ERCC1 codon 118 C→T polymorphism associated with *ERCC1* expression and outcome of FOLFOX-4 treatment in Asian patients with metastatic colorectal carcinoma

Peter Mu-Hsin Chang,[1,2] Cheng-Hwai Tzeng,[1,2] Po-Min Chen,[1,2] Jen-Kou Lin,[1,3] Tzu-Chen Lin,[1,3] Wei-Shone Chen,[1,3] Jeng-Kae Jiang,[1,3] Huann-Sheng Wang[1,3] and Wei-Shu Wang[1,4,5]

[1]School of Medicine, National Yang-Ming University School of Medicine, No.155, Sec.2, Linong Street, Taipei 112; [2]Division of Oncology and Hematology, Department of Medicine, Taipei Veterans General Hospital, No.201, Sec.2, Shih-Pai Road, Taipei 112; [3]Division of Colorectal Surgery, Department of Surgery, Taipei Veterans General Hospital, No.201, Sec.2, Shih-Pai Road, Taipei 112; [4]Department of Medicine, National Yang-Ming University Hospital, No.152, Xin Min Road, I-Lan 260, Taiwan

(Received August 23, 2008/Revised September 29, 2008/Accepted October 11, 2008)

We analyzed the influence of codon 118 C→T polymorphism of *ERCC1* on its protein expression levels, clinicopathological features, and outcome of 168 Chinese patients with metastatic colorectal carcinoma that had been treated with first-line FOLFOX-4 chemotherapy. A high prevalence of C/C genotype was noted (47.6%, *n* = 80; 168 patients in total). A marked increase of ERCC1 protein expression levels was also noted in patients with C/T or T/T genotypes (70% *vs* 20%; *P* < 0.01), which was associated with significantly lower response to FOLFOX-4 (36.4% *vs* 57.5%; p = 0.01), and shorter progression-free (7 months *vs* 13 months; *P* < 0.01) and overall (16 months *vs* 25 months; *P* < 0.01) survival times. By multivariate analysis, this polymorphism was also identified as an independent prognostic factor (*P* = 0.02). These data suggest that Asian populations have a significantly higher prevalence of the C/C genotype in *ERCC1* codon 118, which could be a key determinant for good responses to oxaliplatin-based treatment and favorable outcomes. (*Cancer Sci* 2009; 100: 278–283)

Oxaliplatin, a cytotoxic platinum compound, exerts its cytotoxic effects through the formation of DNA adducts.[1] In combination with 5-FU and leucovorin (LV), oxaliplatin is very effective in the treatment of metastatic colorectal cancer patients.[2] Several proteins of the nucleotide excision repair (NER) pathway, including the excision repair cross-complementing group 1 (*ERCC1*), have been attributed to enhanced repair and tolerance of DNA damage, and the enhanced repair and tolerance of DNA damage may lead to resistance of platinum drugs.[3,4] In fact, overexpression of ERCC1, either at protein or mRNA levels, has been associated with resistance to platinum-based chemotherapy in patients with a variety of malignant diseases, including colorectal carcinoma.[5–9]

ERCC1 is a highly conserved protein, crucial for the removal of DNA adducts caused by platinum compounds.[3,4] *ERCC1* could be up-regulated by transcription factor activator protein 1.[10] And several splicing variants, including the one known as polymorphism of ERCC1, seem to influence the activity of its full-length gene product.[11,12] It has been shown that a common C→T polymorphism at codon 118 of *ERCC1* results in the same amino acid asparagine,[13] but a trend towards higher *ERCC1* mRNA levels as the number of T alleles increase.[14] This polymorphism has been identified as a useful predictor for outcomes in colorectal cancer patients who have been treated with platinum-based chemotherapy. Meanwhile, the median survival time in patients with the C/C genotype has obviously been longer than those with C/T or T/T genotypes.[15,16] But there still exists a contrary report which indicates that the objective response to platinum-based chemotherapy is significantly better in patients with the T/T genotype.[17,18]

