J Gastroenterol 2007; 42:389–394
DOI 10.1007/s00535-007-2017-0

Journal of
Gastroenterology

Ribonucleotide reductase subunit M2 mRNA expression in pretreatment biopsies obtained from unresectable pancreatic carcinomas

Takao Itoi[1], Atsushi Sofuni[1], Noriyoshi Fukushima[2], Fumihide Itokawa[1], Takayoshi Tsuchiya[1], Toshio Kurihara[1], Fuminori Moriyasu[1], Akihiko Tsuchida[3], and Kazuhiko Kasuya[3]

[1]Fourth Department of Internal Medicine, Tokyo Medical University, 6-7-1 Nishishinjuku, Shinjuku-ku, Tokyo 160-0023, Japan
[2]Department of Pathology and Diagnosis, Tokyo Medical University, Tokyo, Japan
[3]Third Department of Surgery, Tokyo Medical University, Tokyo, Japan

Background. Gemcitabine is an efficacious cytotoxic agent used in the treatment of unresectable pancreatic carcinoma (PC). Recently, gemcitabine resistance has been associated with the ribonucleotide reductase subunit M2 (*RRM2*). In this prospective study, we hypothesized that *RRM2* expression in PC biopsy specimens would be a significant predictor of outcome. ***Methods.*** *RRM2* mRNA expression in 35 endoscopic ultrasonography-guided fine needle aspiration biopsy (EUS-FNAB) samples was quantified using real-time quantitative reverse transcription-polymerase chain reaction. ***Results.*** Thirty-one of 35 biopsy specimens could be assessed for *RRM2* expression levels. The mean *RRM2* expression relative to glyceraldehyde-3-phosphate dehydrogenase was 0.248 (range, 0.00739 to 0.858). Eighteen patients (64.5%) had low *RRM2* levels, and 13 patients (35.5%) had high *RRM2* levels with a cutoff of 0.1. The median survival was 8.8 months for patients with low *RRM2* levels and 5.0 months for patients with high levels ($P < 0.05$). In the low *RRM2* expression group, a complete response (CR) was observed in one patient, and a partial response (PR) was observed in eight patients. In contrast, in the high *RRM2* expression group, PR was observed in one patient, and CR was not observed. The overall response rate between the high and low expression groups was significantly different (50.0% vs. 7.7%, $P < 0.05$). ***Conclusions.*** *RRM2* mRNA expression of EUS-FNAB specimens is a key predictive marker of survival in gemcitabine-treated patients with PC.

Key words: pancreatic cancer, *RRM2*, EUS-FNA, predictive marker

Received: August 1, 2006 / Accepted: January 25, 2007
Reprint requests to: T. Itoi

Introduction

Pancreatic carcinoma is the fifth leading cause of cancer death in Japan[1] and the fourth in the United States,[2] and is usually unresectable (80%–90%) at the time of diagnosis despite recent progress in imaging modalities. The prognosis of advanced and metastatic pancreatic carcinoma is dismal, and chemoresistance is a major cause of pancreatic carcinoma treatment failure. Gemcitabine, like S-1, is an efficacious cytotoxic agent[3] that currently has marketing approval in Japan for the treatment of pancreatic carcinoma. However, it is difficult to distinguish between patients who are sensitive or resistant to gemcitabine before chemotherapy.

Recently, the expression and activity of ribonucleotide reductase have been reported to be determinants of gemcitabine chemoresistance in human tumor cells.[4] Ribonucleotide reductase mediates the rate-limiting step in DNA synthesis because it is the only known enzyme that converts ribonucleotides to deoxynucleotides, which are required for DNA polymerization and repair. The ribonucleotide reductase holoenzyme consists of dimerized subunits M1 and 2 (*RRM1* and *RRM2*).[5] Ribonucleotide reductase enzymatic activity is modulated by the levels of *RRM2*.[6] Moreover, overexpression of *RRM2* is associated with resistance to gemcitabine in patients with pancreatic cancers.[7]

Endoscopic ultrasonography-guided fine-needle aspiration biopsy (EUS-FNAB) has been established as a safe and precise procedure for the diagnosis of pancreatic masses.[8,9] Several studies have reported the diagnosis by genetic analysis of samples obtained by EUS-FNAB in patients with pancreatic carcinoma,[10,11] but no reports of the use of this technique for the assessment of treatment options have been published. In this first prospective report, we describe the utility of analyzing the expression of *RRM2* mRNA obtained from pretreatment EUS-FNAB specimens of unresectable pancreatic carcinoma.

