Table V. Correlation between the IC_{50} values for GEM and the mRNA expression levels of genes associated with GEM metabolism.

Gene expressions	Spearman's rank-correlation coefficient (ρ)	P-value
hENT1	0.12	0.67
dCK	-0.025	0.93
RRM1	0.52	0.048[a]
RRM2	0.14	0.63
CDA	-0.34	0.21
hENT1 x *dCK*	0.18	0.52
RRM1 x *RRM2*	0.40	0.14
hENT1 x *dCK*/RRM1 x *RRM2*	-0.27	0.33
RRM1 x *RRM2* x *CDA*	-0.28	0.31
hENT1 x *dCK*/*RRM1* x *RRM2* x *CDA*	-0.21	0.44

[a]P<0.05.

Table VI. Correlation between the IC_{50} values for 5-FU and the mRNA expression levels of genes associated with 5-FU metabolism.

Gene expressions	Spearman's rank-correlation coefficient (ρ)	P-value
TP	0.064	0.82
DPD	0.37	0.17
OPRT	0.086	0.76
TS	0.41	0.12
TP/*DPD*	-0.38	0.16
OPRT/*DPD*	-0.44	0.10
TP x *OPRT*	0.043	0.88
TS x *DPD*	0.55	0.035[a]
TP x *OPRT*/*TS* x *DPD*	-0.48	0.074

[a]P<0.05.

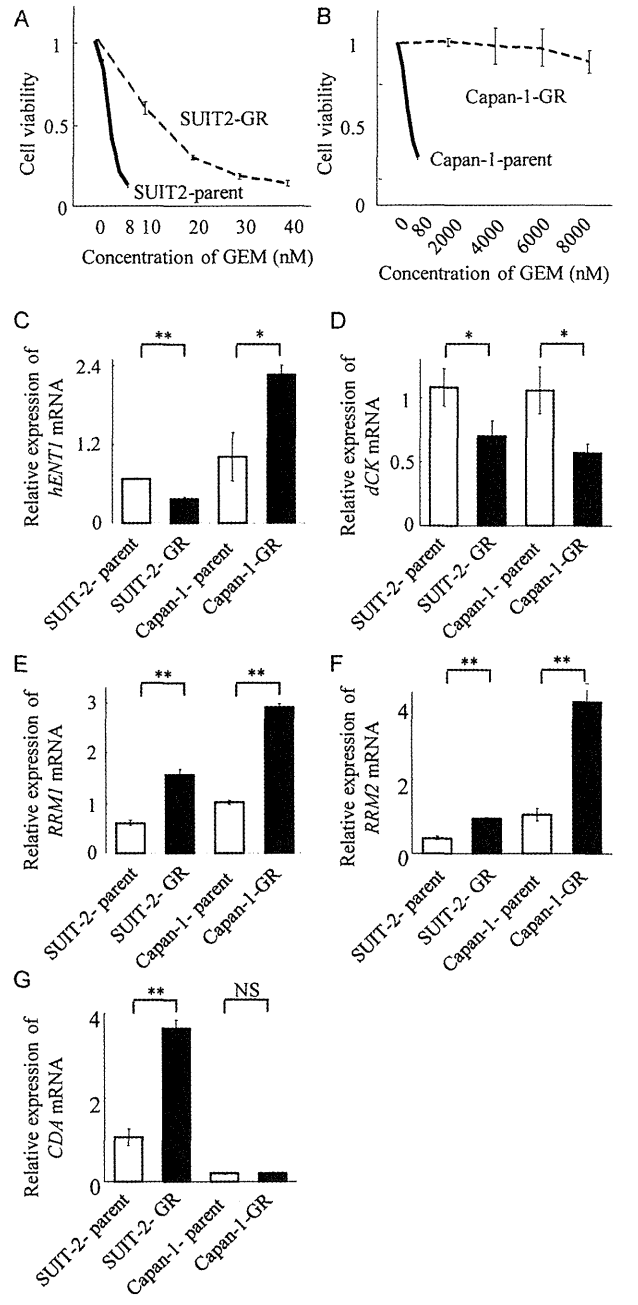

Figure 2. Viability of parental and GEM-resistant cells (SUIT-2-GR and Capan-1-GR) exposed to GEM (A and B). Both GEM-resistant cell lines were significantly more resistant to GEM than the parental cells. Quantitative analyses of *hENT1* (C), *dCK* (D), *RRM1* (E), *RRM2* (F) and *CDA* (G) mRNAs in parental and GEM-resistant cells (SUIT-2-GR and Capan-1-GR). *P<0.05; **P<0.01; NS, not significant.

catalyze another process that yields 5'-fluoro-2'-deoxyuridine-5'-monophosphate, which forms a stable ternary complex with TS to block DNA synthesis and repair (40), there was no significant correlation between the IC_{50} values for 5-FU and the expression levels of *RRM1* or *RRM2*.

Establishment of GEM-resistant pancreatic cancer cells. To investigate the altered expression levels of GEM transport- and metabolism-related genes in GEM-resistant cells, the GEM-resistant pancreatic cancer cells SUIT-2-GR and Capan-1-GR were generated from the parental cell lines (SUIT-2-parent and Capan-1-parent). The GEM IC_{50} values for both of these GEM-resistant cell lines were significantly higher than those of the parental cells (Table VII, Fig. 2A and B; P<0.001). Although SUIT-2-GR cells showed significantly decreased expression levels of *hENT1*, Capan-1-GR cells showed a significant increase in expression (Fig. 2C). The expression levels of *dCK* significantly decreased (Fig. 2D) and those of *RRM1* and *RRM2* significantly increased in both of the GEM-resistant cell lines (Fig. 2E and F). The expression level of *CDA* in SUIT-2-GR cells was significantly higher than that in SUIT-2-parent cells, whereas expressions in the Capan-1-parent and Capan-1-GR cells were very low (and not significantly different) (Fig. 2G). The data regarding the expression levels of *RRM1* in GEM-resistant cells were

Table VII. IC_{50} values of GEM-resistant, 5-FU-resistant and parental cell lines.

Cell line	IC_{50} value	
	GEM (nM)	5-FU (μM)
SUIT-2-parent	3.53±0.062	4.01±0.17
SUIT-2-GR	12.24±1.07	2.12±0.15
SUIT-2-FR	3.03±0.096	7.33±0.24
Capan-1-parent	62.63±6.86	2.35±0.24
Capan-1-GR	> 8000	0.78±0.17
Capan-1-FR	2.69±0.13	7.27±0.63

consistent with the above results in all 15 pancreatic cancer cell lines. Although previous reports show that decreased expression of *hENT1* (20) and increased expression of *CDA* (16), which were only observed in SUIT-2-GR cells, is associated with the development of GEM-resistance, our results from both of the GEM-resistant cells indicated that lower *dCK*, expression, coupled with higher *RRM1* and *RRM2* expressions, are important factors for developing resistance to this agent.

Establishment of 5-FU-resistant pancreatic cancer cells. We generated 5-FU-resistant SUIT-2 (SUIT-2-FR) and Capan-1 (Capan-1-FR) cells by exposure to gradually increasing concentrations of 5-FU. The 5-FU IC_{50} values for both of these resistant cell lines were significantly higher than those of the parental cell lines (Table VII and Fig. 3A and B; P<0.001). As outlined above for the GEM-resistance cells, we measured the expression of genes associated with 5-FU metabolism (Fig. 3C-F). The expression levels of *TP*, *DPD*, *OPRT* and *TS* in SUIT-2-FR cells were significantly higher than those in the parental cells (Fig. 3C-F). Meanwhile, Capan-1-FR cells showed a significant increase in *TP* expression compared with Capan-1-parent cells (Fig. 3C). Although the level of *DPD* expression in Capan-1-FR cells was significantly higher than that in Capan-1-parent cells, it was still extremely low (Fig. 3D) compared with that in the SUIT-2 cell lines. The expression levels of *OPRT* and *TS* in both of the Capan-1 cell lines were almost the same (Fig. 3E and F). Although previous reports showed that increased expressions of *OPRT* and *TS*, which we observed only in SUIT-2-FR cells, were associated with resistance to 5-FU and its prodrugs (32,38,40), our results from both of the 5-FU-resistant cell lines indicated that increased expressions of *TP* and *DPD* are important for developing 5-FU-resistance. We also calculated the *TS* x *DPD* expression level in these cell lines and found that both 5-FU-resistant cell lines showed significantly higher levels of combined expression than the parental cells, although expression levels in Capan-1 cells were lower than those in SUIT-2 cells (Fig. 3G).

GEM-sensitivity of 5-FU-resistant pancreatic cancer cells. To investigate whether there was any cross-resistance to 5-FU and GEM, we examined the sensitivity of 5-FU-resistant cells to GEM. The GEM IC_{50} value of SUIT-2-FR cells was slightly (but significantly) lower than that of the parental cells (Fig. 4A and Table IV; P<0.001); however, there was no significant difference in GEM-sensitivity between Capan-1-FR cells and Capan-1-parent cells (Fig. 4B). This suggests that the acquisition of 5-FU-resistance had no effect on GEM-sensitivity.

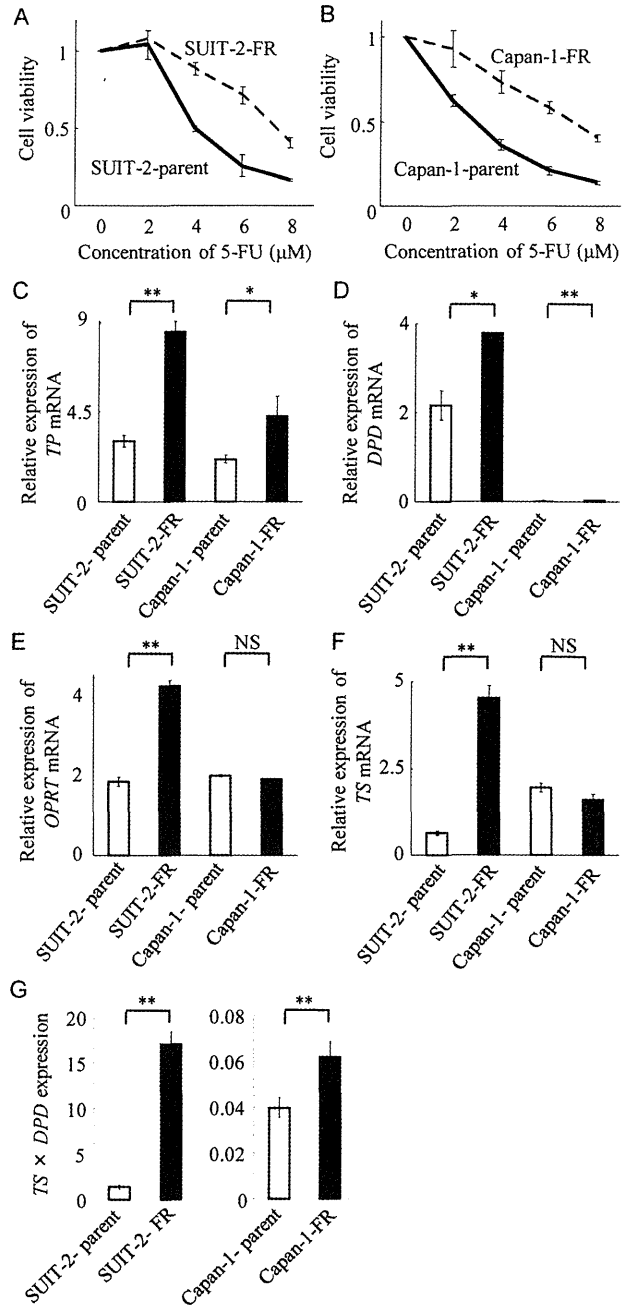

Figure 3. Viability of parental and 5-FU-resistant cells (SUIT-2-FR and Capan-1-FR) exposed to 5-FU (A and B). Both 5-FU-resistant cell lines were significantly more resistant to 5-FU than the parental cells. Quantitative analyses of *TP* (C), *DPD* (D), *OPRT* (E) and *TS* (F) mRNAs in parental and 5-FU-resistant cells (SUIT-2-FR and Capan-1-FR). Combined expression of *TS* x *DPD* in parental and 5-FU-resistant cells (G). *P<0.05; **P<0.01; NS, not significant.

