ERCC1 codon 118 polymorphism is a useful prognostic marker in patients with pancreatic cancer treated with platinum-based chemotherapy

HIROTAKA KAMIKOZURU, HIDEKAZU KURAMOCHI, KAZUHIKO HAYASHI, GO NAKAJIMA and MASAKAZU YAMAMOTO

Department of Surgery, Institute of Gastroenterology, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan

Received December 24, 2007; Accepted February 14, 2008

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). Excision repair cross-complementation group 1 (ERCC1), an excision nuclease located on chromosome 19q13, plays an essential role in NER of DNA, and has been reported to play a major role in the response to platinum-based chemotherapies such as cisplatin and oxaliplatin. 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

Correspondence to: Dr Kazuhiko Hayashi, Department of Surgery, Institute of Gastroenterology, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan
E-mail: hayashi@hosp.net

Key words: ERCC1, codon 118, polymorphism, pancreatic cancer, chemotherapy, platinum

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/m^2 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 alchohol (25:24:1) were added. The suspensions were centrifuged at 14,000 rpm for 8 min in a chilled (8˚C) centrifuge. The upper aqueous phase was removed and combined with glycogen (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

Table I. Demographic and clinical parameters of the 67 patients.

Characteristics	S-1 alone	S-1 + cisplatin
No. of patients	30	37
Gender		
Male	12	24
Female	18	13
Age		
Mean (range)	64 (38-80)	61.8 (42-82)
Status		
Inoperable	1	5
Relapsed	23	27
Palliative surgery	6	5
Prior therapy		
None	15	20
Gemcitabine	10	7
Fluorouracil	2	7
Another chemo-therapeutic agent	0	0
Immunotherapy	3	3
TNM stage before treatment		
IA	0	0
IB	2	3
IIA	2	3
IIB	16	20
III	2	5
IV	8	6

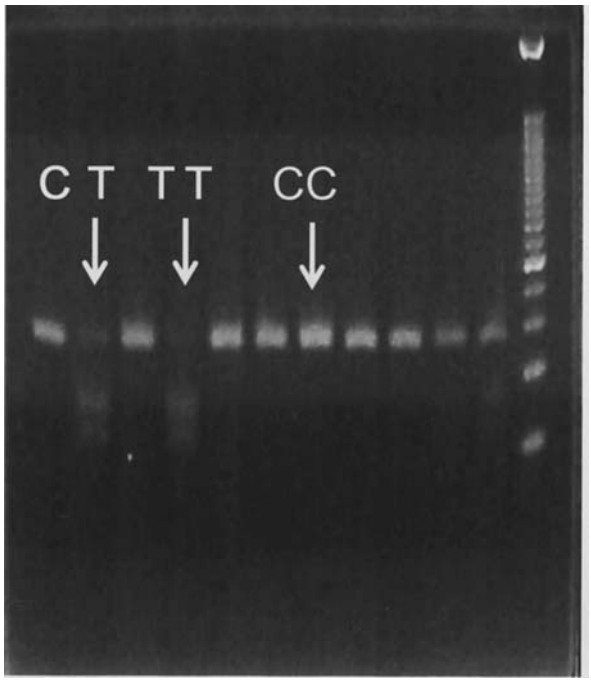

Figure 1. PCR products digested with BsrDI separated by gel electrophoresis. The length of the PCR products was 71 bp. The codon 118 T allele was cleaved by BsrDI in two fragments of 39 and 32 bp, while the C allele was not cleaved.

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 χ^2 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 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).

Table II. Response according to the ERCC1 codon 118 polymorphism.