Materials and methods

Patients

Biopsy specimens from 35 patients with unresectable pancreatic carcinomas (23 patients with stage III and 12 patients with stage IV disease)[12] were obtained at the Fourth Department of Internal Medicine of Tokyo Medical University between December 2003 and October 2005. The eligibility criteria included histologically confirmed, locally advanced, or metastatic pancreatic carcinoma with no prior chemotherapy; clinically measurable or evaluable disease; Southwest Oncology Group scale performance status of 0–2; a life expectancy of greater than 12 weeks; and adequate bone marrow and hepatic and renal function. The EUS-FNAB procedure has been described in previous reports.[11] The first specimen was used for standard histological examination by hematoxylin-eosin staining (Fig. 1), and the second specimen was used for detecting *RRM2* mRNA. The final diagnosis of unresectable pancreatic carcinoma was based on ultrasonography, EUS, and computed tomography imaging studies. All EUS-FNAB procedures were performed by the same investigator (T.I.). This study was carried out in accordance with the institutional review board guidelines, and written informed consent was obtained from all of the patients. All patients were treated with gemcitabine (1000 mg/m2) administered intravenously over 30 min on days 1, 8, and 21 on a 28-day cycle.

Laboratory methods

Total RNA was isolated using the RNeasy Kit (Qiagen, Chatsworth, CA, USA), and DNase treatment was performed using the RNase-Free DNase set (Qiagen), according the manufacturer's instructions. cDNAs were generated using the Superscript II First Strand cDNA Synthesis kit (Invitrogen, Carlsbad, CA, USA). Quantification of *RRM2* cDNA and an internal reference gene (glyceraldehyde-3-phosphate dehydrogenase, GAPDH) was conducted using a fluorescence-based real-time polymerase chain reaction (PCR) method (Taq-Man PCR using an ABI PRISM 7700 Sequence Detection System, Applied Biosystems, Foster City, CA, USA). The following primers were used for real-time PCR. *RRM2*; forward primer, 5′-CTATGGT GAACGTGTTGTAGCCTT-3′; reverse primer, 5′-GTCCTCGTTTCTTGAGCCAGA-3′; TaqMan probe, 5′-FAM-CTGCAGTGGAAGGCATTTTCTTTT CCG-TAMRA-3′ GAPDH; Predeveloped TaqMan Assay Reagents human GAPDH (Applied Biosystems). In brief, cDNA was added into a reaction mixture containing 1 × TaqMan buffer A, 150 nM each primer, 100 nM TaqMan probe, 200 µM dNTP, 3 µM MgCl2, and 0.625 units of AmpliTaqGold, in a final volume of 25 µl. The PCR conditions were 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. Quantification was performed using the relative standard curve method. The standard curve was created automatically by the ABI PRISM 7700 by plotting the threshold cycle (Ct) against each input amount (containing 10^7, 10^6, 10^5, 10^4, 10^3, 10^2, and 10^1 copies) of standard plasmid DNA. The correlation coefficient determined by linear regression (r) for each standard curve was greater than 0.990. The relative amount of each unknown sample was calculated using linear regression analysis from the respective standard curve. A relative target gene expression value for GAPDH as an internal reference gene was used.