To assess the effects of 5-FU-resistance on the expression levels of genes related to GEM transport and metabolism, we measured the expression levels of these genes in 5-FU-resistant and parental cells. SUIT-2-FR cells expressed significantly higher levels of *hENT1*, *dCK*, *RRM1* and *RRM2*, and significantly lower levels of *CDA*, than the parental cells (Fig. 4C-G).

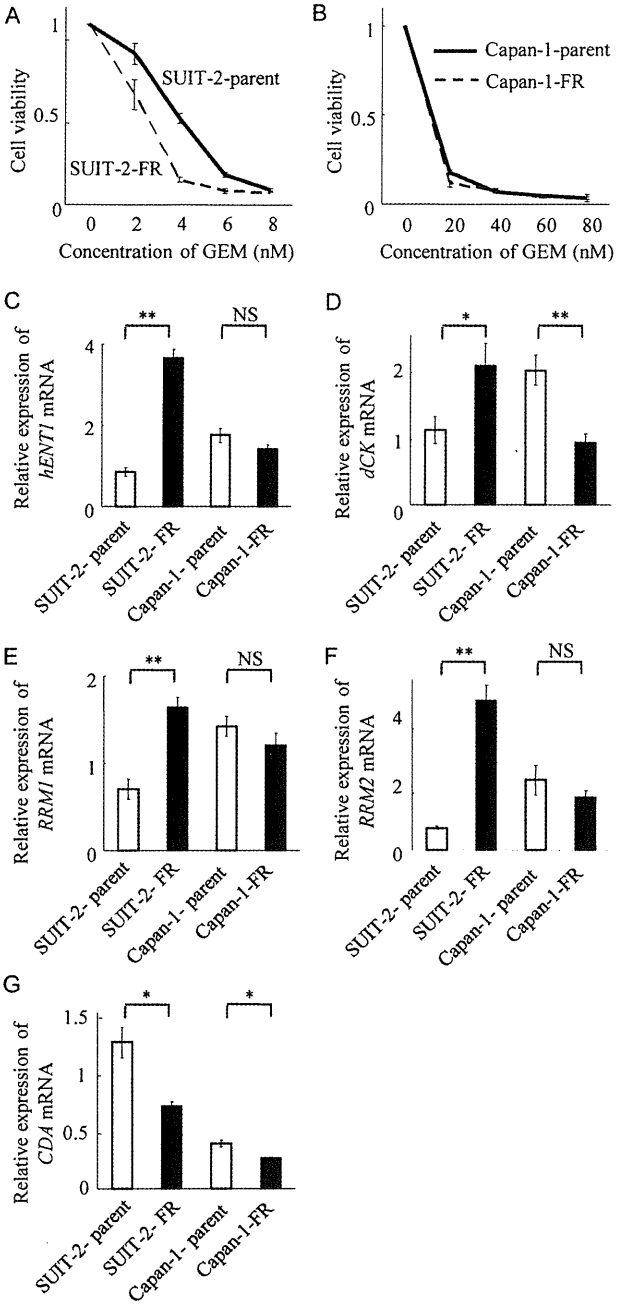

Figure 4. Viability of parental and 5-FU-resistant cells (SUIT-2-FR and Capan-1-FR) exposed to GEM (A and B). SUIT-2-FR cells were slightly, but significantly, more sensitive than SUIT-2-parent cells (A). Quantitative analyses of *hENT1* (C), *dCK* (D), *RRM1* (E), *RRM2* (F) and *CDA* (G) mRNAs in parental and 5-FU-resistant cells (SUIT-2-FR and Capan-1-FR). *P<0.05; **P<0.01; NS, not significant.

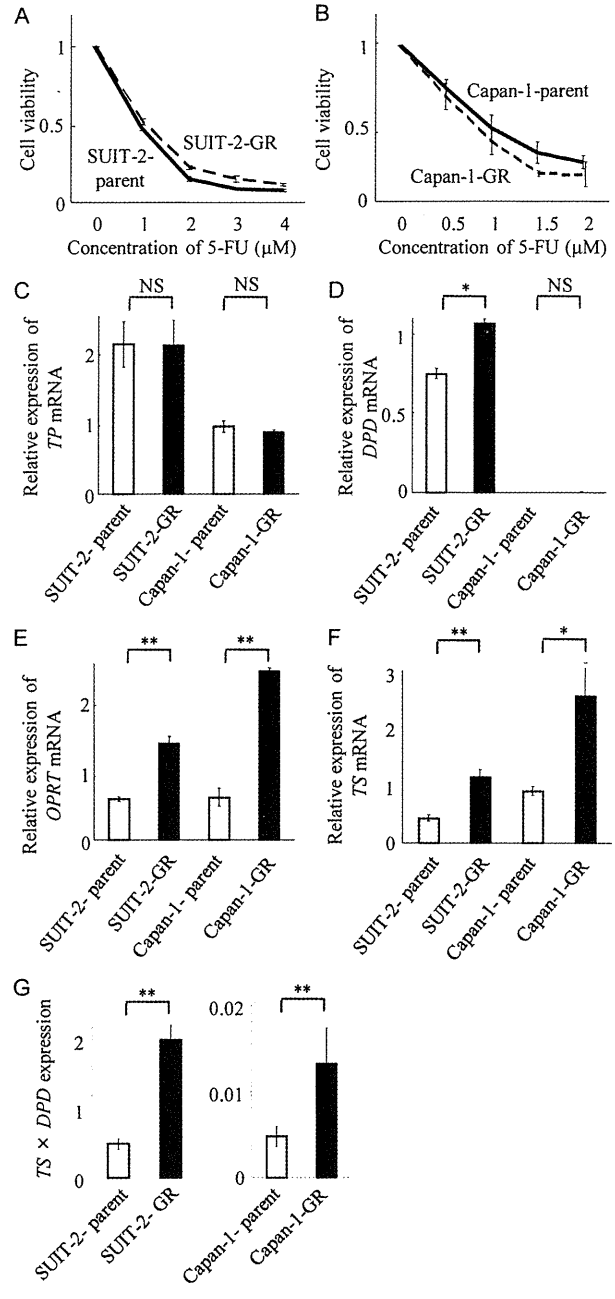

Figure 5. Viability of parental and GEM-resistant cells (SUIT-2-GR and Capan-1-GR) exposed to 5-FU (A and B). Quantitative analyses of *TP* (C), *DPD* (D), *OPRT* (E) and *TS* (F) mRNAs in parental and GEM-resistant cells (SUIT-2-GR and Capan-1-GR). Combined expression of *TS* x *DPD* in parental and GEM-resistant cells (G). *P<0.05; **P<0.01; NS, not significant.

Meanwhile, Capan-1-FR cells showed no significant changes in expression levels of *hENT1*, *RRM1* and *RRM2* compared with the parental cells (Fig. 4C, E and F), although they did show significantly decreased expressions of *dCK* and *CDA* (Fig. 4D and G). Despite significantly increased expressions of *RRM1* and *RRM2*, SUIT-2-FR cells did not become resistant to GEM. These results suggest that there may be a substantial number of patinets who become sensitive to GEM (via increased expressions of *dCK* and *hENT1*) after developing resistance to 5-FU.

5-FU sensitivity of GEM-resistant pancreatic cancer cells. We also investigated the sensitivity to 5-FU of GEM-resistant cells and found that GEM-resistant cells had similar levels of 5-FU-sensitivity to the parental cells (Fig. 5A and B). These data suggest that GEM-resistance did not affect 5-FU-sensitivity.

Similarly, we measured the expression levels of the genes related to 5-FU metabolism in GEM-resistant cell lines (Fig. 5C-F). Although there was no significant change in the expression level of *TP*, the levels of *OPRT*, *TS* and *TS* x *DPD* expression were significantly higher in both of the GEM-resistant cell lines than in parental cells (Fig. 5C, E-G).

SUIT-2-GR cells showed significantly higher level of *DPD* expression than the SUIT-2-parent cells, whereas the expression levels in Capan-1-GR and Capan-1-parent cells were too low to compare (Fig. 5D). Although GEM-resistant cells showed significant increases in *DPD*, *OPRT*, *TS* and *TS* x *DPD* expression levels (as observed in 5-FU-resistant cells) (Fig. 3C-G), they did not become resistant to 5-FU. This suggests that increased expression of *TP* may be essential for the development of 5-FU-resistance in both cell lines.

Discussion

Although GEM-based chemotherapy is still the standard palliative chemotherapy for pancreatic cancer (5,11), the efficacy of GEM as a single agent is limited, and clinicians are often torn when faced with GEM-refractory patients. To improve the prognosis of patients with pancreatic cancer, much effort has been put into developing other effective first- and second-line chemotherapy regimens such as 5-FU prodrugs, used alone or in combination with GEM; however, their therapeutic effects are modest or disappointing. Therefore, individualized chemotherapy based on the gene expression profiles of the individual's own cancer tissues would be a helpful strategy for selecting those patients that are likely to respond to treatment (20,33). Many studies of the mechanisms of GEM and 5-FU metabolism have suggested that certain genes/proteins are associated with sensitivity to these drugs (12-15,17-26,39,40). However, to our knowledge, there is no study evaluating acquired cross-resistance between GEM and 5-FU and its correlation with gene expression.

In the present study, we analyzed the IC_{50} values for GEM and 5-FU in 15 pancreatic cancer cell lines and found no correlation between sensitivity to either drug. Moreover, we evaluated sensitivity to these agents using pancreatic cancer cell lines resistant to either GEM or 5-FU and found that these GEM- or 5-FU-resistant cells acquired no cross-resistance to the other agent. These data suggest that first line chemotherapy using either GEM- or 5-FU may not promote resistance to the other drug and confirm that combination therapy, or second-line chemotherapy using one or other of the drugs, may be a useful strategy for treating pancreatic cancer. Notably, SUIT-2-FR cells showed slightly (but significantly) higher sensitivity to GEM than parental cells. However, recent clinical studies have not shown striking results with second-line chemotherapy (41-43); therefore, further investigation is needed to select the best agents for first- or second-line chemotherapy for pancreatic cancer.

To evaluate whether any changes occurred in cells that developed resistance to GEM or 5-FU, we also analyzed the expression levels of the genes associated with transport and metabolism in 15 pancreatic cancer cell lines and GEM- or 5-FU resistant cells. Regarding GEM, the present data suggest that lower expression of *dCK*, coupled with higher expressions of *RRM1* and *RRM2* may be important factors for developing the resistance to this drug. Akita *et al* (44) demonstrated that only patients with low levels of RRM1 expression derive significant benefit from GEM in terms of preventing disease recurrence. Therefore, RRM1 expression may contribute to GEM-resistance in pancreatic cancer. However, SUIT-2-FR cells did not become resistant to GEM, despite increased *RRM1* expression levels, suggesting that quantification of several genes and a combined evaluation of the results may be needed if individualized chemotherapy based on gene expression profiles is to be used in a clinical setting.

The data regarding 5-FU-resistant cells suggest that higher *TP*, *DPD* and *TS* x *DPD* expressions may be important factors for developing the resistance to this drug. There was no significant change in 5-FU-sensitivity in GEM-resistant cells expressing higher levels of *DPD*, *OPRT*, *TS* and *TS* x *DPD*, suggesting that increased expression levels of *TP* may be essential for the development of 5-FU-resistance. Increased *TP* expression was initially reported to be correlated with increased sensitivity to 5-FU, possibly due to increased synthesis of 2'-deoxy-5'-fluorouridine (FUDR) (45). However, higher TP expression was also reported to correlate with a poor response to 5-FU-based treatment and shorter survival times in colorectal (45) and pancreatic cancer (39) patients, although there are conflicting results (46). TP is identical to platelet-derived endothelial cell growth factor (PD-ECGF) in terms of its pro-angiogenic activity; therefore, the activity of this enzyme is used as a prognostic indicator (47). Conversely, TP is also an enzyme that metabolizes the 5-FU prodrug, capecitabine (N4-pentoxycarbonyl-5'-5-fluorocytidine). This is an attractive novel fluoropyrimidine analogue with great clinical potential. It is metabolized in the liver and tumor tissues to 5'-deoxy-5-fluorouridine (5'-DFUR) by CDA. 5'-DFUR is then converted to 5-FU by TP (26,46). Because TP is highly expressed in tumor tissues relative to host cells, capecitabine can be selectively activated in tumor tissues, suggesting that TP may contribute to capecitabine sensitivity (26,31). Additionally, CDA is also associated with GEM-resistance due to its ability to inactivate GEM (48). Therefore, capecitabine may be a potent drug for treating GEM-resistant patients showing high *CDA* expression, or for 5-FU-resistant patients showing high *TP* expression. However, further studies are needed to elucidate the correlation between capecitabine-sensitivity and *CDA* and/or *TP* expression.