	Global Responder/global	C/T+T/T Responder/global	C/C Responder/global	p-value
S-1 alone	3/30 (10.0%)	2/18 (11.1%)	1/12 (8.3%)	0.802
S-1 + cisplatin	7/37 (18.9%)	4/10 (40%)	3/27 (11.1%)	0.058
Global	10/67 (14.9%)	6/28 (21.4%)	4/39 (10.2%)	

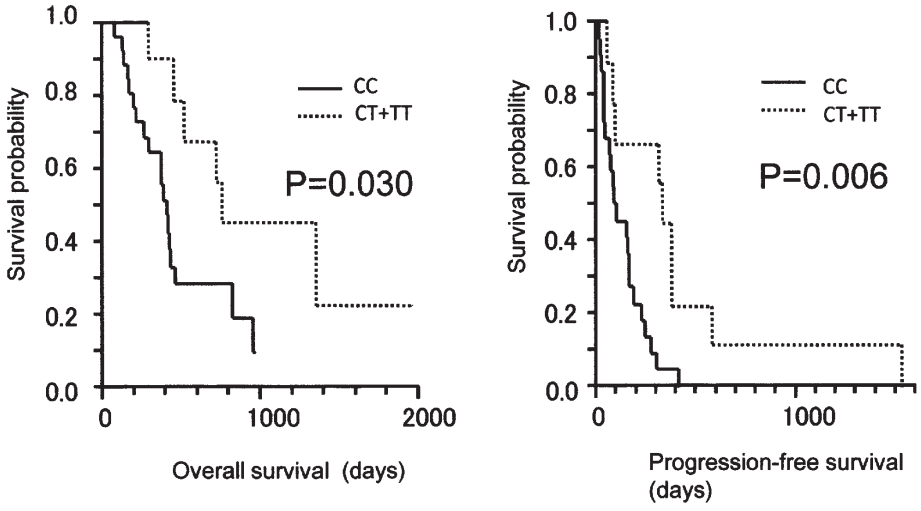

Figure 2. Kaplan-Meier survival curve for patients treated with cisplatin/S-1 combination. Patients with at least one T allele (CT+TT) had a better PFS (P=0.006) and OS (P=0.030) than those with CC genotype.

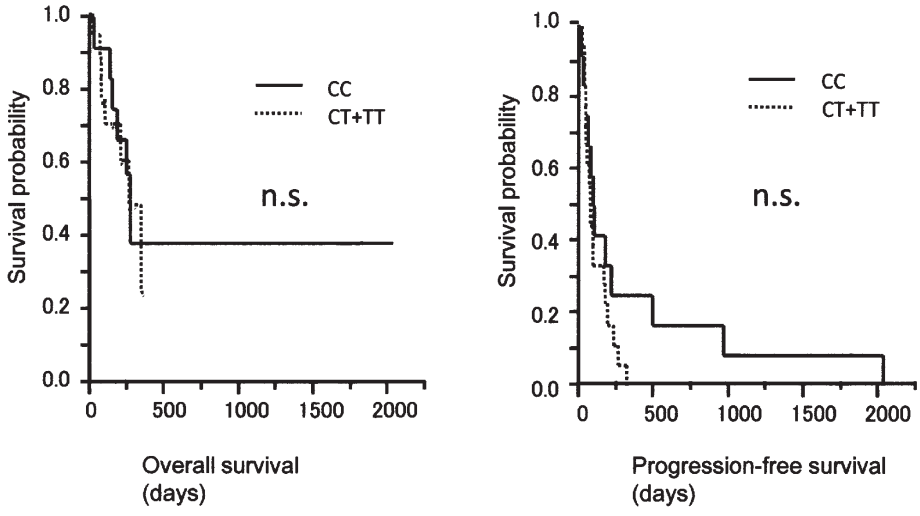

Figure 3. Kaplan-Meier survival curve for patients treated with S-1 alone. There were no statistically significant differences in either PFS or OS between ERCC1 codon 118 genotypes.

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

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 intra-tumoral 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.

Acknowledgements

We thank Dr Koji Tanaka for his excellent primer designing, and Mr. Chris W.P. Reynolds of the International Medical Communications Center of Tokyo Medical University for his careful review of this manuscript.