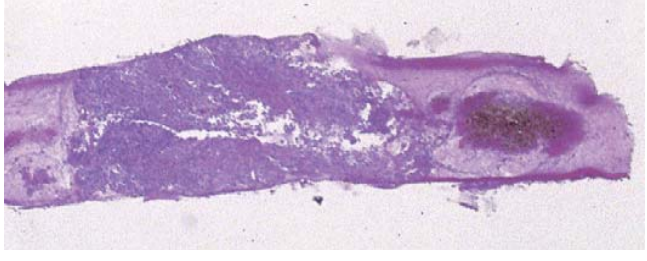

Fig. 1. Photomicrograph of representative biopsy specimen samples obtained by endoscopic ultrasonography-guided fine-needle aspiration biopsy using a 19-gauge needle (×20)

Efficacy assessment

Standard tumor response criteria were used to determine an objective tumor response. A complete response (CR) was defined as the disappearance of all measurable and evaluable disease for at least 4 weeks without the appearance of any new lesions. A partial response (PR) indicated a reduction of >50% in the sum of the products of the greatest perpendicular dimensions of all measurable lesions for at least 4 weeks without the appearance of any new lesions. Stable disease (SD) corresponded to a decrease of <50% in the sum of the product of the greatest perpendicular dimensions of measurable lesions or an increase of <25% in the sum of the products of the greatest perpendicular dimensions of measurable disease for a minimum of 3 months. Progressive disease (PD) was defined as an increase of >25% in the sum of the products of measurable lesions, the appearance of new lesions, or deterioration of any evaluable disease. Survival was measured from the time of initiation of therapy until death. Response duration was defined as the time from documentation of response to the first observation of progressive disease. Patients were treated until disease progression or the occurrence of unacceptable toxicity without second-line chemotherapy for gemcitabine.

Statistical analysis

Quantitative PCR analyses yield values that are expressed as ratios between two absolute measurements (gene of interest: internal reference gene). We used the maximal χ-squared method to determine a cutoff value to segregate patients into groups with low or high transcript levels. To determine the *P* value, we used bootstrap-like simulations to estimate the distribution of a maximal χ-squared statistic. The Kaplan-Meier test for survival and time to progression was used. The log-rank test was applied to compare survival and time to progression between subgroups. A value of $P < 0.05$ was considered to indicate statistical significance. All analyses were performed with the SPSS software package, version 10.0.5 (SPSS, Chicago, IL, USA).

Results

Patient characteristics

A total of 31 out of 35 pancreatic carcinomas could be assessed for *RRM2* expression levels. Two cases were not quantifiable because of fibrotic or necrotic tumor tissue, and two cases were omitted owing to the presence of renal cell carcinoma and malignant lymphoma. The clinical characteristics of the 31 patients are shown in Table 1 (20 patients with stage III and 11 patients with stage IV). Median patient age was 66 years; 16 patients were male; and 93.5% of patients had a performance status of 0 or 1. The pancreatic masses were located in the head of the pancreas in 15 patients and in the body/tail in 16 patients. The 12 patients with masses in the head of the pancreas had obstructive jaundice. The EUS-FNAB procedure was performed without any procedure-related complication.

Table 1. Clinical patient and gene characteristics[a]

No. of patients	31
Age, years (range)	66.0 (30–81)
Sex	
Male	16 (51.6%)
Female	15 (48.4%)
Performance status	
0–1	29 (93.5%)
2	2 (6.5%)
Icterus	12 (38.7%)
Location	
Head	15 (48.4%)
Body/Tail	16 (51.6%)
Histology	
Adenocarcinoma	30 (96.8%)
Adenosquomous carcinoma	1 (3.2%)
TNM cancer staging	
Stage III	20 (64.5%)
Stage IV	11 (35.5%)
RRM2[b] mRNA, mean (range)	0.248 (0.00739–0.858)
RRM2 mRNA	
≤ 0.1	18 (58.1%)
>0.1	13 (41.9%)
No. of chemotherapy cycles, median (range)	3.8 (2–11)