In addition to codon 118 C→T, different polymorphisms of *ERCC1* may play a role in regulating its expression in different cell types. For example, *ERCC1* C8092A polymorphism, located in the 3′-untranslated region, is thought to affect mRNA stability.[19] Previous reports suggested that the *ERCC1* C8092A polymorphism was associated with outcomes in advanced head and neck squamous cell carcinoma, non-small cell lung cancer, and epithelial ovarian cancer patients.[20–22]

Ethnic differences do exist between enzymes that are involved in the targeting and metabolism of certain chemotherapeutic drugs that may affect the efficacy and toxicity of treatment in patients of different ethnic origins. For example, the prevalence of homozygous triple-repeat polymorphism in the 5′-enhancer region of the thymidylate synthase gene (*TSER*) is very common in Chinese patients, which may account for an impaired response to fluoropyrimidine regimens.[23] The UGT1A1*28 polymorphism is rare in Asian populations,[24] but the UGT1A1*6 polymorphism is very common in Asian populations, which leads to altered risk of developing severe neutropenia after being treated with irinotecan.[25] It has been shown that Asian populations seem to have a higher ratio of C allele component compared to Caucasian patients, but the sample size is quite small.[15] In a recent study a significantly higher ratio (58.2%) of the homozygous C/C genotype was found in 67 Japanese patients with pancreatic cancer.[26] Whether the percentage of those with the C/C genotype in *ERCC1* codon 118 is higher among Chinese patients may account for an increased susceptibility to platinum-based chemotherapy is of interest and deserves further studies.

Based on these earlier findings, we propose that codon 118 C→T polymorphism of *ERCC1* may correlate with ERCC1 protein expression and clinical outcomes of oxaliplatin-based chemotherapy in metastatic colorectal cancer patients. To examine the ethnic differences of this polymorphism among Asian populations and its influence on ERCC1 protein expression levels and outcomes of first-line FOLFOX-4 treatment, a study has been conducted.

[5]To whom correspondence should be addressed. E-mail: 11313@ilan.tpg.gov.tw

doi: 10.1111/j.1349-7006.2008.01031.x

Table 1. Clinicopathological features of metastatic colorectal cancer patients with or without *ERCC1* codon 118 C→T polymorphism ($n = 168$)

Characteristics	C/C (wild-type) (%)	C/T or T/T (%)	*P*-values
All patients	80 (100)	88 (100)	
Age (years)			
<50	36 (45.0)	35 (39.8)	0.49
≥50	44 (55.0)	53 (60.2)	
Gender			
Male	47 (58.8)	58 (65.9)	0.34
Female	33 (41.2)	30 (34.1)	
Performance status			
0	58 (72.5)	62 (70.5)	0.77
1, 2	22 (27.5)	26 (29.5)	
Primary tumor			
Colon	56 (70.0)	61 (69.3)	0.92
Rectum	24 (30.0)	27 (30.7)	
Histological differentiation			
Well/moderate	63 (78.8)	72 (81.8)	0.62
Poorly/unknown	17 (21.2)	16 (18.2)	
Invasive extent			
T1–2	23 (28.8)	20 (22.7)	0.37
T3–4	57 (71.2)	68 (77.3)	
Lymph node involvement			
N0	21 (26.2)	32 (36.4)	0.16
N1–3	59 (73.8)	56 (63.6)	
Serum CEA level (ng/mL)			
≤6	13 (16.2)	12 (13.6)	0.64
>6	67 (83.8)	76 (86.4)	
Grade 3/4 oxaliplatin-neuropathy			
Presence	15 (18.8)	18 (20.5)	0.78
Absence	65 (81.2)	70 (79.5)	
TSER 28-bp polymorphism			
2R/2R	1 (1.2)	0 (0)	0.91
2R/3R	26 (32.5)	29 (33.0)	
3R/3R	53 (66.3)	59 (67.0)	

CEA, carcinoembryonic antigen; TSER 28-bp polymorphism, germ-line polymorphisms of the number of 28-base pair tandemly repeated sequences in the 5′-enhancer region of the thymidylate synthase gene.

