Science; contract grant number: 97-15