[a] Numbers in parentheses indicate percentages except when specified as a range

[b] *RRM2*, ribonucleotide reductase subunit M2

Patients received a median of 3.8 cycles (range, 2–11) of chemotherapy. The mean total dose of gemcitabine used was 16530 mg (range, 7800–52800 mg). CR was observed in one patient (3.2%), PR was observed in nine patients (29.3%), and SD in 11 patients (35.5%), whereas ten patients (32.3%) developed PD, resulting in an overall response rate of 32.3% [95% confidence interval (CI) 0.17–0.51]. Twenty-one of the 31 patients studied had died and ten were alive at the completion of the study. The overall survival time for all 31 patients was 7.8 months (95% CI, 4.3–10.2 months) (Fig. 2). The therapy was well tolerated; grade 3–4 neutropenia (no grade 3–4 infection), thrombocytopenia (no bleeding), nausea, asthenia, and alopecia were seen in 20%, 6.7%, 13.3%, 6.7%, and 6.7% of patients, respectively.

RRM2 *mRNA expression and clinical outcome*

The mean *RRM2* expression relative to the GAPDH internal reference gene was 0.248 (range, 0.00739 to 0.858) (Table 1) (Fig. 3A, B). The median *RRM2* expression was 0.101. Eighteen patients (58.1%) had low *RRM2* levels, and 13 patients (41.9%) had high *RRM2* levels, using a cutoff of 0.1. The median survival time was 8.8 months for the 18 patients with low *RRM2* levels and 5.0 months for the 13 patients with high levels ($P = 0.0104$) (Fig. 4). Thirteen stage III cases and five stage IV cases were included in the group showing low *RRM2* expression. In contrast, seven stage III cases and six stage IV cases were included in the group showing high *RRM2* expression. There was no statistical significance in the proportion of Stage III to Stage IV disease between low and high *RRM2* expression ($P = 0.499$). Response to chemotherapy between high and low *RRM2* expression is shown in Table 2. In the low *RRM2* expression group, CR was observed in one patient, PR was observed in eight patients, and SD in eight patients, whereas one patient developed PD. In contrast, in the high *RRM2* expression group, PR was observed in one, CR in none, and SD in three patients, and PD was observed in nine patients. The overall response rate between high and low expression groups was significantly different (50.0% vs. 7.7%, $P = 0.013$).