Materials and Methods

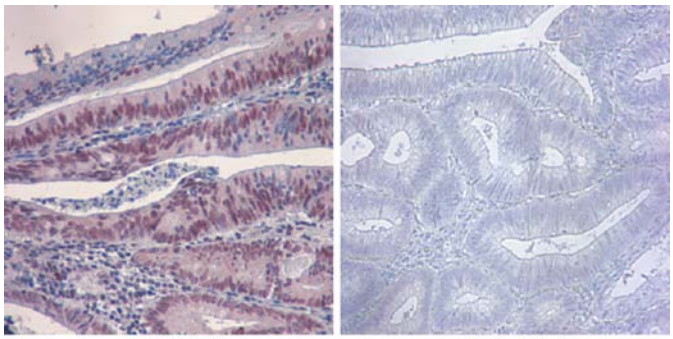
Patient characteristics. To understand the impact of *ERCC1* codon 118 C→T polymorphism on its protein expression levels and treatment outcomes of oxaliplatin-based chemotherapy, we examined 184 consecutive Chinese patients with unresectable metastatic colorectal cancer, who had received FOLFOX-4 as first-line treatment, from June 2003 to December 2006. Among them, 168 patients were enrolled and analyzed (patients' characteristics are shown in Table 1). The remainder were excluded. Excluded patients lacked measurable lesions ($n = 5$), had not had primary tumors removed and thus accurate T and N stages could not be determined ($n = 4$), had died before blood sampling ($n = 3$), were unwilling to participate ($n = 2$), or were lost to follow-up ($n = 2$). The FOLFOX-4 regimen consisted of oxaliplatin (Sanofi-Aventis, Paris, France) (85 mg/m^2, 1-h infusion, day 1) and LV (200 mg/m^2, 2-h infusion, days 1 and 2), before the administration of bolus 5-FU (400 mg/m^2, days 1 and 2) and infusional 5-FU (600 mg/m^2, 22-h infusion immediately after bolus 5-FU, days 1 and 2) every 2 weeks. Patients with or without *ERCC1* codon 118 C→T polymorphism were followed up at a similar intensity with a median duration of 18 months.

The responses of treatment were evaluated on the basis of standard RECIST criteria. Patients with complete response (CR), partial response (PR), or stable disease remained in the protocol until progressive disease or unacceptable toxicity was documented. Common toxicities were assessed at baseline, and after two, four, and six courses of treatment according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC). Treatment was delayed until recovery if grade 3–4 toxicity occurred, and the doses of oxaliplatin and 5-FU were reduced by 20% in subsequent cycles. In the case of intolerable toxicity or failure of front-line FOLFOX-4, treatment was discontinued, and irinotecan-based or fluoropyrimidine-only regimens were subsequently administered according to the physicians' decision. During treatments, chest X-ray, ultrasonography of the abdomen, or computed tomography was conducted every 2 months. An institutional review board approved this study and informed consent was given by all patients before blood testing for genotyping.

Examination of *ERCC1* codon 118 C→T polymorphism. Genomic DNA was extracted from patients' leukocytes obtained via 0.5 mL whole blood using standard phenol-chloroform procedures subject to *ERCC1* codon 118 C→T-testing. The codon 118 C→T polymorphism of the *ERCC1* was examined by the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method as previously described.[15] 0.1 μg genomic DNA, forward primer 5′-GCA GAG CTC ACC TGA GGA AC-3′ and reverse primer 5′-GAG GTG CAA GAA GAG GTG GA-3′ were used for PCR amplification. After initial denaturation at 95°C for 5 min, the reaction was carried out at 95°C denaturation for 1 min, 65°C annealing for 1 min, and 72°C extension for 1 min for a total of 40 cycles. PCR products, after being digested by the BsrD1 restriction enzyme (New England Biolabs, Beverly, MA, USA) at 60°C for 16 h, were separated on 4% Nusieve ethidium bromide-stained agarose gels. The RFLP analysis of the resultant 208-bp fragment led to C/C (208 bp), C/T (208, 128, 80 bp), as well as T/T (128, 80 bp) genotypes.

