Abstract. Excision repair cross-complementation 1 (ERCC1) has been reported to play a major role in the response to platinum-based therapies. It has recently been proposed that a synonymous polymorphism at codon 118 converting a common codon usage (AAC) to an infrequent one (AAT) may impair ERCC1 translation and to affect the response to cisplatin chemotherapy. We analyzed the association between this polymorphism and clinical outcome in 67 pancreatic cancer patients treated with cisplatin and S-1 or with S-1 alone. ERCC1 codon 118 polymorphism was analyzed using PCR-RFLP. Thirty-nine of the patients (58.2%) were homozygous for AAC codon, 7 (10.4%) were homozygous for AAT codon, and 21 (31.3%) were heterozygous. Among those treated with cisplatin and S-1, no significant difference in objective response rate was observed between genotypes. However, the patients with one or two AAT codons had a significantly longer progression-free survival (PFS) and overall survival (OS) than those homozygous for AAC allele (PFS: 338 days vs 106 days, p=0.006, OS: 763 days vs 415 days, p=0.030). In contrast, no significant difference in PFS or OS was observed between genotypes among the patients treated with S-1 alone. ERCC1 polymorphism may be a useful prognostic marker in patients with pancreatic cancer treated with platinum-based chemotherapy.

Introduction

Pancreatic cancer is the fourth leading cause of cancer death in United States (Cancer facts and findings 2005. American Cancer Society, Atlanta, 2005) and fifth in Japan (Ministry of Health, Labor and Welfare: Population Survey Report 2005. http://www.mhlw.go.jp/toukei/saikin/hw/jinkou/kakutei05/hyo7.html). Most patients receive diagnoses at an advanced stage, and the 5-year survival rate is <5% (1,2). It is well known that gemcitabine has been used as a standard therapy for pancreatic cancer (2,3) for a decade.

The oral drug regimen S-1 (TS-1 Taiho Pharmaceutical Co., Tokyo, Japan), which uses the 5-fluorouracil prodrug tegafur and two modulators, 5-chloro-2,4-dihydroxypyridine and potassium oxonate (4), was approved in Japan for pancreatic cancer in August 2006, and several clinical trials have been reported (5). Clinical trials using S-1 have shown promising results in various solid tumors. Response rates of 35-50% were reported for single agent S-1 use for gastric cancer, colon cancer (6,7), and breast cancer (Sano et al, Proc ASCO 19: abs. 404, 2000). Moreover, Koizumi et al reported a 74% response rate in a phase I/II study of S-1 and cisplatin in advanced gastric cancer patients (8). On the basis of these data, we have been using S-1 for the treatment of patients with advanced pancreatic cancer, either as a single agent or in combination with cisplatin since 2000, and have reported an excellent response rate (20.0% as a single agent and 57.1% in combination with cisplatin) and tolerable toxicity (9). Addition of cisplatin to S-1 seemed to be very effective for the treatment of pancreatic cancer although the toxicities were clearly enhanced in combination therapy (9).

The nucleotide excision repair (NER) pathway is the only known mechanism for the removal of bulky, helix-distorting DNA adducts, such as those generated by platinum-based chemotherapy, and it synthesizes a correct strand (10,11). ERCC1 forms a heterodimer with xeroderma pigmentum F (XPF) and executes a 5' incision into the damaged DNA strand, a process that is the last of several steps to remove the platinum-damaged DNA lesion (12-15). Therefore, high levels of intratumoral ERCC1 mRNA are expected to be associated with resistance to platinum-based chemotherapies. As expected, several studies revealed...
that higher ERCC1 mRNA levels are related to resistance to the platinum-based chemotherapies of ovarian, gastric, and colon cancers and non-small cell lung cancers (16-19). A synonymous polymorphism of ERCC1 codon 118 converting a common codon usage (AAC) to an infrequent one (AAT), both coding asparagine, has been reported (20). Since Yu et al, reported that ERCC1 mRNA levels were reduced in human ovarian cancer cells with a variant allele, and that those cells were inferior in recovery from platinum damage to cells with wild-type allele (21), several studies have assessed the relation between ERCC1 codon 118 polymorphism and sensitivity to a platinum agent in ovarian cancers, colorectal cancers, gastric cancers, and non-small cell lung cancers (22-31). It is possible for ERCC1 codon 118 polymorphism to be a biomarker for assessing the sensitivity to a platinum-based therapy, although it is still a matter of controversy whether either a wild-type allele or a mutant-type allele affect the platinum resistance. As far as we know, no report has been published with respect to the relationship between pancreatic cancer and this ERCC1 codon 118 polymorphism.

The aim of our study was to assess whether this polymorphism had an effect on tumor response or survival time in patients with advanced or relapsed pancreatic cancer treated with S-1 together with cisplatin.

**Patient s and methods**

**Patient population.** Sixty-seven patients with inoperable or relapsed pancreatic cancer were assessed [36 male and 31 female; median age 62.7 (range 38-82)]. All patients were treated at the Institute of Gastroenterology, Tokyo Women's Medical University from August 2000 to March 2007. Thirty-seven patients out of 67 were treated with S-1 twice daily for 21 days in combination with cisplatin 30 mg/m² on days 1 and 8, followed by a 2-week period rest. Thirty patients treated with S-1 alone twice daily for 28 days were assessed as a control group (Table I).