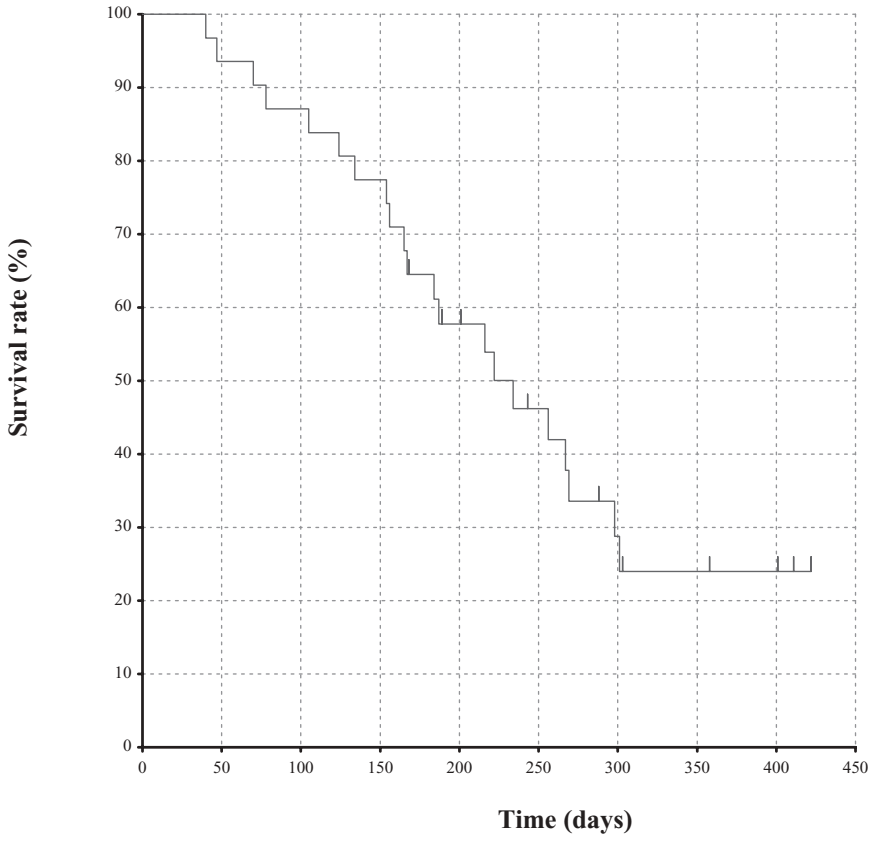

Fig. 2. Kaplan-Meier estimates of overall survival. Median survival for 31 patients was 7.8 months

Table 2. Response to gemcitabine between the high and low *RRM2* expression groups

	RRM2 > 0.1 (n = 13)	*RRM2* ≤ 0.1 (n = 18)
Complete response	0	1
Partial response	1	8
Stable disease	3	8
Progressive disease	9	1

RRM2, ribonucleotide reductase subunit 2

Discussion

In this prospective study, we investigated the utility of analyzing the expression of the *RRM2* gene to predict chemosensitivity to gemcitabine in patients with pancreatic carcinoma. This study showed that *RRM2* mRNA levels may affect survival. The median survival of the patients with low *RRM2* expression was significantly longer than those with high *RRM2* expression. Interestingly, CR could be achieved only in patients with low *RRM2* expression, and the overall response rate was significantly higher also in these patients than in the patients with high *RRM2* expression. These data suggest that it is worthwhile evaluating pretreatment *RRM2* expression in unresectable pancreatic carcinoma patients. Previous reports on gemcitabine monotherapy described survival times similar to the present data.[3,13–18] Although *RRM2* expression was not examined, these reports may contain cases of both high and low *RRM2* expression, leading to variations in chemosensitivity to gemcitabine.

In this study, we measured *RRM2* expression, although the ribonucleotide reductase holoenzyme consists of dimerized *RRM1* and *RRM2*. Duxbury et al.[7] reported that *RRM2* enhanced pancreatic adenocarcinoma chemoresistance to gemcitabine in vitro. We planned this prospective study on the basis of their data. There have not been any clinical studies on ribonucleotide reductase overexpression in conjunction with gemcitabine-based chemotherapy for pancreatic carcinoma. To the best of our knowledge, although the current study is preliminary, this is the first clinical trial of *RRM2* expression in patients with pancreatic carcinoma using EUS-FNAB pretreatment biopsy specimens. Although there is no report on *RRM1* expression in patients with pancreatic cancer, *RRM1* gene expression was described as a crucial predictive marker of survival in non-small cell lung cancer patients receiving gemcitabine-based chemotherapy.[19–21] In particular, *RRM1* levels influenced time to progression and survival in the