In conclusion, we found no cross-resistance between GEM and 5-FU, even in pancreatic cancer cell lines that developed resistance to the other drug. These results suggest that it may be possible to use either of these drugs as second-line chemotherapy in patients with pancreatic cancer that has developed resistance to one of these agents. In addition, quantitative analyses of *RRM1*, *TP*, *DPD* and *TS* may be a potent strategy for developing individualized chemo-therapeutic regimens.

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References

1. Li D, Xie K, Wolff R and Abbruzzese JL: Pancreatic cancer. Lancet 363: 1049-1057, 2004.

2. Matsuno S, Egawa S, Fukuyama S, *et al*: Pancreatic Cancer Registry in Japan: 20 years of experience. Pancreas 28: 219-230, 2004.
3. Carpelan-Holmstrom M, Nordling S, Pukkala E, *et al*: Does anyone survive pancreatic ductal adenocarcinoma? A nationwide study re-evaluating the data of the Finnish Cancer Registry. Gut 54: 385-387, 2005.
4. Wagner M, Redaelli C, Lietz M, Seiler CA, Friess H and Buchler MW: Curative resection is the single most important factor determining outcome in patients with pancreatic adenocarcinoma. Br J Surg 91: 586-594, 2004.
5. Burris HA 3rd, Moore MJ, Andersen J, *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 15: 2403-2413, 1997.
6. Colucci G, Labianca R, Di Costanzo F, *et al*: Randomized phase III trial of gemcitabine plus cisplatin compared with single-agent gemcitabine as first-line treatment of patients with advanced pancreatic cancer: the GIP-1 study. J Clin Oncol 28: 1645-1651, 2010.
7. Cunningham D, Chau I, Stocken DD, *et al*: Phase III randomized comparison of gemcitabine versus gemcitabine plus capecitabine in patients with advanced pancreatic cancer. J Clin Oncol 27: 5513-5518, 2009.
8. Kindler HL, Niedzwiecki D, Hollis D, *et al*: Gemcitabine plus bevacizumab compared with gemcitabine plus placebo in patients with advanced pancreatic cancer: phase III trial of the cancer and leukemia group B (CALGB 80303). J Clin Oncol 28: 3617-3622, 2010.
9. Moore MJ, Goldstein D, Hamm J, *et al*: Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 25: 1960-1966, 2007.
10. Carmichael J: The role of gemcitabine in the treatment of other tumours. Br J Cancer 78 (Suppl 3): S21-S25, 1998.
11. Oettle H, Post S, Neuhaus P, *et al*: Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. JAMA 297: 267-277, 2007.
12. Garcia-Manteiga J, Molina-Arcas M, Casado FJ, Mazo A and Pastor-Anglada M: Nucleoside transporter profiles in human pancreatic cancer cells: role of hCNT1 in 2',2'-difluorodeoxycytidine-induced cytotoxicity. Clin Cancer Res 9: 5000-5008, 2003.
13. Fukunaga AK, Marsh S, Murry DJ, Hurley TD and McLeod HL: Identification and analysis of single-nucleotide polymorphisms in the gemcitabine pharmacologic pathway. Pharmacogenomics J 4: 307-314, 2004.
14. Plunkett W, Huang P, Searcy CE and Gandhi V: Gemcitabine: preclinical pharmacology and mechanisms of action. Semin Oncol 23: 3-15, 1996.
15. Heinemann V, Xu YZ, Chubb S, *et al*: Cellular elimination of 2',2'-difluorodeoxycytidine 5'-triphosphate: a mechanism of self-potentiation. Cancer Res 52: 533-539, 1992.
16. Eda H, Ura M, K FO, Tanaka Y, Miwa M and Ishitsuka H: The antiproliferative activity of DMDC is modulated by inhibition of cytidine deaminase. Cancer Res 58: 1165-1169, 1998.
17. Blackstock AW, Lightfoot H, Case LD, *et al*: Tumor uptake and elimination of 2',2'-difluoro-2'-deoxycytidine (gemcitabine) after deoxycytidine kinase gene transfer: correlation with in vivo tumor response. Clin Cancer Res 7: 3263-3268, 2001.
18. Duxbury MS, Ito H, Zinner MJ, Ashley SW and Whang EE: RNA interference targeting the M2 subunit of ribonucleotide reductase enhances pancreatic adenocarcinoma chemosensitivity to gemcitabine. Oncogene 23: 1539-1548, 2004.
19. Fujita H, Ohuchida K, Mizumoto K, *et al*: Gene expression levels as predictive markers of outcome in pancreatic cancer after gemcitabine-based adjuvant chemotherapy. Neoplasia 12: 807-817, 2010.
20. Giovannetti E, Del Tacca M, Mey V, *et al*: Transcription analysis of human equilibrative nucleoside transporter-1 predicts survival in pancreas cancer patients treated with gemcitabine. Cancer Res 66: 3928-3935, 2006.
21. Mackey JR, Mani RS, Selner M, *et al*: Functional nucleoside transporters are required for gemcitabine influx and manifestation of toxicity in cancer cell lines. Cancer Res 58: 4349-4357, 1998.
22. Nakahira S, Nakamori S, Tsujie M, *et al*: Involvement of ribonucleotide reductase M1 subunit overexpression in gemcitabine resistance of human pancreatic cancer. Int J Cancer 120: 1355-1363, 2007.
23. Ohhashi S, Ohuchida K, Mizumoto K, *et al*: Down-regulation of deoxycytidine kinase enhances acquired resistance to gemcitabine in pancreatic cancer. Anticancer Res 28: 2205-2212, 2008.
24. Rosell R, Felip E, Taron M, *et al*: Gene expression as a predictive marker of outcome in stage IIB-IIIA-IIIB non-small cell lung cancer after induction gemcitabine-based chemotherapy followed by resectional surgery. Clin Cancer Res 10: 4215-4219, 2004.
25. Spratlin J, Sangha R, Glubrecht D, *et al*: The absence of human equilibrative nucleoside transporter 1 is associated with reduced survival in patients with gemcitabine-treated pancreas adenocarcinoma. Clin Cancer Res 10: 6956-6961, 2004.
26. Guichard SM, Macpherson JS, Mayer I, *et al*: Gene expression predicts differential capecitabine metabolism, impacting on both pharmacokinetics and antitumour activity. Eur J Cancer 44: 310-317, 2008.
27. Okusaka T, Funakoshi A, Furuse J, *et al*: A late phase II study of S-1 for metastatic pancreatic cancer. Cancer Chemother Pharmacol 61: 615-621, 2008.
28. Shirasaka T, Shimamato Y, Ohshimo H, *et al*: Development of a novel form of an oral 5-fluorouracil derivative (S-1) directed to the potentiation of the tumor selective cytotoxicity of 5-fluorouracil by two biochemical modulators. Anticancer Drugs 7: 548-557, 1996.
29. Tatsumi K, Fukushima M, Shirasaka T and Fujii S: Inhibitory effects of pyrimidine, barbituric acid and pyridine derivatives on 5-fluorouracil degradation in rat liver extracts. Jpn J Cancer Res 78: 748-755, 1987.
30. Shirasaka T, Shimamoto Y and Fukushima M: Inhibition by oxonic acid of gastrointestinal toxicity of 5-fluorouracil without loss of its antitumor activity in rats. Cancer Res 53: 4004-4009, 1993.
31. Ishikawa T, Sekiguchi F, Fukase Y, Sawada N and Ishitsuka H: Positive correlation between the efficacy of capecitabine and doxifluridine and the ratio of thymidine phosphorylase to dihydropyrimidine dehydrogenase activities in tumors in human cancer xenografts. Cancer Res 58: 685-690, 1998.
32. Kuramochi H, Hayashi K, Uchida K, *et al*: High intratumoral dihydropyrimidine dehydrogenase mRNA levels in pancreatic cancer associated with a high rate of response to S-1. Cancer Chemother Pharmacol 63: 85-89, 2008.
33. Danesi R, De Braud F, Fogli S, Di Paolo A and Del Tacca M: Pharmacogenetic determinants of anti-cancer drug activity and toxicity. Trends Pharmacol Sci 22: 420-426, 2001.
34. Sato N, Mizumoto K, Beppu K, *et al*: Establishment of a new human pancreatic cancer cell line, NOR-P1, with high angiogenic activity and metastatic potential. Cancer Lett 155: 153-161, 2000.
35. Ohuchida K, Mizumoto K, Murakami M, *et al*: Radiation to stromal fibroblasts increases invasiveness of pancreatic cancer cells through tumor-stromal interactions. Cancer Res 64: 3215-3222, 2004.
36. Ohuchida K, Mizumoto K, Ogura Y, *et al*: Quantitative assessment of telomerase activity and human telomerase reverse transcriptase messenger RNA levels in pancreatic juice samples for the diagnosis of pancreatic cancer. Clin Cancer Res 11: 2285-2292, 2005.
37. Nakano Y, Tanno S, Koizumi K, *et al*: Gemcitabine chemoresistance and molecular markers associated with gemcitabine transport and metabolism in human pancreatic cancer cells. Br J Cancer 96: 457-463, 2007.
38. Ichikawa W, Uetake H, Shirota Y, *et al*: Both gene expression for orotate phosphoribosyltransferase and its ratio to dihydropyrimidine dehydrogenase influence outcome following fluoropyrimidine-based chemotherapy for metastatic colorectal cancer. Br J Cancer 89: 1486-1492, 2003.
39. Miyake K, Imura S, Yoshizumi T, Ikemoto T, Morine Y and Shimada M: Role of thymidine phosphorylase and orotate phosphoribosyltransferase mRNA expression and its ratio to dihydropyrimidine dehydrogenase in the prognosis and clinicopathological features of patients with pancreatic cancer. Int J Clin Oncol 12: 111-119, 2007.
40. Allen WL and Johnston PG: Role of genomic markers in colorectal cancer treatment. J Clin Oncol 23: 4545-4552, 2005.
41. Androulakis N, Syrigos K, Polyzos A, *et al*: Oxaliplatin for pretreated patients with advanced or metastatic pancreatic cancer: a multicenter phase II study. Cancer Invest 23: 9-12, 2005.
42. Demols A, Peeters M, Polus M, *et al*: Gemcitabine and oxaliplatin (GEMOX) in gemcitabine refractory advanced pancreatic adenocarcinoma: a phase II study. Br J Cancer 94: 481-485, 2006.

43. Kulke MH, Blaszkowsky LS, Ryan DP, *et al*: Capecitabine plus erlotinib in gemcitabine-refractory advanced pancreatic cancer. J Clin Oncol 25: 4787-4792, 2007.
44. Akita H, Zheng Z, Takeda Y, *et al*: Significance of RRM1 and ERCC1 expression in resectable pancreatic adenocarcinoma. Oncogene 28: 2903-2909, 2009.
45. Metzger R, Danenberg K, Leichman CG, *et al*: High basal level gene expression of thymidine phosphorylase (platelet-derived endothelial cell growth factor) in colorectal tumors is associated with nonresponse to 5-fluorouracil. Clin Cancer Res 4: 2371-2376, 1998.
46. Ackland SP and Peters GJ: Thymidine phosphorylase: its role in sensitivity and resistance to anticancer drugs. Drug Resist Updat 2: 205-214, 1999.
47. Ishikawa F, Miyazono K, Hellman U, *et al*: Identification of angiogenic activity and the cloning and expression of platelet-derived endothelial cell growth factor. Nature 338: 557-562, 1989.
48. Bengala C, Guarneri V, Giovannetti E, *et al*: Prolonged fixed dose rate infusion of gemcitabine with autologous haemopoietic support in advanced pancreatic adenocarcinoma. Br J Cancer 93: 35-40, 2005.