Examination of number of 28-bp tandemly repeated sequence in the 5′-enhancer region of the thymidylate synthase gene. Since 5-FU was used in combination with oxaliplatin for treating these patients, and germ-line polymorphisms of the number of the 28-bp tandemly repeated sequence in the TSER remarkably affect the response and survival of colorectal cancer patients who receive 5-FU,[27] the influences of this polymorphism on patients with or without *ERCC1* codon 118 C→T polymorphism deserve further studies. Genomic DNA was prepared from patients' leukocytes accordingly and a set of primers for amplification of the TSER was used according to a method previously described.[28] The sequences of the forward and reverse primers are 5′-GTG GCT CCT GCG TTT CCC CC-3′ and 5′-CCA AGC TTG GCT CCG AGC CGG CCA CAG GCA TGG CGC GG-3′, respectively. Amplification was performed for 30 cycles, including denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min between the initial denaturation at 94°C for 2 min and a final extension at 72°C for 5 min. Finally, the amplified DNA fragments were analyzed by eletrophoresis on a 4% agarose gel to determine the number of the 28-bp tandemly repeated sequence over the TSER.

Immunohistochemical (IHC) staining. To examine the influence of codon 118 C→T polymorphism of the *ERCC1* on ERCC1 protein expression, we obtained paraffin-embedded colorectal tumor tissues from 60 colorectal cancer patients, who agreed to release tumor tissues for examination. These tissues were subjected to IHC staining. Tumor tissue sections were stained with a mouse monoclonal anti-*ERCC1* antibody (clone SPM243) (Spring Bioscience, Fremont, CA, USA), using a streptavidin-biotin-immunoperoxidase kit (BioGenex, San Roman, CA, USA) according to the manufacturer's instructions. An experienced pathologist examined these slides microscopically, and both the



	ERCC1(+)	ERCC1(–)	
C/C (n = 30)	6	24	P^* < 0.01
C/T (n = 22)	15	7	$P^{\#}$ = 0.72
T/T (n = 8)	6	2	

Fig. 1. Representative immunohistochemical staining patterns of *ERCC1* in patients' tumor tissues, original magnification ×400. Tumor tissues from 60 colorectal cancer patients were stained with a mouse monoclonal anti-*ERCC1* antibody, using a streptavidin-biotin-immunoperoxidase kit according to the manufacturer's instructions. Positive *ERCC1* staining is defined as intense nuclear signals. The statistical difference between the correlation between *ERCC1* codon 118 statuses and *ERCC1* nuclear staining patterns was determined by χ^2-test. *P** indicates the difference in *ERCC1* expression between patients with or without the T allele; while *P#* defines the difference in *ERCC1* expression between patients with the C/T or T/T genotypes.

intensity and distribution of IHC staining signals were analyzed. Only nuclear immunoreactivity was considered positive for *ERCC1*. The staining intensity was graded on a scale of 0–3. The percentage of stained tumor cells (0–100%) was counted and final semiquantitative scores were calculated by intensity multiplying by distribution ranging from 0 to 300. The median value therefore is used as the cut-off point for separating *ERCC1*-positive from -negative tumors.

Statistical analysis and survival curve plotting. All statistical analyses were performed using SPSS software (SPSS for Windows, version 14.0; SPSS, Chicago, IL, USA). The progression-free and overall survival curves were also plotted, using the Kaplan–Meier product limit method, and the statistical differences in survival among subgroups were compared by log-rank test. The correlations between *ERCC1* condon 118 statuses and ERCC1 protein expression, clinicopathological characteristics and response to FOLFOX-4 treatment were analyzed separately. The statistical differences of these correlations were determined by χ^2-test. To assess the independent prognostic values of this polymorphism, we used Cox proportional hazards regression analysis (multivariate) which included *ERCC1* codon 118 status and other clinicopathological parameters. Two-sided *P*-values of less than 0.05 were considered statistically significant.