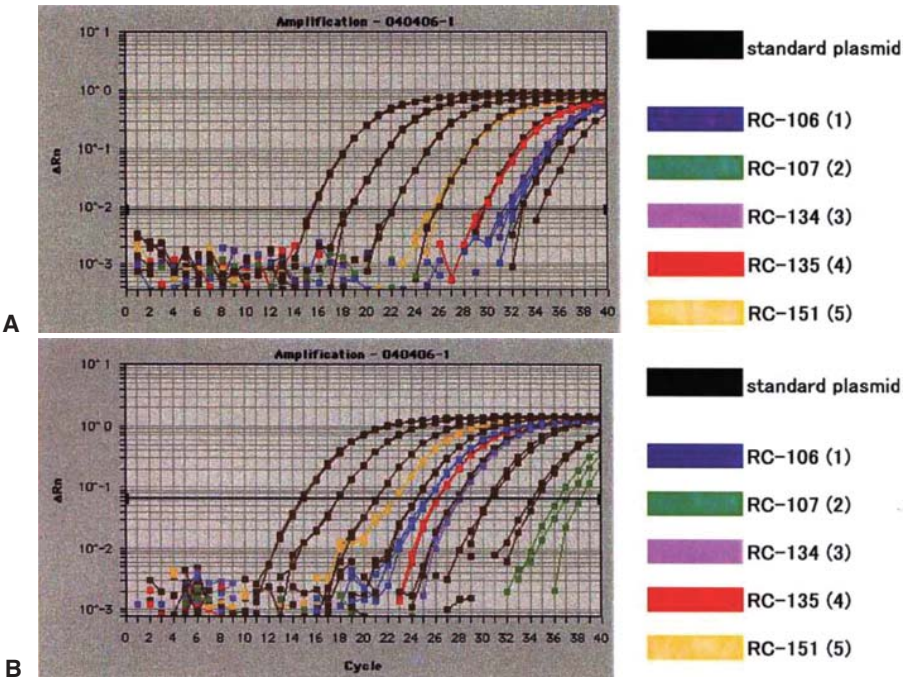

Fig. 3A,B. Ribonucleotide reductase subunit M2 (*RRM2*) mRNA quantification was performed using the relative standard curve method. The standard curve was created automatically by the ABI PRISM 7700 by plotting the threshold cycle against each input amount (containing 10^7, 10^6, 10^5, 10^4, 10^3, 10^2, and 10^1 copies) of standard plasmid DNA. A *RRM2* mRNA expression value (**A**) with glyceraldehyde-3-phosphate dehydrogenase (**B**) as an internal reference gene was used

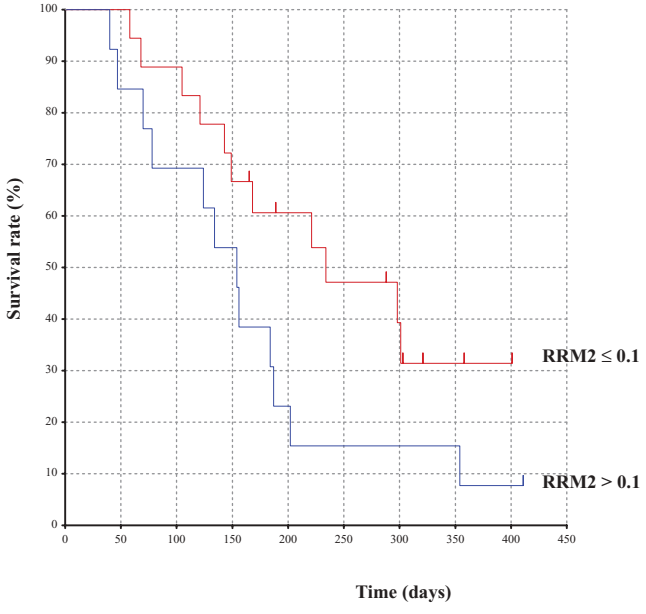

Fig. 4. Kaplan-Meier estimates of time to survival according to *RRM2* mRNA expression. The median survival was 8.8 months for the 18 patients with low *RRM2* levels (>0.1) and 5.0 months for the 13 patients with high levels (≤0.1)

gemcitabine/cisplatin arm (8.4 months vs. 2.7 months, $P = 0.009$; 13.7 months vs. 3.6 months, $P = 0.02$, respectively).[20] These data suggest that genetic testing of *RRM1* mRNA expression levels can and should be used to personalize chemotherapy in patients with non-small cell lung cancer. We should examine not only *RRM2* expression but also *RRM1* expression to clarify chemosensitivity to gemcitabine in the future.

To date, several gemcitabine-based combination chemotherapy regimens have undergone considerable testing for advanced pancreatic carcinoma[14–18] and it seems at present that gemcitabine-based combination chemotherapy may become the first-line treatment for pancreatic carcinoma. Thus, it is very important to predict chemosensitivity to gemcitabine prior to treatment.