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ORIGINAL ARTICLE – TRANSLATIONAL RESEARCH AND BIOMARKERS

MicroRNA Expression as a Predictive Marker for Gemcitabine Response after Surgical Resection of Pancreatic Cancer

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ABSTRACT

Background. To improve the prognosis of patients after resection of pancreatic cancer, the most appropriate and efficient treatment should be provided to specific subsets of patients. Our aim was to identify promising microRNAs as markers to predict responses to gemcitabine in patients with resected pancreatic cancer.

Methods. Two gemcitabine-resistant pancreatic cancer cell lines were established, and global microRNA expression analyses was performed by quantitative reverse transcription–polymerase chain reaction (qRT-PCR). Eleven miRNAs were selected as putative predictive markers and analyzed by means of macrodissected formalin-fixed, paraffin-embedded samples obtained from 90 patients with or without gemcitabine treatment after resection of pancreatic cancer.

Results. We identified 24 microRNAs whose expression was altered in gemcitabine-resistant cells. qRT-PCR analyses showed that patients with high miR-142-5p and miR-204 expression had significantly longer survival times than those with low miR-142-5p ($P = 0.0077$) and miR-204 ($P = 0.0054$) expression in the gemcitabine-treated group. This was not seen in the nontreated group. Multivariate analyses showed that miR-142-5p expression was an independent prognostic marker only in patients treated with gemcitabine ($P = 0.034$).

Conclusions. miR-142-5p is a promising predictive marker for gemcitabine response in patients with resected pancreatic cancer.

Pancreatic cancer is the fourth most common cause of tumor-related death in the industrialized world.[1,2] Only 10–20% of pancreatic cancer patients are candidates for surgery at the time of presentation, and fewer than 20% of patients who undergo curative resection are alive after 5 years.[3,4] A few recent reports have described the successful use of adjuvant chemotherapies such as 5-fluorouracil and gemcitabine.[5,6] However, not all patients benefit from such adjuvant chemotherapy, and we cannot predict which patients will benefit most from this treatment. Therefore, to improve the prognosis of patients with resected pancreatic cancer, we need to identify specific markers that can predict responses to adjuvant therapy. Such a personalized therapy, based on the predictive markers, may provide the most appropriate and efficient treatment for a specific subset of patients.

Gemcitabine is a deoxycytidine analog with antitumor activity that bears a resemblance, both structurally and metabolically, to arabinosyl cytosine.[7] Gemcitabine is widely accepted as the first-line treatment for patients with advanced or resected pancreatic cancer.[6,8] However, recent reports showed that the complete plus partial response rate and the disease control rate in advanced pancreatic cancer are 8.0–13.5% and 49.2–62.1%, respectively, even with combination-treatment arms.[9,10] The data suggest that approximately half of patients with resected pancreatic cancer do not benefit from gemcitabine-based combination therapies. Therefore, predictive markers are needed to select those patients who may benefit most from gemcitabine-based therapy. So far, research into the mechanism of

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resistance to gemcitabine has led to identification of several candidate predictive markers. These include genes related to gemcitabine metabolism and transport, such as deoxycytidine kinase, ribonucleotide reductase, and human equilibrative nucleoside transporter-1.[11,12] However, the usefulness of such markers in a clinical setting remains unclear because of difficulties in evaluating their protein or mRNA levels. Even when well-established methods are used, immunohistochemical evaluation and its interpretation are different between laboratories.[13] Also, accurate quantitative analyses of mRNA from clinical samples are often difficult as a result of degradation. Therefore, we need more reliable methods-based biomarkers to predict responses to gemcitabine.

MicroRNAs (miRNAs) are small noncoding RNA gene products of approximately 22 nt that are found in a variety of organisms. They play key roles in regulating the translation and degradation of mRNAs through base pairing to partially complementary sites, predominantly in the 3′-untranslated regions of mRNAs.[14,15] Because microRNAs are well preserved, even in formalin-fixed, paraffin-embedded (FFPE) samples, the quantitative analysis of miRNA in many types of clinical sample is accurate and reliable.[16,17] Therefore, miRNAs are promising molecular markers.

There is increasing evidence that miRNAs are mutated or differentially expressed in many types of cancers, and thus are also potential diagnostic markers.[18–20] The expression levels of several miRNAs, such as miR-21, miR-196a-2, miR-155, and miR-210 in pancreatic cancer, are known to correlate with a poor prognosis.[19,21,22] Thus, there is a possibility that specific miRNAs that can be used as predictive markers for a gemcitabine response exist.

Here, to identify predictive markers, we established two gemcitabine-resistant pancreatic cancer cell lines and performed global miRNA expression analyses. We then further investigated 11 selected miRNAs as potential predictive markers using macrodissected FFPE samples derived from 90 patients with resected pancreatic cancer.

MATERIALS AND METHODS

Cell Lines and Establishment of Gemcitabine-Resistant Cells

Two human pancreatic cancer cell lines, SUIT-2 and CAPAN-1 (the gift of Dr. H. Iguchi, National Shikoku Cancer Center, Matsuyama, Japan), were used in this study. Gemcitabine-resistant cells were generated by exposing these cell lines to gradually increasing concentrations of gemcitabine. The initial concentration of gemcitabine was 1 nM, which did not seem to affect the proliferation of either the SUIT-2 or CAPAN-1 cells. When the cells had adapted to the drug, the concentration of gemcitabine was gradually increased by 10–100 nM per week to a final concentration of 200 nM (SUIT-2) and 1 μM (CAPAN-1). Gemcitabine was dissolved in phosphate-buffered saline and added to the cell culture media.

Propidium Iodide Assay

Cell proliferation was evaluated by measuring the fluorescence intensity of propidium iodide as described previously.[23] The fluorescence intensity corresponding to the total cells was measured with a CytoFluor multiwell plate reader (PerSeptive Biosystems, Framingham, MA, USA). The results were converted to percentage survival rates by comparing treated cells with untreated cells.

miRNA Expression Profiling

Expression profiles for the 365 mature miRNAs were obtained by quantitative polymerase chain reaction (qPCR) using the TaqMan Array Human MiRNA (TLDA) or TaqMan MiRNA Assays (Applied Biosystems; ABI) on an ABI Prism 7900HT according to the manufacturer's instructions. Mature miRNAs were normalized to RNU44 and are expressed as fold changes relative to controls.

Patients and Pancreatic Tissues

From 1992 through 2008, a total of 104 patients underwent pancreatic resection for pancreatic cancer at the Department of Surgery and Oncology, Kyushu University Hospital (Fukuoka, Japan). Survival was measured from the time of pancreatic resection, and death was the end point. The follow-up data for 103 cases were available. Thirteen of the 103 patients were excluded from the present study because they had received combination therapy with gemcitabine and other chemotherapeutic agents such as S1(tegafur/gimestat/potassium oxonate).

Our final study series consisted of 90 patients with resected pancreatic cancer with available follow-up data. The patients (57 men and 32 women) had a median age of 65 years (range, 36–86 years). The median observation time for overall survival was 14.7 months, ranging 0.5–108 months. Sixty-three patients died during follow-up; the other patients were alive and censored.

All resected specimens were fixed in formalin and embedded in paraffin for pathological diagnosis. All tissues adjacent to the specimens were evaluated histologically according to the criteria of the World Health Organization.[24] Diagnoses were confirmed independently by two pathologists with regard to the pathological features of all cases. Tumor stage was assessed according to the International Union Against Cancer classification.[25] Clinicopathological

characteristics of the tumors are shown in Table 1. The study was approved by the Ethics Committee of Kyushu University and was conducted according to the Ethical Guidelines for Human Genome/Gene Research enacted by the Japanese government and the Helsinki Declaration.

Macrodissection

After a review of representative hematoxylin and eosin-stained slides, 4–7 sections (5 μm thick) were cut from FFPE blocks for macrodissection. Adjacent normal tissues, including normal acinar tissues and adipose tissues, in the sections were removed macroscopically with a scalpel. Only the cancerous parts were used for the isolation of miRNA.

TABLE 1 Correlation between median OS and clinical and pathological factors in patients with resected pancreatic cancer

Factor	*n*	OS (95% CI)	*P* value
Age			0.675
≥65 years	47	19 (13.07–27)	
<64 years	42	19 (12–26)	
Sex			0.7544
Male	57	23 (13.07–30.17)	
Female	32	14 (10–24.2)	
pT category			0.0019
pTl/pT2/pT3	55	23 (16.30–43)	
p4	33	12 (9.43–19)	
pN category			0.0070
pN0	26	43 (16.93–)	
pN1	62	13.27 (11.6–22)	
Histological grade			0.0804
G1	19	31 (9–)	
G2	33	23 (14–30.16)	
G3	35	12 (10–23)	
Residual tumor			<0.0001
R0	53	26 (19–45)	
R1	34	12 (9–13.73)	
Vessel invasion			0.0204
Positive	57	13.73 (12–23)	
Negative	31	27 (15–)	
Lymphatic invasion			0.3004
Positive	76	15 (12–24.2)	
Negative	18	27 (14.53–)	
Neural invasion			0.6849
Positive	73	16.93 (13.07–26)	
Negative	15	23 (4.7–)	
Adjuvant therapy			0.0382
Yes	59	23 (14.53–30.17)	
No	30	12 (7.7–24.2)	

OS overall survival, *CI* confidence interval

Isolation of miRNA

miRNA was extracted from the macrodissected FFPE samples with the RNeasy FFPE kit (Qiagen, Tokyo, Japan) using a method modified from the manufacturer's instructions. Briefly, macrodissected FFPE sections were deparaffinized with xylene, washed with ethanol, and dried. Lysis buffer and proteinase K were added to the dried sections. The sections were incubated and Binding buffer was then added to the lysate and transferred to a gDNA Eliminator spin column (Qiagen) to remove genomic DNA. After eliminating DNA, 100% ethanol was added to the flow-through. After mixing, the samples were transferred to an RNeasy MinElute column (Qiagen), which binds total RNA. After washing, the purified RNA was eluted with 50 μl of RNase-free water.

Quantitative Reverse Transcription–Polymerase Chain Reaction

Quantitative reverse transcription–polymerase chain reaction (qRT-PCR) was performed in a Chromo4 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA) using TaqMan MicroRNA Reverse Transcription Kit and TaqMan Universal PCR Master Mix (Applied Biosystems, Tokyo, Japan). For the measurement of miRNA expression, we performed two-step qRT-PCR with specific primers for the indicated miRNAs (designed by Applied Biosystems) following the manufacturer's protocol. Each sample was run in triplicate. The level of miRNA expression was calculated from a standard curve constructed by using small RNAs from CAPAN-1 cells. The expression levels of the indicated miRNAs were normalized to those of RNU6B. The accuracy and integrity of the PCR products were confirmed with an Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Palo Alto, CA, USA).

Statistical Analysis

A data mining technique provided by the SAS Institute was used to split gene expression in high- and low-level groups on the basis of a platform that recursively partitions data according to a relationship between the *X* and *Y* values, creating a tree of partitions (recursive descent partition analysis).[26] By searching all possible cuts, it finds a set of cut points of *X* values (gene expression) that best predict the *Y* value (survival time). These data splits are done, recursively forming a tree of decision rules until the desired fit is reached; the most significant split is determined by the largest likelihood ratio chi-square statistic. In either case, the split is chosen to maximize the difference in the responses between the two branches of the split.