The protocol was reviewed and approved by an institutional review board and an ethics committee before study activation, and informed consent was obtained from each patient according to the institutional regulations.

**Clinical evaluation and response criteria.** Response criteria were based on the response evaluation criteria in solid tumors (32). Response was assessed by computer assisted tomography in liver, lymph node, lung metastases, as well as in primary lesions. To be classified as a responder, a tumor had to have a 30% reduction of the indicator lesion without growth of other disease or the appearance of new lesions. Overall survival (OS) was calculated as the period from the initiation of chemotherapy to death. Progression-free survival (PFS) was calculated as the period from the initiation of chemotherapy to the day the treating physician determined disease progression.

**DNA extraction, PCR, and genotyping.** In 61 patients, formalin-fixed, paraffin-embedded (FFPE) samples were used to obtain genomic DNA, and peripheral bloods were used in 6 patients. FFPE tissues were cut into serial sections with a thickness of 10 μm. Tissues were dissected from the slide using a scalpel. Tissue samples were heated at 92°C for 30 min in 4 mol/l DTT-GITC/sarc [4 mol/l guanidinium isothiocyanate, 50 mmol/l Tris-HCl (pH 7.5), 25 mmol/l EDTA; Invitrogen, Carlsbad, CA]. To the tissue suspensions, 600 μl of freshly prepared phenol/chloroform/isoamyl alcohol (25:24:1) were added. The suspensions were centrifuged at 14,000 rpm for 7 min in a chilled (8°C) centrifuge. The upper aqueous phase was removed and combined with glycerogen (10 μl) and 300 μl of isopropanol. The tubes were placed at -20°C for 30-45 min to precipitate the DNA. After centrifugation at 14,000 rpm for 7 min in a chilled (8°C) centrifuge, the supernatant was carefully poured off and the pellet was resuspended in 30 μl of purified water.

DNA from the peripheral blood was extracted using QIAamp® DNA blood mini kit (Qiagen, Valencia, CA). The genomic region containing the codon 118 in ERCC1 was amplified by PCR. The sequences of the primer used were as
follows: forward 5'-GCAATCCCGTACTGAAGTTCGT-3', reverse 5'-CAGCACATAGTCGGGAATTACGT-3'. The PCR condition used were 1 cycle for 2 min at 50˚C, 10 min at 95˚C, and then 46 cycles consisting of 15 sec at 95˚C, and 1 min at 64˚C. PCR products (10 µl) were digested with BsrDI which specifically cuts the codon 118 T allele, for 4 h at 65˚C in a final volume of 20 µl with 2 U of BsrDI in a 10 mmol/l Tris-HCl buffer at pH 7.9 containing 50 mmol/l NaCl, 1 mmol/l DTT, and 20 μg bovine serum albumin (24). Digestion products were analyzed by electrophoresis on 4% E-Gel® (Invitrogen, Carlsbad, CA) (Fig. 1).

Statistical analysis. The χ² test for independence was used to assess the association between ERCC1 codon 118 genotype and response to chemotherapy. The association between the genetic polymorphism and OS or PFS was estimated using the Kaplan-Meier method, and the p-value was assessed using the log-rank test. Because of the small number of patients with TT genotype, we combined TT and CT genotype groups; including at least one variant allele, and compared them with the CC genotype group, without including a variant type allele in the analysis. All analysis was performed with JMP software version 5.0.1 (SAS Institute Inc., Cary, NC, USA).

Results

Polymorphism of ERCC1 codon 118. The ERCC1 codon 118 genotypes were classified into AAT-homozygotes (TT), AAC-homozygotes (CC), AAC/AAT-heterozygotes (CT). The frequencies of TT, CC, CT genotypes were 7 (10.4%), 39 (58.2%), and 21 (31.3%), respectively.

Overall survival, progression-free survival, response and ERCC1 codon 118 polymorphism. The overall objective response rate to S-1 alone was 10.0%, and was 18.9% when cisplatin was used in combination. The objective response rate to the cisplatin/S-1 combination is greater in patients with at least one T allele (TT+CT) than in those with CC genotype, but it did not reach a significant difference (40% vs. 11.1%; p=0.058) (Table II).

For patients treated with the cisplatin/S-1 combination, those with TT+CT genotype had a better PFS and OS when compared with those with the CC genotype (PFS median: 338 vs. 106 days; p=0.006. OS median: 763 vs. 415 days; p=0.030) (Fig. 2). However, there were no statistically significant differences in either PFS or OS depending on ERCC1 genotype for those treated with S-1 alone (PFS: 84 vs. 114 days; P=0.171, OS: 270 vs. 280 days; P=0.670) (Fig. 3).