Currently, with the progress of EUS-FNAB procedures, the necessary samples can be obtained easily, and the diagnostic accuracy for pancreatic solid masses is at least 85%.[8,9] One of the important aspects of this study is the genetic analysis of EUS-FNAB specimens in combination with routine diagnosis. Interestingly, in this study, two samples were omitted owing to the presence of renal cell carcinoma and malignant lymphoma. These data suggest that pretreatment biopsy specimens by EUS-FNAB in patients with pancreatic masses should be obtained, not only to test for gemcitabine chemoresistance but also to confirm a diagnosis of adenocarcinoma to ensure that the appropriate treatment is administered. In the present study, we examined only *RRM2* expression, but in the near future it may be necessary to examine the chemosensitivity of a range of drugs using the EUS-FNAB procedure.

In this study, there were some cases in which survival time did not correlate with *RRM2* expression. Gemcitabine chemosensitivity depends not only on *RRM2* expression but also on several other factors. Recently,

3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP, Triapine; Vion Pharmaceuticals, New Haven, CT, USA) was described as a new inhibitor of *RRM2*.[22] A Phase I trial of 3-AP in combination with gemcitabine for advanced cancers, including pancreatic carcinoma, is underway.[23,24] A preliminary study suggested that there was prolonged stabilization of disease or decreases in serum tumor markers associated with stable disease though there were no objective responses.

In conclusion, the results from this prospective study demonstrate that *RRM2* mRNA expression levels may correlate with gemcitabine sensitivity. In the near future, a large number of prospective randomized chemotherapy trials will be scheduled. These data suggest that *RRM2* is a novel, informative biomarker for predicting and monitoring the responses of pancreatic adenocarcinoma patients to gemcitabine.

Acknowledgments. This research was supported in part by a grant from the Japanese Gastroenterological Endoscopic Society. The authors also thank Hirotsugu Kawashima, Naoki Nagao, and Yuichi Nagakawa for their valuable help.