Categorical variables were compared by a chi-square test (Fisher's exact probability test). Survival curves were constructed using the Kaplan-Meier product-limit method and compared by the log rank test. To evaluate the independent prognostic factors associated with patient survival (with or without gemcitabine treatment), multivariate Cox proportional hazard regression analysis was used, with miR-142-5p expression, miR-34a expression, pN status, and/or residual tumor status (R factor) as covariates. Statistical significance was defined as a P value of <0.05. All statistical analyses were performed by JMP 7.01 software (SAS Institute, Cary, NC, USA).

RESULTS

Establishment of Gemcitabine-Resistant Pancreatic Cancer Cells

Gemcitabine-resistant SUIT-2 and CAPAN-1 cells were generated by exposure to gradually increasing concentrations of gemcitabine. The final concentration of gemcitabine was 200 nM for SUIT-2 cells and 1 μM for CAPAN-1 cells. The viability of the parental SUIT-2 cells significantly decreased to less than 20% after treatment with 10 nM gemcitabine, while the viability of gemcitabine-resistant SUIT-2 cells remained unchanged after treatment with 10 nM gemcitabine. The viability of the parental CAPAN-1 cells significantly decreased to less than 10% after treatment with 1 μM gemcitabine, while the viability of gemcitabine-resistant CAPAN-1 cells remained unchanged after treatment with 1 μM gemcitabine.

miRNA Differentially Expressed between Parent and Gemcitabine-Resistant Cells

We used the TaqMan miRNA array to obtain the 365 miRNA expression profiles from the parent and gemcitabine-resistant pancreatic cancer cell lines. The results show that the expression of 10 miRNAs was more than 2-fold higher in both SUIT-2 and CAPAN-1 gemcitabine-resistant cells compared with the parental cells. We also identified 14 miRNAs in both SUIT-2 and CAPAN-1 gemcitabine-resistant cells that were downregulated to less than 30% of that in the parental cells.

To confirm our TaqMan miRNA array data, we subjected 11 of the differentially expressed miRNAs (5 upregulated miRNAs: miR-9, miR-27a, miR-424, miR-449b, and miR-34a; 6 downregulated miRNAs: miR-152, miR-181c, miR-518b, miR-125a, miR-142-5p, and miR-204), which were selected on the basis of expression levels and fold changes as promising candidate of clinical markers, to triplicate qRT-PCR analysis, and obtained consistent results (data not shown).

Analysis of miRNA Expression in Relation to Survival Time of Patients with Pancreatic Cancer after Curative Resection

Conventional prognostic factors, such as pT category, pN category, R factor, positive vessel invasion, and adjuvant therapies, including 5-fluorouracil or gemcitabine, reached significance for overall survival (Table 1). We also found that the patients treated with gemcitabine had a better prognosis than those without, although the difference was not significant ($P = 0.058$).

Next, to investigate the correlation between gemcitabine response and miRNA expression identified by miRNA expression profiling, patients with resected pancreatic cancer were divided into two groups: patients who were treated with gemcitabine and patients who were not. Within each group, we investigated the correlation between the expression levels of the 11 miRNAs identified by miRNA expression profiling and the prognosis of patients with resected pancreatic cancer. miRNA expression was also divided into high- and low-level groups by recursive descent partition analysis, as described by Hoffmann et al.[26]

First, we investigated the correlation between prognosis and the six downregulated miRNAs: miR-152, miR-181c, miR-518b, miR-125a, miR-142-5p, and miR-204. For the gemcitabine group, the high miR-142-5p and miR-204 patients had a significantly longer survival time than the low miR-142-5p and miR-204 patients (Figs. 1 and 2, $P = 0.0077$ for miR-142-5p; $P = 0.0054$ for miR-204). The median survival time was 45 months and 33 months, respectively, for the high miR-142-5p and high miR-204 patients, and 16.3 months in both the low miR-142-5p and low miR-204 patients (Table 2). We also found that the high miR-125a patients had significantly longer survival times than the low miR-125a patients when the Wilcoxon test was used ($P = 0.035$), although the difference was not statistically significant when the log rank test was used (Table 2, $P = 0.085$). However, for the nongemcitabine group, there were no differences in the survival times between the high and low miR-142-5p, miR-204, or miR-125a patients (Table 2). Analysis of both the gemcitabine and nongemcitabine groups did not revealed any significant differences in survival time between the high and low miR-152, miR-181c, and miR-518b patients (data not shown).

Next, we investigated the correlation between prognosis and the six upregulated miRNAs: miR-9, miR-27a, miR-424, miR-449b, and miR-34a. For the nongemcitabine group, the high miR-34a patients had a significantly longer survival times than the low miR-34a patients (Table 2, $P = 0.012$), while there were no significant differences in survival time between the high and low miR-34a patients in the gemcitabine group (Table 2, $P = 0.175$). The median

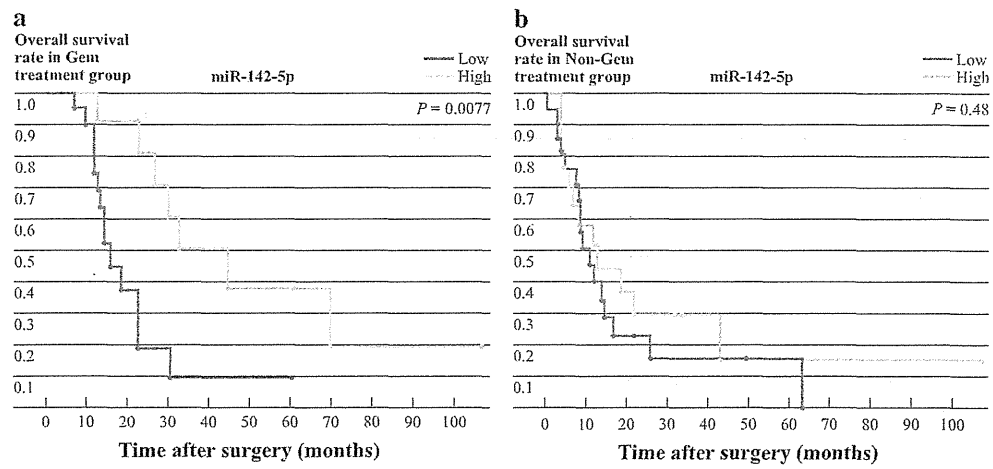

FIG. 1 Correlation between the expression levels of miR-142-5p identified by miRNA expression profiling and the prognosis of patients with resected pancreatic cancer in the gemcitabine and nongemcitabine groups. The levels of miRNA expression were normalized against RNU6B. High miR-142-5p expression was significantly associated with longer survival times in the gemcitabine group ($P = 0.0077$), but not in the nongemcitabine group ($P = 0.48$)

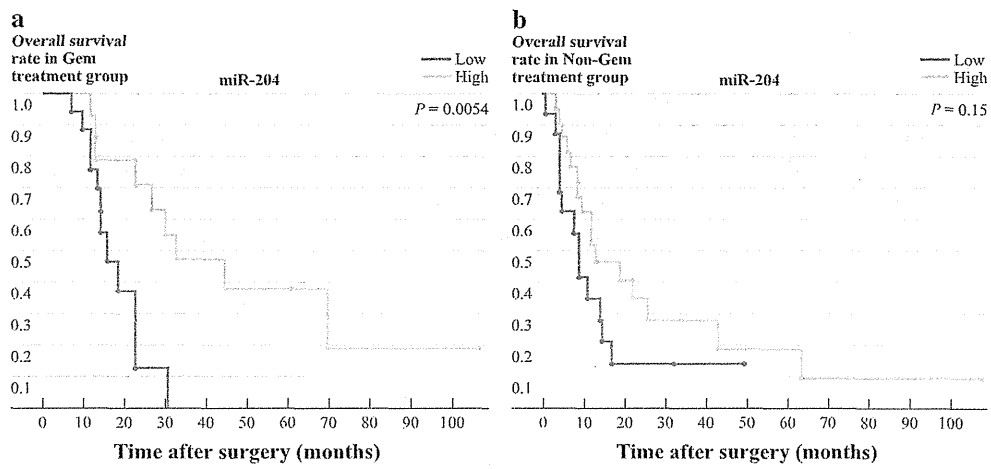

FIG. 2 The correlation between the expression levels of miR-204 identified by miRNA expression profiling and the prognosis of patients with resected pancreatic cancer in the gemcitabine and nongemcitabine groups. The levels of miRNA expression were normalized against RNU6B. High miR-204 expression was significantly associated with longer survival times in the gemcitabine group ($P = 0.0054$), but not in the nongemcitabine group ($P = 0.15$)

TABLE 2 Correlation between median overall survival and microRNA expression in patients with resected pancreatic cancer

Factor	Overall survival (95% CI)		*P* value
	High	Low	
miR-142-5p			
Gem group	45 (23–)	16.3 (12–23)	0.0077
Non-Gem group	13.07 (4.7–43)	11 (7.7–14.7)	0.4880
miR-204			
Gem group	33 (13.27–)	16.3 (12–23)	0.0054
Non-Gem group	13.07 (8.47–26)	9 (4–14.7)	0.1527
miR-125a			
Gem group	30.17 (19–)	14.57(12–31)	0.0854
Non-Gem group	12 (8.47–16.93)	14(3–)	0.8990
miR-34a			
Gem group	27 (20.87–47.9)	16.3 (12–)	0.1754
Non-Gem group	16.9 (9–24.4)	8.5 (3–12)	0.0127

CI confidence interval, *Gem* gemcitabine

survival time was 16.9 months in the high miR-34a patients and 8.5 months in the low miR-34a patients in the nongemcitabine group (Table 2). We also found no significant differences in survival between the high and low miR-9, miR-27a, miR-424, and miR-449b patients in the gemcitabine and nongemcitabine groups (data not shown).

Multivariate Analysis of miR-142-5p and miR-34a Expression in Relation to Survival Time of Patients with or without Gemcitabine Treatment after Curative Resection

In three miRNAs identified as potential prognostic markers in the gemcitabine group, miR-142-5p was the best candidate and was the most specific for the gemcitabine group compared with the nongemcitabine group. Therefore, we focused on miR-142-5p as the most promising predictive marker for gemcitabine. Also, univariate analysis of the gemcitabine group showed that the only significant clinical factors were pN status (pN1 vs. pN0), and R factor (R1 vs. R0). Multivariate survival analysis, based on the Cox proportional hazard model, was performed by using miR-142-5p expression (high versus low), pN status, and R factor. Overall survival time was significantly dependent on miR-142-5p expression (Table 3, $P = 0.034$), but not on pN

TABLE 3 Multivariate survival analysis (Cox regression model) of clinical prognostic factors and miR-142-5p expression in patients with gemcitabine treatment

Factor	Relative risk	95% CI	*P* value
miR-142-5p	3.109	1.087–10.01	0.0340
pN status	2.055	0.646–9.219	0.2395
R factor	2.197	0.823–5.904	0.1146

CI confidence interval

status ($P = 0.24$) or R factor ($P = 0.11$). In the gemcitabine group, miR-142-5p expression was an independent prognostic marker for pancreatic cancer patients, with a relative risk of 3.109 (Table 3).

Univariate analysis of the nongemcitabine group showed that the only significant clinical factor was pN status. Therefore, multivariate survival analysis was performed by using miR-34a expression and pN status. The results show that the overall survival time was significantly dependent on both miR-34a expression and pN status (Table 4, $P = 0.0103$ for miR-34a; $P = 0.0035$ for pN status). In the nongemcitabine group, miR-34a expression was an independent prognostic marker for pancreatic cancer patients with a relative risk of 2.920 (Table 4).

DISCUSSION

The present miRNA profiling study using two gemcitabine-resistant pancreatic cancer cell lines and their parent cell lines identified 24 miRNAs candidates, which were up- or downregulated in gemcitabine resistant cells. The present results also showed that high miR-142-5p and miR-204 patients had statistically significantly longer survival times than the low miR-142-5p and miR-204 patients in the gemcitabine group, but not in nongemcitabine group, although further examination is needed because the number of patients in the nongemcitabine group is too small to conclude that there were no differences in survival between high and low expression patients in the nongemcitabine group. Liu et al. reported that miR-142-5p was repressed in human lung cancer, and the transfection of miR-142-5p significantly repressed lung cancer cell growth.[27] miR-204 has been also reported to be downregulated in intrahepatic cholangiocarcinoma, and the level of miR-204 expression was inversely correlated with that of Bcl-2 expression, possibly leading to chemotherapeutic drug-triggered apoptosis.[28] Taken together, these data suggest that miR-142-5p and miR-204 are promising predictive markers for chemotherapeutic responses in patients with resected pancreatic cancer.