References

1. Matsuno H, Isaji S, Imaizumi T, *et al*: Summary of the pancreas cancer registration for 20 years. Suizo 18: 97-169, 2003.
2. Saif MW: Pancreatic cancer: highlights from the 42nd annual meeting of the American Society of Clinical Oncology, 2006. JOP 7: 337-348, 2006.
3. 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: randomized trial. J Clin Oncol 15: 2403-2413, 1997.
4. Shirasaka T and Taguchi T: Timeline from discovery of 5-FU to development of an oral anticancer agent S-1 and its drug concept. Jpn J Cancer Chemother 33: 4-18, 2006.
5. Ueno H, Okusaka T, Ikeda M, Takezako Y and Morizane C: An early phase II study of S-1 in patients with metastatic pancreas cancer. Oncology 68: 171-178, 2005.
6. Sakata Y, Ohtsu A, Horikoshi N, Sugimachi K, Mitachi Y and Taguchi T: Late phase II study of novel oral fluoropyrimidine anticancer drug S-1 (1 M tegafur-0.4 M gimestat-1 M ostat potassium) in advanced gastric cancer patients. Eur J Cancer 34: 1715-1720, 1998.
7. Ohtsu A, Baba H, Sakata Y, Mitachi Y, Horikoshi N, Sugimachi K and Taguchi T: Phase II study of S-1, a novel oral fluoropyrimidine derivative, in patients with metastatic colorectal carcinoma. Br J Cancer 83: 141-145, 2000.
8. Koizumi W, Tanabe S, Saigenji K, *et al*: Phase I/II study of S-1 combined with cisplatin in patients with advanced gastric cancer. Br J Cancer 89: 2207-2212, 2003.
9. Hayashi K, Imaizumi T, Uchida K, Kuramochi H and Takasaki K: High response rate in patients with pancreatic cancer using the novel oral fluoropyrimidine S-1. Oncol Rep 9: 1355-1361, 2002.
10. Kartalou M and Essigmann JM: Mechanisms of resistance to cisplatin. Mutat Res 478: 23-43, 2001.
11. Reardon JT, Vaisman A, Chaney SG and Sancar A: Efficient nucleotide excision repair of cisplatin, oxaliplatin, and bis-aceto-amine-dichloro-cyclohexylamine-platinum (IV) (JM216) platinum intrastrand DNA diadducts. Cancer Res 59: 3968-3971, 1999.
12. Reed E: Platinum-DNA adduct, nucleotide excision repair and platinum based anti-cancer chemotherapy. Cancer Treat Rev 24: 331-344, 1998.
13. Reed E: ERCC1 and clinical resistance to platinum-based therapy. Clin Cancer Res 11: 6100-6102, 2005.
14. Sancer A and Reardon JT: Nucleotide excision repair in *E coli* and man. Adv Protein Chem 69: 43-71, 2004.
15. De Laat WL, Jaspers NG J and Hoeijmakers JH: Molecular mechanism of nucleotide excision repair. Genes Dev 13: 768-785, 1999.
16. Dabholkar M, Vionnet J, Bostick-Burton F, Yu JJ and Reed E: Messenger RNA levels of XPAC and ERCC1 in ovarian cancer tissue correlate with response to platinum based chemotherapy. J Clin Invest 94: 703-708, 1994.
17. Metzger R, Leichman CG, Danenberg KD, *et al*: ERCC1 mRNA levels complement thmidylate synthase mRNA levels in predicting response and survival for gastric cancer patients receiving combination cisplatin and fluorouracil chemotherapy. J Clin Oncol 16: 309-316, 1998.
18. Shirota Y, Stoehmacher J, Brabender J, *et al*: ERCC1 and thymidylate synthase mRNA levels predict survival for colorectal cancer patients receiving combination oxaliplatin and fluorouracil chemotherapy. J Clin Oncol 19: 4298-4304, 2001.
19. Lord RV, Brabender J, Gandara D, *et al*: Low ERCC1 expression correlates with prolonged survival after cisplatin plus gemcitabine chemotherapy in non-small cell lung cancer. Clin Cancer Res 8: 2286-2291, 2002.
20. Yu JJ, Mu C, Lee KB, *et al*: A nucleotide polymorphism in ERCC1 in human ovarian cancer cell lines and tumor tissues. Mutat Res 382: 13-20, 1997.