References

1. The Editorial Board of the Cancer Statistics in Japan. Cancer Statistics in Japan 2001. Tokyo: Foundation for Promotion of Cancer Research.
2. Greenlee RT, Hill-Harmon MB, Thun TM. Cancer statistics 2001. CA Cancer J Clin 2001;51:15–36.
3. Storniolo AM, Enas NH, Brown CA, Voi M, Rothenberg ML, Schilsky R. An investigational new drug treatment program for patients with gemcitabine: results for over 3000 patients with pancreatic carcinoma. Cancer 1999;85:1261–8.
4. Goan YG, Zhou B, Hu E, Mi S, Yen Y. Overexpression of ribonucleotide reductase as a mechanism of resistance to 2,2-difluorodeoxycytidine in the human KB cancer cell line. Cancer Res 1999;59:4204–7.
5. Nutter LM, Cheng Y. Nature and properties of mammalian ribonucleotide diphosphate reductase. In Cory JG, Cory AH, editors. Inhibitors of ribonucleotide diphosphate reductase activity. New York: Pergamon Press; 1989. p. 37–54.
6. Eriksson S, Martin DW Jr. Ribonucleotide reductase in cultured mouse lymphoma cells. Cell cycle-dependent variation in the activity of subunit protein M2. J Bio Chem 1981;256;9436–40.
7. Duxbury MS, Ito H, Zinner MJ, Ashley SW, Whang EE. RNA interference targeting the M2 subunit of ribonucleotide reductase enhances pancreatic adenocarcinoma chemosensitivity to gemcitabine. Oncogene 2003;8:1–10.
8. Wiersema MJ, Vilmann P, Giovannini M, Chang KJ, Wiersema LM. Endosonography-guided fine-needle aspiration biopsy: diagnostic accuracy and complication assessment. Gastroenterology 1997;112:1087–95.
9. Chang KJ, Nguyen PM, Erickson RA, Durban TE, Katz KD. The clinical utility of endoscopic ultrasound-guided fine-needle aspiration in the diagnosis and staging of pancreatic carcinoma. Gastrointest Endosc 1997;45:387–93.
10. Tada M, Komatsu Y, Kawabe T, Sasahira N, Isayama H, Toda N, et al. Quantitative analysis of *K-ras* gene mutation in pancreatic tissue obtained by endoscopic ultrasonography-guided fine needle aspiration: clinical utility for diagnosis of pancreatic tumor. Am J Gastroenterol 2002;97:2263–70.
11. Itoi T, Takei K, Sofuni A, Itokawa F, Tsuchiya T, Kurihara T, et al. Immunohistochemical analysis of p53 and MIB-1 in tissue specimens obtained from endoscopic ultrasonography-guided fine needle aspiration biopsy for the diagnosis of solid pancreatic masses. Oncol Rep 2005;13;229–34.
12. Exocrine pancreas. In: American Joint Committee on Cancer, editors. AJCC Cancer Staging Manual. 6th ed. New York: Springer; 2002. p. 157–64.
13. Burris HA 3rd, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, 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 1977;15:2403–13.
14. Hidalgo M, Castellano D, Paz-Ares L, Gravalos C, Diaz-Puente M, Hitt R, et al. Phase I–II study of gemcitabine and fluorouracil as a continuous infusion in patients with pancreatic cancer. J Clin Oncol 1999;17:585–92.
15. Scheithauer W, Kornek GV, Raderer M, Hejna M, Valencak J, Miholic J, et al. Phase II trial of gemcitabine, epirubicin and granulocyte colony-stimulating factor in patients with advanced pancreatic adenocarcinoma. Br J Cancer 1999;80:1797–802.
16. Heinemann V, Wilke H, Mergenthaler HG, Clemens M, Konig H, Illiger HJ, et al. Gemcitabine and cisplatin in the treatment of advanced or metastatic pancreatic cancer. Ann Oncol 2000;11:1399–403.
17. Rocha Lima CM, Green MR, Rotche R, Miller WH Jr, Jeffrey GM, Cisar LA, et al. Irinotecan plus gemcitabine results in no survival advantage compared with gemcitabine monotherapy in patients with locally advanced or metastatic pancreatic cancer despite increased tumor response rate. J Clin Oncol 2004;22:3776–83.
18. Van Cutsem E, van de Velde H, Karasek P, Oettle H, Vervenne WL, Szawlowski A, et al. Phase III trial of gemcitabine plus tipifarnib compared with gemcitabine plus placebo in advanced pancreatic cancer. J Clin Oncol 2004;22:1430–8.
19. Bepler G, Sharma S, Cantor A, Gautam A, Haura E, Simon G, et al. RRM1 and PTEN as prognostic parameters for overall and disease-free survival in patients with non-small-cell lung cancer. J Clin Oncol 2004;22:1878–85.
20. Rosell R, Danenberg KD, Alberola V, Bepler G, Sanchez JJ, Camps C, et al. Ribonucleotide reductase messenger RNA expression and survival in gemcitabine/cisplatin-treated advanced non-small cell lung cancer patients. Clin Cancer Res 2004;10:1318–25.
21. Rosell R, Scagliotti G, Danenberg KD, Lord RV, Bepler G, Novello S, et al. Transcripts in pretreatment biopsies from a three-arm randomized trial in metastatic non-small-cell lung cancer. Oncogene 2003;22:3548–53.
22. Finch RA, Liu M, Grill SP, Rose WC, Loomis R, Vasquez KM, et al. Triapine (3-aminopyridine-2-carboxaldehyde-thiosemicarbazone): a potent inhibitor of ribonucleotide reductase activity with broad spectrum antitumor activity. Biochem Pharmacol 2000;59:983–91.
23. Wadler S, Makower D, Clairmont C, Lambert P, Fehn K, Sznol M. Phase I and pharmacokinetic study of the ribonucleotide reductase inhibitor, 3-aminopyridine-2-carboxaldehyde thiosemicarbazone, administered by 96-hour intravenous continuous infusion. J Clin Oncol 2004;22:1553–63.
24. Yen Y, Margolin K, Doroshow J, Fishman M, Johnson B, Clairmont C, et al. A phase I trial of 3-aminopyridine-2-carboxaldehyde thiosemicarbazone in combination with gemcitabine for patients with advanced cancer. Cancer Chemother Pharmacol 2004;54:331–42.