TABLE 4 Multivariate survival analysis (Cox regression model) of clinical prognostic factors and miR-34a expression in patients without gemcitabine treatment

Factor	Relative risk	95% CI	*P* value
miR-34a	2.920	1.303–6.295	0.0103
pN status	2.957	1.410–6.812	0.0035

CI confidence interval

In neuroblastomas, miR-34a was generally expressed at lower levels in unfavorable primary tumors, and the reintroduction of miR-34a results in a dramatic reduction in cell proliferation.[29] miR-34a expression was decreased in 9 of 25 (36%) colon cancers, and transient introduction of miR-34a suppressed the in vitro and in vivo growth of colon cancer.[30] In non-small-cell lung cancer, the miR-34 family was downregulated in tumors compared with normal tissues, and low levels of miR-34a expression correlated with a high probability of relapse.[31] These data suggest that miR-34a functions as a potential tumor suppressor. Our results show that miR-34a is a favorable prognostic marker in patients without gemcitabine treatment after resection. We also found no correlation between miR-34a expression and survival time in patients treated with gemcitabine, possibly suggesting that pancreatic cancers with low levels of miR-34a are more sensitive to gemcitabine treatment than those with high levels of miR-34a, although larger studies are needed to confirm this.

In conclusion, miR-142-5p expression is correlated with survival time in patients treated with gemcitabine after surgical resection of pancreatic cancer, but not in patients without gemcitabine treatment. miR-142-5p would be a promising predictive marker for gemcitabine treatment in patients with resected pancreatic cancer, although further examination are needed to analyze the functional role of these microRNAs. In the present study, we used FFPE samples to measure the indicated miRNAs. Measurement of such miRNAs may be possible using plasma, serum, and pancreatic fluids, suggesting that these miRNAs may be useful in predicting the effects of chemotherapy for unresectable pancreatic cancer and of neoadjuvant chemotherapy for resectable pancreatic cancer, where it is difficult to obtain tissues samples without the use of invasive procedures.

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CONFLICT OF INTEREST None.

REFERENCES

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. *CA Cancer J Clin.* 2007;57:43–66.
2. Hirata K, Egawa S, Kimura Y, et al. Current status of surgery for pancreatic cancer. *Dig Surg.* 2007;24:137–47.
3. Hawes RH, Xiong Q, Waxman I, Chang KJ, Evans DB, Abbruzzese JL. A multispecialty approach to the diagnosis and management of pancreatic cancer. *Am J Gastroenterol.* 2000; 95:17–31.
4. Matsuno S, Egawa S, Fukuyama S, et al. Pancreatic Cancer Registry in Japan: 20 years of experience. *Pancreas.* 2004;28: 219–30.
5. Neoptolemos JP, Stocken DD, Friess H, et al. A randomized trial of chemoradiotherapy and chemotherapy after resection of pancreatic cancer. *N Engl J Med.* 2004;350:1200–10.
6. Oettle H, Post S, Neuhaus P, et al. Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. *JAMA.* 2007;297:267–77.
7. Heinemann V, Hertel LW, Grindey GB, Plunkett W. Comparison of the cellular pharmacokinetics and toxicity of 2′,2′-difluorodeoxycytidine and 1-beta-D-arabinofuranosylcytosine. *Cancer Res.* 1988;48:4024–31.
8. Burris HA III, Moore MJ, Andersen J, 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.
9. Moore MJ, Goldstein D, Hamm J, et al. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol.* 2007;25:1960–6.
10. Van Cutsem E, Vervenne WL, Bennouna J, et al. Phase III trial of bevacizumab in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer. *J Clin Oncol.* 2009;27: 2231–7.
11. Farrell JJ, Elsaleh H, Garcia M, et al. Human equilibrative nucleoside transporter 1 levels predict response to gemcitabine in patients with pancreatic cancer. *Gastroenterology.* 2009;136: 187–95.
12. Nakano Y, Tanno S, Koizumi K, et al. Gemcitabine chemoresistance and molecular markers associated with gemcitabine transport and metabolism in human pancreatic cancer cells. *Br J Cancer.* 2007;96:457–63.
13. Wolff AC, Hammond ME, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J Clin Oncol.* 2007;25:118–45.
14. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. *Science.* 2001;294:853–8.
15. Lau NC, Lim LP, Weinstein EG, Bartel DP. An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans. Science.* 2001;294:858–62.
16. Li J, Smyth P, Flavin R, et al. Comparison of miRNA expression patterns using total RNA extracted from matched samples of formalin-fixed paraffin-embedded (FFPE) cells and snap frozen cells. *BMC Biotechnol.* 2007;7:36.
17. Xi Y, Nakajima G, Gavin E, et al. Systematic analysis of microRNA expression of RNA extracted from fresh frozen and formalin-fixed paraffin-embedded samples. *RNA.* 2007;13: 1668–74.
18. Lebanony D, Benjamin H, Gilad S, et al. Diagnostic assay based on hsa-miR-205 expression distinguishes squamous from nonsquamous non-small-cell lung carcinoma. *J Clin Oncol.* 2009;27: 2030–7.
19. Bloomston M, Frankel WL, Petrocca F, et al. MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. *JAMA.* 2007;297: 1901–8.
20. Xie Y, Todd NW, Liu Z, et al. Altered miRNA expression in sputum for diagnosis of non-small cell lung cancer. *Lung Cancer.* 2010;67:170–6.
21. Greither T, Grochola L, Udelnow A, Lautenschlager C, Wurl P, Taubert H. Elevated expression of microRNAs 155, 203, 210 and 222 in pancreatic tumours associates with poorer survival. *Int J Cancer.* 2010;126(1):73–80.
22. Dillhoff M, Liu J, Frankel W, Croce C, Bloomston M. MicroRNA-21 is overexpressed in pancreatic cancer and a potential predictor of survival. *J Gastrointest Surg.* 2008;12:2171–6.
23. Ohuchida K, Mizumoto K, Murakami M, et al. Radiation to stromal fibroblasts increases invasiveness of pancreatic cancer cells through tumor-stromal interactions. *Cancer Res.* 2004;64: 3215–22.
24. Hamilton R, Gray C, Belanger SA, et al. Cardiac risk assessment before the use of stimulant medications in children and youth: a joint position statement by the Canadian Paediatric Society, the Canadian Cardiovascular Society and the Canadian Academy of Child and Adolescent Psychiatry. *J Can Acad Child Adolesc Psychiatry.* 2009;18:349–55.
25. Sobin C, Gutierrez M, Alterio H. Polymorphisms of delta-aminolevulinic acid dehydratase (ALAD) and peptide transporter 2 (PEPT2) genes in children with low-level lead exposure. *Neurotoxicology.* 2009;30:881–7.
26. Hoffmann AC, Mori R, Vallbohmer D, et al. High expression of HIF1a is a predictor of clinical outcome in patients with pancreatic ductal adenocarcinomas and correlated to PDGFA, VEGF, and bFGF. *Neoplasia.* 2008;10:674–9.
27. Liu X, Sempere LF, Galimberti F, et al. Uncovering growth-suppressive microRNAs in lung cancer. *Clin Cancer Res.* 2009; 15:1177–83.
28. Chen L, Yan HX, Yang W, et al. The role of microRNA expression pattern in human intrahepatic cholangiocarcinoma. *J Hepatol.* 2009;50:358–69.
29. Welch C, Chen Y, Stallings RL. MicroRNA-34a functions as a potential tumor suppressor by inducing apoptosis in neuroblastoma cells. *Oncogene.* 2007;26:5017–22.
30. Tazawa H, Tsuchiya N, Izumiya M, Nakagama H. Tumor-suppressive miR-34a induces senescence-like growth arrest through modulation of the E2F pathway in human colon cancer cells. *Proc Natl Acad Sci USA.* 2007;104:15472–7.
31. Gallardo E, Navarro A, Vinolas N, et al. miR-34a as a prognostic marker of relapse in surgically resected non-small-cell lung cancer. *Carcinogenesis.* 2009;30:1903–9.

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Follow-up study after resection of intraductal papillary mucinous neoplasm of the pancreas; special references to the multifocal lesions and development of ductal carcinoma in the remnant pancreas

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KEYWORDS:
IPMN;
Multifocal;
Pancreatic ductal adenocarcinoma

Abstract

BACKGROUND: Frequency and characteristics of metachronous occurrence of multifocal intraductal papillary mucinous neoplasms (IPMNs) or distinct pancreatic ductal adenocarcinomas (PDACs) in the remnant pancreas during follow-up evaluation after pancreatectomy for IPMNs have not been well known. The aim of this study was to investigate the outcomes after resection of IPMNs, especially focusing on the metachronous occurrence of multifocal IPMNs and distinct PDACs.

METHODS: Medical records of 172 patients who underwent resection of IPMNs were reviewed retrospectively, and the data regarding the occurrence of metachronous IPMNs or PDACs in the remnant pancreas during a mean postoperative follow-up period of 64 months were collected.

RESULTS: The incidence including synchronous and metachronous multifocal occurrence of IPMNs was 20% (34 of 172), and that of distinct PDACs was 9.9% (17 of 172). Ten metachronous IPMNs developed in the remnant pancreas after a mean time of 23 postoperative months (range, 12–84 mo), and 2 with main duct IPMNs (both were carcinoma in situ) required remnant pancreatectomy. Six distinct PDACs developed in the remnant pancreas after a mean time of 84 postoperative months (range, 12–150 mo). Four of them were found to have a tumor with a size of less than 2 cm, whereas the remaining 2 PDACs were found to be unresectable more than 10 years after resection of IPMNs.

CONCLUSIONS: Intense long-term follow-up evaluation is necessary for the early detection of metachronous occurrence of distinct PDACs as well as malignant IPMNs after resection of IPMNs.

Recent intense investigations regarding intraductal papillary mucinous neoplasms (IPMNs) of the pancreas have provided us with much information about their unique clinical and biological characteristics.[1–16] One of them is that IPMNs have a wide spectrum from adenoma to invasive

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carcinoma, and therefore it is important to determine the malignant potential of IPMNs preoperatively. We recently were able to treat most patients with IPMNs appropriately according to international consensus guidelines,[1] which suggest several predictive factors for malignant IPMNs, and, as a result, outcomes after the treatment of IPMNs seem to be satisfactory.[2–5]

There are 2 types of recurrence after resection of IPMNs: metastatic or local recurrence of invasive carcinoma and metachronous multifocal occurrence of IPMNs in the remnant pancreas. In addition, recent reports have shown that distinct pancreatic ductal adenocarcinomas (PDACs) occur synchronously or metachronously during management of IPMNs, with a frequency of approximately 10%, and IPMNs thus have been recognized recently as one of the predictors for PDAC.[6–11] Several reports have shown the long-term outcome after resection of IPMNs; however, they have not distinguished such various types of recurrent diseases.[3–5] Therefore, the frequency and characteristics of metachronous occurrence of multifocal IPMNs or distinct PDACs in the remnant pancreas after pancreatectomy for IPMNs have been unclear, and we do not know how frequent and how long we should check the patients after resection of IPMNs. The aim of this study was to investigate the outcomes after resection of IPMNs, especially focusing on the metachronous occurrence of multifocal IPMNs and concomitant PDACs.