Discussion

In this study, ERCC1 codon 118 polymorphism was strongly associated with PFS and OS for pancreatic cancer patients treated with a cisplatin/S-1 combination, but not for those treated with S-1 alone. Those patients treated with the cisplatin/S-1 combination who had at least one T allele (CT genotype+TT genotype) had better PFS and OS than those with CC genotype. On the other hand, among patients treated with S-1 alone, there were no differences in either PFS or OS depending on the ERCC1 genotype. This result indicates that ERCC1 polymorphism can be a useful biomarker for cisplatin, but not for fluoropyrimidine (e.g., S-1).
Although the objective response to the cisplatin/S-1 combination is greater in patients with CT+TT genotypes than in those with CC genotype, the response rates did not show a significant difference. Our objective response rates in this study were 10% in patients with S-1 alone and 18.9% in those with S-1/cisplatin, and they were lower than the data we reported previously, 20.0% in S-1 alone and 57.1% in S-1/cisplatin (9). We assume that the reason for this discrepancy is because most patients in this study were relapsed patients, as we used surgical specimens for analysis, so that a larger portion of the patients already had a history of adjuvant chemotherapy. This lower response rate may have resulted in the lack of statistically significant difference between the response rate and ERCC1 genotype in this study.

Platinum compounds are widely used chemotherapeutic agents against solid tumors, but are rarely used against pancreatic cancer as a single agent. The contribution of cisplatin to survival time is controversial, as it was reported that the addition of cisplatin to 5-FU leads to a higher objective response rate, but could not prolong the survival time in patients with gastric cancer (33). However, a recent randomized phase III study demonstrated that gastric cancer patients treated with cisplatin/S-1 showed significantly longer survival times than those treated with S-1 alone (Narahara et al, J Clin Oncol Proc ASCO 5: abs. 4514, 2007), verified the contribution of cisplatin to survival time. With respect to pancreatic cancer, gemcitabine has been the standard chemotherapeutic agent (2,3), and several clinical trials of cisplatin combined with gemcitabine have been reported. The combination of cisplatin and gemcitabine tended to yield longer PFS and OS, but the difference did not reach statistical significance (34). We reported that cisplatin/S-1

![Figure 2. Kaplan-Meier survival curve for patients treated with cisplatin/S-1 combination. Patients with at least one T allele (CT+TT) had a better PFS (P=0.006) and OS (P=0.030) than those with CC genotype.](image1)

![Figure 3. Kaplan-Meier survival curve for patients treated with S-1 alone. There were no statistically significant differences in either PFS or OS between ERCC1 codon 118 genotypes.](image2)
combination therapy showed promising activity against pancreatic cancer, though toxicity was higher in patients with cisplatin/S-1 than those treated with S-1 alone (9). Thus it is beneficial to select a group of patients with a higher possibility of sensitivity to cisplatin/S-1 in order to avoid unnecessary chemotherapy.

NER is the pathway that repairs bulky platinum-DNA damage, and ERCC1 is a critical NER component (10-15). Several clinical studies have reported an association between expression levels of ERCC1 mRNA and response to platinum compound in patients with ovarian, gastric, colorectal, and especially non-small cell lung cancers; higher levels of ERCC1 mRNA were associated with resistance to platinum-based chemotherapy, and low levels of ERCC1 mRNA were associated with better clinical outcomes after platinum-based chemotherapy (16-19). Since a silent mutation at ERCC1 codon 118 in exon 4, namely, C to T transition from wild-type (AAC) to variant-type (AAT), both of which code asparagines, had been reported (20), the association between ERCC1 codon 118 polymorphism and ERCC1 mRNA expression levels were investigated. In in vitro studies using various human ovarian carcinoma cell lines, Yu et al reported that ERCC1 mRNA was reduced in cells with variant type ERCC1 codon 118 polymorphism as compared to cells with the wild-type, and cells with the variant type were less proficient at cisplatin-DNA adduct than cells with the wild-type (21). In contrast, Park et al reported that patients with metastatic colorectal cancer with C allele had lower intratumoral ERCC1 mRNA levels than patients without C allele (Park et al, Proc Am Assoc Cancer Res abs. 1591, 2002). We assume that these differences were due to the absence of larger studies. Which genotype is associated with higher mRNA levels and/or better prognosis with the patients that received platinum compound is still a controversial topic (21-31). Some reported environmental factors such as smoking interaction might modulate and mask the genetic effect of polymorphism (35-37).

Although measuring mRNA expression levels is a desirable method according to previous studies (16-19), collecting sufficient tumor samples from pancreatic cancer patients is not easy because only 5-25% of pancreatic cancer patients are candidates for resection (38). Additionally, since the pancreas is located in the retroperitoneal space and is a functionally delicate organ, endoscopic ultrasound-guided fine-needle aspiration biopsy, which is not a simple technique, is required in order to obtain tumor samples from inoperable patients. With respect to pancreatic cancer, accessing DNA polymorphism is a reasonable method because tumor tissue is not always necessary, normal tissue or even peripheral blood can be used to obtain DNA.

To our knowledge, this is the first report which shows the relationship between ERCC1 codon 118 polymorphism and survival benefit in pancreatic cancer patients treated with a platinum compound. Our findings suggest that this ERCC1 codon 118 polymorphism may play an important role in selecting a suitable group of patients more likely to benefit from platinum compound treatment. To confirm our result, larger prospective clinical trials are hoped for.

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