21. Yu JJ, Lee KB, Mu C, Li Q, Abernathy TV, Bostick-Bruton F and Reed E: Comparison of two human ovarian carcinoma cell lines (A2780/CP70 and MCAS) that are equally resistant to platinum, but differ at codon 118 of the ERCC1 gene. Int J Oncol 16: 555-560, 2000.
22. Kang S, Ju W, Kim JW, *et al*: Association between excision repair cross-complementation group 1 polymorphism and clinical outcome of platinum-based chemotherapy in patients with epithelial ovarian cancer. Exp Mol Med 38: 320-324, 2006.
23. Park DJ, Zhang W, Stoehlmacher J, *et al*: ERCC1 gene polymorphism as a predictor for clinical outcome in advanced colorectal cancer patients treated with platinum-based chemotherapy. Clin Adv Hematol Oncol 1: 162-166, 2003.
24. Viguier J, Boige V, Miquel C, *et al*: ERCC1 codon 118 polymorphism is a predictive factor for the tumor response to oxaliplatin/5-fluorouracil combination chemotherapy in patients with advanced colorectal cancer. Clin Cancer Res 11: 6212-6217, 2005.
25. Stoehlmacher J, Park DJ, Zhang W, Yang D, Groshen S, Zahedy S and Lenz HJ: A multivariate analysis of genomic polymorphisms: prediction of clinical outcome to 5-FU/oxaliplatin combination chemotherapy in refractory colorectal cancer. Br J Cancer 91: 344-354, 2004.
26. Ruzzo A, Graziano F, Loupakis F, *et al*: Pharmacogenetic profiling in patients with advanced colorectal cancer treated with first-line FOLFOX-4 chemotherapy. J Clin Oncol 25: 1247-1254, 2007.
27. Ruzzo A, Graziano F, Kawakami K, *et al*: Pharmacogenetic profiling and clinical outcome of patients with advanced gastric cancer treated with palliative chemotherapy. J Clin Oncol 24: 1883-1891, 2006.
28. Isla D, Sarries C, Rosell R, *et al*: Single nucleotide polymorphisms and outcome in docetaxel-cisplatin-treated advanced non-small-cell lung cancer. Ann Oncol 15: 1194-1203, 2004.
29. Ryu JS, Hong YC, Han HS, *et al*: Association between polymorphisms of ERCC1 and XPD and survival in non-small-cell lung cancer patients treated with cisplatin combination chemotherapy. Lung Cancer 44: 311-316, 2004.
30. Su D, Ma S, Liu P, *et al*: Genetic polymorphisms and treatment response in advanced non-small cell lung cancer. Lung Cancer 56: 281-288, 2007.
31. Zhou W, Gurubhagavatula S, Liu G, *et al*: Excision repair cross-complementation group 1 polymorphism predicts overall survival in advanced non-small cell lung cancer patients treated with platinum-based chemotherapy. Clin Cancer Res 10: 4939-4943, 2004.
32. Therasse P, Arbuck SG, Eisenhauer EA, *et al*: New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 92: 205-216, 2000.
33. Ohtsu A, Shimada Y, Shirao K, *et al*: Randomized phase III trial of fluorouracil alone versus fluorouracil plus cisplatin versus uracil and tegaful plus mitomycin in patients with unresectable, advanced gastric cancer: The Japan Clinical Oncology Group Study (JCOG 9205). J Clin Oncol 21: 54-59, 2003.
34. Heinemann V, Quietzsch D, Gieseler F, *et al*: Randomized phase III trial of gemcitabine plus cisplatin compared with gemcitabine alone in advanced pancreatic cancer. J Clin Oncol 24: 3946-3952, 2006.
35. Zhou W, Liu G, Park S, *et al*: Gene-smoking interaction associations for the ERCC1 polymorphisms in the risk of lung cancer. Cancer Epidemiol Biomarkers Prev 14: 491-496, 2005.
36. Park SY, Hong YC, Kim JH, Kwak SM, Cho JH, Lee HL and Ryu JS: Effect of ERCC1 polymorphisms and the modification by smoking on the survival of non-small cell lung cancer patients. Med Oncol 23: 489-498, 2006.
37. Ryu JS, Viguier J and Praz F: Genetic effect of ERCC1 codon 118 polymorphism and confounding factors. Clin Cancer Res 12: 4784-4785, 2006.
38. Martin JL, Harvey HA, Lipton A and Martin R: Combined chemoradiotherapy for unresectable pancreatic cancer. Am J Clin Oncol 22: 309-314, 1999.