Patients and Methods

Medical records of 172 patients who underwent pancreatectomy for IPMN at the Department of Surgery and Oncology at Kyushu University Hospital between 1987 and 2010 were reviewed retrospectively. There were 105 men and 67 women, with a mean age of 66 years (range, 33–85 y). Surgical indication was determined based on the suggestions described in the international consensus guidelines[1]; all the cases of main-duct IPMNs, and branch-duct IPMNs with symptoms such as abdominal pain, jaundice, and pancreatitis, the presence of mural nodule, cyst size 30 mm or larger, and dilation of the main pancreatic duct throughout the study period. However, several patients with branch-duct IPMNs without any of the earlier-listed factors underwent pancreatectomy at the initial period of this study. IPMNs were classified into main-duct and branch-duct types based on the preoperative radiologic findings as described in our previous reports.[15,16] The lesions having no predictive factors usually were left in the remnant pancreas in the patient with multifocal IPMNs. Pathologic results were determined by the World Health Organization criteria published in 2010[17]: low-grade, intermediate-grade, and high-grade dysplasia (carcinoma in situ), and invasive carcinoma. The highest degree of pathologic grade was determined in multifocal lesions. Staging of PDAC was defined according to the Japanese General Rules for pancreatic cancer.[18] In all cases, resection margins of the pancreas were checked histologically by frozen sections during surgery as well as permanent sections postoperatively. Pancreatectomy would be completed for negative margin status of main pancreatic duct in frozen section, whereas additional resection would be considered for the presence of tumor cells at the cut margin of the main pancreatic duct.

Follow-up evaluation by physical examination and radiologic studies was performed according to the pathologic results. Routine radiologic examination included computed tomography (CT) and/or magnetic resonance imaging (MRI)/magnetic resonance cholangiopancreatography (MRCP). If there were positive findings in these modalities such as the presence of a new lesion, dilation of the main pancreatic duct, or morphologic changes of residual IPMNs, endoscopic ultrasound sonography (EUS) and endoscopic retrograde pancreatography (ERP) with pancreatic juice cytology were added. EUS-guided fine-needle aspiration has not been performed to date at our institution because of the apprehension of seeding of the tumor cells when malignant. Surveillance was performed every 6 months for noninvasive IPMNs (low- to high-grade dysplasia) using CT and MRI/MRCP during the initial 2 years after surgery, and every 12 months after that. Recently, surveillance for noninvasive IPMNs has been changed to alternating CT and MRI/MRCP every 6 months. For invasive IPMNs and IPMNs with distinct PDACs, surveillance was performed every 3 months using CT during the initial 2 years to focus on the detection of recurrent metastatic/local diseases, and then every 6 months using CT and MRI/MRCP after that for the detection of metachronous IPMNs/PDACs as well as recurrent diseases. Our recent surveillance flow chart is shown in Fig. 1. Recurrent IPMNs were defined as metastatic lesions in the liver, lung, or distant lymph node; dissemination; or local recurrence owing to microscopic residual cancer cells. Metachronous occurrence of IPMNs or PDACs was defined as new occurrence of the tumor in the remnant pancreas after complete resection of the tumor. In such cases, surgical margin of the

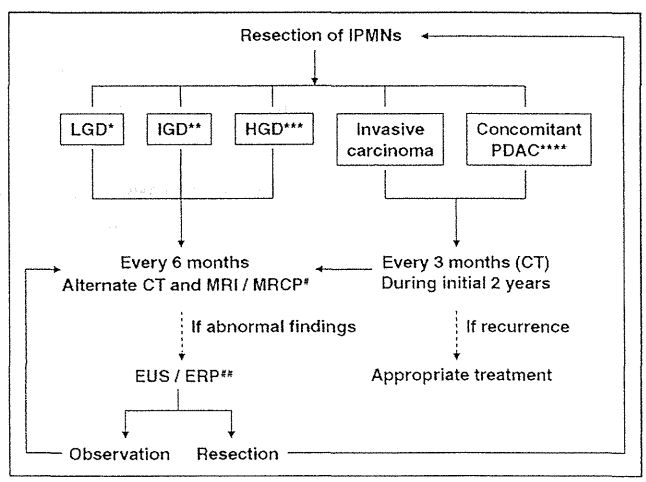

Figure 1 Flow diagram of surveillance after resection of IPMNs of the pancreas according to pathologic grade. HGD, high-grade dysplasia; IGD, intermediate-grade dysplasia; LGD, low-grade dysplasia.

Table 1 Characteristics of 172 patients with intraductal papillary mucinous neoplasms (IPMNs) of the pancreas

	LGD (n = 77)	IMGD (n = 33)	HGD (n = 26)	Invasive carcinoma (n = 36)	Total (n = 172)
At the initial assessment and operation					
Location (pancreas head/pancreas body to tail)	35/42	8/25	16/10	25/11	84/88
Type (Main duct/Branch duct)	8/69	7/26	13/13	16/20	44/128
Synchronous multifocal IPMNs*	13 (10)	4 (0)	2 (1)	5 (5)	24 (16)
Synchronous distinct PDACs	9	2	0	3	14
During follow-up period					
Recurrent IPMNs	0	0	0	16	16
Recurrent PDAC	6	1	0	2	9
Metachronous IPMNs†	1	5 (1)	3 (1)	1	10 (2)
Metachronous distinct PDACs††	4 (3)	1	0	1	6 (3)
Comprehensive assessments					
Multifocal IPMNs	14	9	5	6	34
Distinct PDACs††	13 (3)	3	0	4	17 (3)
Disease-specific survival rate (%) (5-/10-year)	94/91	96/89	100/100	31/24	83/78

LGD = low grade dysplasia; IMGD = intermediate grade dysplasia; HGD = high grade dysplasia; PDAC = pancreatic ductal adenocarcinoma.
*The number in parenthesis indicates the number of patients having residual IPMNs in the remnant pancreas after operation.
†The number in parenthesis indicates the number of patients who underwent remnant total pancreatectomy for metachronous IPMNs.
††The number in parenthesis indicates the number of patients having both synchronous and metachronous PDACs, and thus, a total of 17 patients had distinct PDACs synchronously and/or metachronously.

pancreas at the initial surgery was free from tumor cells, and the new lesion usually developed apart from the surgical margin.

Death related to invasive IPMNs or concomitant PDACs was considered disease specific, and a disease-specific survival rate was calculated using the Kaplan–Meier method.

Results

Table 1 shows the characteristics of the study population. There were 44 main-duct and 128 branch-duct types. Twenty patients (12%) had a history of pancreatitis preoperatively. There were 77 patients with low-grade dysplasia, 33 patients with intermediate-grade dysplasia, 26 patients with high-grade dysplasia, and 36 patients with invasive carcinoma. Eight branch-duct IPMNs did not have any malignant predictors described in consensus guidelines,[1] all of which were low-grade dysplasia, whereas the other 164 had at least one of the predictors. Seventy-seven patients had pathologically mural nodules, and 45 of them were malignant (58%; 20 noninvasive and 25 invasive carcinomas). The mean follow-up period of this study population was 64 months (range, 1–283 mo), and 5-year/10-year disease-specific survival rates of low-grade dysplasia, intermediate-grade dysplasia, high-grade dysplasia, invasive carcinoma, were 94%/91%, 96%/89%, 100%/100%, and 31%/24%, respectively (Table 1).

There were 24 multiple IPMNs at the time of initial diagnosis (Table 1). Complete resection of IPMNs including 5 cases of total pancreatectomy was achieved in 156 patients, whereas IPMNs were left in the remnant pancreas in the remaining 16 patients (Table 1). In the latter group, 14 lesions were branch-duct IPMNs without any predictive factor for malignancy described in the consensus guidelines,[1] and the remaining 2 were low-grade dysplasia left at the cut margin of the main pancreatic duct. IPMNs and synchronous concomitant PDACs were resected in 14 patients. In these 14 patients with PDACs, there was 1 patient in stage 0, 4 patients in stage I, 2 patients in stage II, 5 patients in stage III, and 2 patients in stage IVa.

There were 16 and 9 patients having local or distant metastatic recurrence after resection of invasive IPMNs and distinct PDACs, respectively, and there was no recurrence in low- to high-grade dysplasia of IPMNs (Table 1). Ten metachronous IPMNs occurred in the remnant pancreas after a mean of 23 postoperative months (range, 12–84 mo). They were 6 branch-duct type and 4 main-duct types. In all 10 patients, the pancreatic cut margins were negative for tumor cells at the initial surgery. All 6 branch-duct IPMNs were small size cysts without any sign of mural nodule or clinical symptom, and did not require resection. Two of the 4 main-duct IPMNs were resected by remnant total pancreatectomy, and their pathologic results were both noninvasive carcinomas. The other 2 metachronous main-duct IPMNs were observed without resection because of the absence of informed consent.

Of 16 patients with IPMN left in place in the remnant pancreas, 2 patients had recurrence of invasive IPMNs. The other 14 lesions showed no morphologic change during the mean follow-up period of 47 months (range, 6–156 mo). No patients had a metachronous occurrence of new IPMNs in the remnant pancreas in this group. The prevalence of multifocal IPMNs including both synchronous and metachronous in this study population was 20% (34 of 172) (Table 1).

All 14 IPMNs with concomitant PDACs were branch-duct type. In these patients, 3 metachronous PDACs developed after resection of low-grade dysplasia of IPMNs and concomitant PDACs (Table 1). In addition, 3 PDACs metachronously occurred after resection of IPMNs. Therefore, occurrence of PDACs after resection of IPMNs was observed in 6 patients after a mean time of 84 postoperative months (range, 12–150 mo), and thus the prevalence of distinct PDACs in the resected IPMNs was 9.9% (17 of 172) (Table 1). Three patients with metachronous PDACs had branch-duct IPMNs as well, and thus all 17 patients who had distinct PDACs had branch-duct IPMNs. By contrast, metachronous PDACs developed irrespective of the pathologic grade and tumor location (head or body to tail) of IPMNs and there seemed to be no predisposing time point after the initial surgery. Three of 6 metachronous PDACs were found by CT with a tumor size of less than 2 cm, whereas the remaining 2 PDACs were found as unresectable cancers with liver metastases after more than 10 years after resection of IPMNs. The intervals between a prior normal imaging study and detection of PDACs in 2 patients with unresectable PDACs were 8 and 13 months. The remaining 1 PDAC (noninvasive) was diagnosed by pancreatic juice cytology obtained by ERP, which was detected by neither CT nor MRI/MRCP.

Fifty-three extrapancreatic malignancies were found in 47 patients (27%) before (n = 28), at the time of (n = 18), and after initial surgery (n = 7). There were 16 colorectal, 11 gastric, 6 prostate, 4 gynecologic, 3 lung, 3 bladder, 2 breast, 2 renal, and 6 other malignancies.

Comments

The present study focusing on the metachronous occurrence of IPMNs or distinct PDACs in the remnant pancreas after resection of IPMNs has shown that strict surveillance would provide a chance to detect noninvasive IPMNs and early stage PDACs. Those metachronous lesions developed even long term after initial surgery irrespective of pathologic grade, and thus all the patients should be strictly followed up for a long time after resection of IPMNs. By contrast, there still have been unresolved issues regarding diagnostic modalities and intervals of surveillance.

The incidence of multifocal occurrence of IPMNs in this study is consistent with that in previous reports, ranging from 14.6% to 36%.[3–5] In patients with synchronous multifocal IPMNs, the international consensus guidelines[1] recommend that only those having predictive factors for malignancy should be resected, and those without predictive factors might be left in the remnant pancreas. We basically have followed the recommendation of the guidelines,[1] and we have not experienced any cases showing morphologic changes in IPMNs left in the remnant pancreas during the follow-up period. We also have experienced metachronous occurrence of IPMNs after compete resection of initial IPMNs, and all of them could be managed surgically or nonsurgically. Surgical management for multifocal IPMNs described in the guidelines[1] seem to be appropriate; however, long-term strict follow-up evaluation is necessary to detect metachronous occurrence of IPMNs.

Several recent reports[9–11] have investigated the development of distinct PDACs during follow-up evaluation of branch-duct IPMNs, which are not an indication for surgery, and reported an incidence ranging from 4.5% to 8%. This report focuses on the metachronous occurrence of PDACs in the remnant pancreas after resection of IPMNs. Uehara et al[7] found 5 PDACs during follow-up evaluation of 60 cases of branch-duct IPMNs, and 4 of them were resectable. Thus, branch-duct IPMNs would be a good predictor for early detection of PDACs during observations of small branch-duct IPMNs or after resection of branch-duct IPMNs with malignant predictors.

By contrast, some metachronous PDACs were found as unresectable cancers more than 10 years after the initial surgery for IPMNs. A noteworthy fact is that annual examination was not sufficient to detect all the cases of resectable PDACs. This finding suggests the possibility of the rapid growth of distinct PDACs. Therefore, we are now checking all the patients on follow-up evaluation at least every 6 months by imaging even long term after resection of IPMNs, as shown in Fig. 1. However, prospective surveillance is necessary to confirm whether this algorism would be adequate or not.

One of the metachronous PDACs was found as carcinoma in situ by pancreatic juice cytology obtained during ERP, although CT and MRI showed no morphologic abnormality. ERP is not usually included in the routine follow-up modalities in our department; however, the present case shows the use of pancreatic juice cytology for the detection of early pancreatic cancer during management of IPMNs. EUS fine-needle aspiration cytology recently has taken the place of pancreatic juice cytology by ERP in terms of definitive pathologic diagnosis of pancreatic cancer.[19] By contrast, we recently reported a patient with multiple IPMNs with concomitant noninvasive and invasive PDACs, which were detected by pancreatic juice cytology during preoperative ERP.[20] In this patient, multiple PDACs could not be detected by either CT/MRI or EUS, and we consider that pancreatic juice cytology during ERP has an important role to detect early pancreatic cancers, which cannot be detected by regular imaging modalities. Further investigation is necessary to determine whether ERP should be included in routine follow-up diagnostic modalities, and how frequently ERP should be performed during follow-up evaluation of the remnant pancreas after resection of IPMNs.

Another important issue during management of IPMNs is the occurrence of extrapancreatic malignancies. The prevalence of extrapancreatic malignancies in this study is consistent with those in previous reports, ranging from 24% to 32%,[1,21] and various organs seem to be involved. Therefore, careful attention should be paid to extrapancreatic diseases

as well as metachronous diseases in the remnant pancreas during surveillance after resection of IPMNs.

Recent advances in molecular biology have provided many insights into biological behaviors of IPMNs as well as PDACs,[22–26] however, molecular analyses regarding genetic changes in multiple occurrences of IPMNs and PDACs in the same pancreas have not been used to date. Others recently have shown that patients with IPMNs often have extrapancreatic tumors[1,21] and therefore patients with IPMNs might have some systemic genetic abnormalities causing carcinogenesis. Those types of investigations would provide some hints for the early detection of PDACs during management of IPMNs.

In conclusion, intense long-term, follow-up evaluation is necessary for the early detection of metachronous occurrence of IPMNs as well as distinct PDACs after resection of IPMNs.

References

1. Tanaka M, Chari S, Adsay V, et al. International Association of Pancreatology. International consensus guidelines for management of intraductal papillary mucinous neoplasms and mucinous cystic neoplasms of the pancreas. Pancreatology 2006;6:17–32.
2. Tanaka M. Controversies in the management of pancreatic IPMN. Nat Rev Gastroenterol Hepatol 2011;8:56–60.
3. Salvia R, Fernández-del Castillo C, Bassi C, et al. Main-duct intraductal papillary mucinous neoplasms of the pancreas: clinical predictors of malignancy and long-term survival following resection. Ann Surg 2004;239:678–87.
4. Rodriguez JR, Salvia R, Crippa S, et al. Branch-duct intraductal papillary mucinous neoplasms: observations in 145 patients who underwent resection. Gastroenterology 2007;133:72–9.
5. Partelli S, Fernandez-del Castillo C, Bassi C, et al. Invasive intraductal papillary mucinous carcinomas of the pancreas: predictors of survival and the role of lymph node ratio. Ann Surg 2010;251:477–82.
6. Yamaguchi K, Ohuchida J, Ohtsuka T, et al. Intraductal papillary-mucinous tumor of the pancreas concomitant with ductal carcinoma of the pancreas. Pancreatology 2002;2:484–90.
7. Uehara H, Nakaizumi A, Ishikawa O, et al. Development of ductal carcinoma of the pancreas during follow-up of branch duct intraductal papillary mucinous neoplasms of the pancreas. Gut 2008;57:1561–5.
8. Ingkakul T, Sadakari Y, Ienaga J, et al. Predictors of the presence of concomitant invasive ductal carcinoma in intraductal papillary mucinous neoplasm of the pancreas. Ann Surg 2010;251:70–5.
9. Tanno S, Nakano Y, Koizumi K, et al. Pancreatic ductal adenocarcinomas in long-term follow-up patients with branch duct intraductal papillary mucinous neoplasms. Pancreas 2010;39:36–40.
10. Tanno S, Nakano Y, Sugiyama Y, et al. Incidence of synchronous and metachronous pancreatic carcinoma in 168 patients with branch duct intraductal papillary mucinous neoplasms. Pancreatology 2010;10:173–8.
11. Sawai Y, Yamao K, Bhatia V, et al. Development of pancreatic cancers during long-term follow-up of side-branch intraductal papillary mucinous neoplasms. Endoscopy 2010;42:1077–84.
12. Sugiyama M, Atomi Y. Intraductal papillary mucinous tumors of the pancreas: imaging studies and treatment strategies. Ann Surg 1998;228:685–91.
13. Schmidt CM, White PB, Waters JA, et al. Intraductal papillary mucinous neoplasms: predictors of malignant and invasive pathology. Ann Surg 2007;246:644–54.
14. Furukawa T, Klöppel G, Volkan Adsay N, et al. Classification of types of intraductal papillary-mucinous neoplasm of the pancreas: a consensus study. Virchows Arch 2005;447:794–9.
15. Sadakari Y, Ienaga J, Kobayashi K, et al. Cyst size indicates malignant transformation in branch duct intraductal papillary mucinous neoplasm of the pancreas without mural nodules. Pancreas 2010;39:232–6.
16. Sadakari Y, Ohuchida K, Nakata K, et al. Invasive carcinoma derived from the nonintestinal type intraductal papillary mucinous neoplasm of the pancreas has a poorer prognosis than that derived from the intestinal type. Surgery 2010;147:812–7.
17. Adsay NV, Kloppel G, Fukushima N, et al. Intraductal papillary-mucinous neoplasms of the pancreas. In: Bosman FT, Carneiro F, Hruban RH, et al, eds. World Health Organization Classification of Tumors, Pathology and Genetics of Tumors of the Digestive System. Lyon, France: IARC Press; 2010:304–13.
18. Japan Pancreas Society. Classification of Pancreatic Carcinoma. 2nd ed. Revised in English. Tokyo: Kanehara; 2002.
19. Wakatsuki T, Irisawa A, Bhutani MS, et al. Comparative study of diagnostic value of cytologic sampling by endoscopic ultrasonography-guided fine-needle aspiration and that by endoscopic retrograde pancreatography for the management of pancreatic mass without biliary stricture. J Gastroenterol Hepatol 2005;20:1707–11.
20. Mori Y, Ohtsuka T, Tsutsumi K, et al. Multifocal pancreatic ductal adenocarcinomas concomitant with intraductal papillary mucinous neoplasms of the pancreas, detected by intraoperative pancreatic juice cytology. A case report. JOP 2010;11:389–92.
21. Reid-Lombardo KM, Mathis KL, Wood CM, et al. Frequency of extrapancreatic neoplasms in intraductal papillary mucinous neoplasm of the pancreas: implications for management. Ann Surg 2010;251:64–9.
22. Sato N, Goggins M. Epigenetic alterations in intraductal papillary mucinous neoplasms of the pancreas. J Hepatobiliary Pancreat Surg 2006;13:280–5.
23. Ohuchida K, Mizumoto K, Yu J, et al. S100A6 is increased in a stepwise manner during pancreatic carcinogenesis: clinical value of expression analysis in 98 pancreatic juice samples. Cancer Epidemiol Biomarkers Prev 2007;16:649–4.
24. Miyasaka Y, Nagai E, Yamaguchi H, et al. The role of the DNA damage checkpoint pathway in intraductal papillary mucinous neoplasms of the pancreas. Clin Cancer Res 2007;13:4371–7.
25. Fritz S, Fernandez-del Castillo C, Mino-Kenudson M, et al. Global genomic analysis of intraductal papillary mucinous neoplasms of the pancreas reveals significant molecular differences compared to ductal adenocarcinoma. Ann Surg 2009;249:440–7.
26. Nakata K, Nagai E, Ohuchida K, et al. REG4 is associated with carcinogenesis in the "intestinal" pathway of intraductal papillary mucinous neoplasms. Mod Pathol 2009;22:460–8.

Pancreatic Ductal Adenocarcinoma Derived From IPMN and Pancreatic Ductal Adenocarcinoma Concomitant With IPMN

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Objectives: Pancreatic ductal adenocarcinoma (PDAC) may derive from an intraductal papillary mucinous neoplasm (IPMN) of the pancreas or may develop in the pancreatic duct apart from IPMN. The purpose of this study was to define the clinicopathological features of these 2 entities and compare them with those of ordinary PDAC.

Methods: Of 765 patients who had surgical resection for IPMN, 122 were diagnosed as having PDAC derived from IPMN and 31 with PDAC concomitant with IPMN. In addition, 7605 patients with PDAC who were registered in the Japan Pancreas Society pancreatic cancer registry were compared with the above patients.

Results: Pancreatic ductal adenocarcinomas derived from IPMN and concomitant with IPMN were significantly smaller, less invasive, and less extensive than ordinary PDAC. The median survival of patients with the 2 conditions was significantly longer than for those with ordinary PDAC when compared overall or when limited to TS2 (2.0 cm < tumor size ≤ 4.0 cm) or TS3 (4.0 cm < tumor size ≤ 6.0 cm) cases.

Conclusions: These findings suggest that PDAC concomitant with IPMN and PDAC derived from IPMN may have more favorable biological behaviors or be diagnosed earlier than ordinary PDAC.

Key Words: IPMN, PDAC concomitant with IPMN, PDAC derived from IPMN

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Intraductal papillary mucinous neoplasm (IPMN) is characterized by papillary proliferation of atypical mucinous epithelium in the pancreatic ductal system, and the affected pancreatic ducts are often cystically dilated.[1,2] Intraductal papillary mucinous neoplasm is a spectrum of diseases ranging from adenoma, to in situ carcinoma, to invasive carcinoma (minimally invasive carcinoma and invasive carcinoma derived from IPMN).[3] On the other hand, pancreatic ductal adenocarcinoma (PDAC) develops independently of IPMN in the pancreatic duct.[4,5] When PDAC originates in the vicinity of IPMN, the distinction between PDAC derived from IPMN and PDAC concomitant with IPMN is sometimes difficult to make. In this collective series, we developed a definition of the 2 conditions and analyzed the incidence of the conditions in patients with IPMN. In addition, we compared the clinicopathological features between (1) ordinary PDAC and PDAC derived from IPMN and (2) ordinary PDAC and PDAC concomitant with IPMN.

MATERIALS AND METHODS

The Japan Pancreas Society (JPS) formed a committee to solve the clinical and pathological problems associated with PDAC derived from IPMN and PDAC concomitant with IPMN. The committee (Drs H. Maguchi, K. Hanada, Y. Noda, M. Tada, and A. Nakaizumi as internists; Drs K. Yamaguchi, T. Hatori, Y. Shimizu, and T. Nakagori as surgeons; and Drs Y. Kato, T. Furukawa, B. Nobukawa, and S. Ban as pathologists) discussed the definition of PDAC derived from IPMN and PDAC concomitant with IPMN and proposed a new definition of 3 categories (PDAC derived from IPMN, PDAC concomitant with IPMN, and PDAC of undetermined relationship with IPMN) based on the topological relationship of the 2 conditions and the presence or absence of a histological transition (Fig. 1) between the conditions as follows:

PDAC Derived From IPMN

Pancreatic ductal adenocarcinoma is evidently derived from IPMN, based on the findings of radiologic images and macroscopic or microscopic findings, and a histological transition is present between IPMN and PDAC.

PDAC Concomitant With IPMN

Intraductal papillary mucinous neoplasm is obviously different from PDAC, according to the radiologic images and macroscopic or microscopic findings.