Results

A significantly higher frequency of the C/C genotype in *ERCC1* codon 118 was identified in Asian populations. We found that the incidences of codon 118 C/C, C/T, and T/T were 47.6% (n = 80), 39.9% (n = 67), and 12.5% (n = 21), respectively, in CRC patients. To our interest, in comparison with Caucasian populations in previous studies,[15,17] a high percentage (~50%) of the C/C genotype in our patients was clearly demonstrated, indicating the existence of ethnic difference for this polymorphism.

Table 2. Response to FOLFOX-4 treatment in metastatic colorectal cancer patients with different *ERCC1* codon 118 status (n = 168)

Response	C/C (wild-type) (%)	C/T or T/T (%)	*P*-values*
All patients enrolled	80 (100)	88 (100)	
OR (CR + PR)	46 (57.5)	32 (36.4)	0.01
CR	5 (6.3)	3 (3.4)	
PR	41 (51.2)	29 (33.0)	
SD	28 (35.0)	36 (40.9)	
PD	6 (7.5)	20 (22.7)	

CR, complete remission; OR, overall response; PD, progressive disease, PR, partial remission; SD, stable disease.
**P*-values represent the comparison of overall response rates between patients with different *ERCC1* codon 118 polymorphisms.

***ERCC1* codon 118 C→T polymorphism was associated with increased ERCC1 protein expression levels in patients' tumor tissues.** Since the codon 118 C→T polymorphism of the *ERCC1* gene was associated with a trend towards higher mRNA levels as the number of T alleles increased,[14] increased ERCC1 protein expression was proposed in patients with this polymorphism. As shown in Fig. 1, the intensive nuclear signals stood for positive staining for *ERCC1*. Very interestingly, a marked increase in ERCC1 protein expression was indeed observed in patients with C/T or T/T genotypes, as the percentage of positive *ERCC1* staining in patients with or without this polymorphism was 70% and 20%, respectively (P < 0.01; Fig. 1). Later on, we examined whether there was a gene–dose effect of this polymorphism on its protein expression by comparing patients with C/T (n = 22) or T/T (n = 8) genotypes. Although a marked increase in ERCC1 protein expression was observed in patients with the T allele; no between-groups difference between the C/T or T/T genotypes on *ERCC1* expression was found (P = 0.72; Fig. 1). Therefore, we divided the patients into two groups, with or without T allele, for subsequent clinicopathological analysis.

***ERCC1* codon 118 C→T polymorphism lead to a poor response and unfavorable prognosis of patients treated with FOLFOX-4, without affecting the incidence of severe peripheral sensory neuropathy.** As shown in Table 2, patients with C/T or T/T genotypes had a significantly lower response to FOLFOX-4 treatment (36.4% *vs* 57.5%, P = 0.01). Accordingly, a shorter progression-free (7 months *vs* 13 months; P < 0.01) as well as overall (16 months *vs* 25 months; P < 0.01) survival time was observed in patients with the C/T or T/T genotypes (Fig. 2). Concordantly, a significantly shorter progression-free (9 months *vs* 13 months; P < 0.01) as well as overall (16 months *vs* 25 months; P < 0.01) survival time was found in patients with positive *ERCC1* IHC staining in colorectal tumor tissues (Fig. 1), which was consistent with previous findings.[9] By adjusted analysis, this polymorphism was further identified as an independent prognostic factor (P = 0.02; Table 3).

Later on, we supposed that patients with this polymorphism, by enhanced excision repair of platinum-DNA lesions, might account for a lower incidence of severe oxaliplatin-related peripheral sensory neuropathy. However, as shown in Table 1, the incidence of grade 3 or 4 neuropathies after six courses of FOLFOX-4 treatment was very similar in both patients groups (18.8% *vs* 20.5%; P = 0.78).

TSER 28-bp polymorphism was not associated with *ERCC1* codon 118 polymorphism. The differences in the number of the 28-bp tandemly repeated sequence in the TSER of patients with or without *ERCC1* polymorphisms were analyzed as shown in Table 1. The percentages of patients with 2R/2R, 2R/3R, and 3R/3R were 1.2%, 32.5%, and 66.3%, respectively, in patients with the C/C genotype. These were very similar to the 0, 33.0%,

doi: 10.1111/j.1349-7006.2008.01031.x

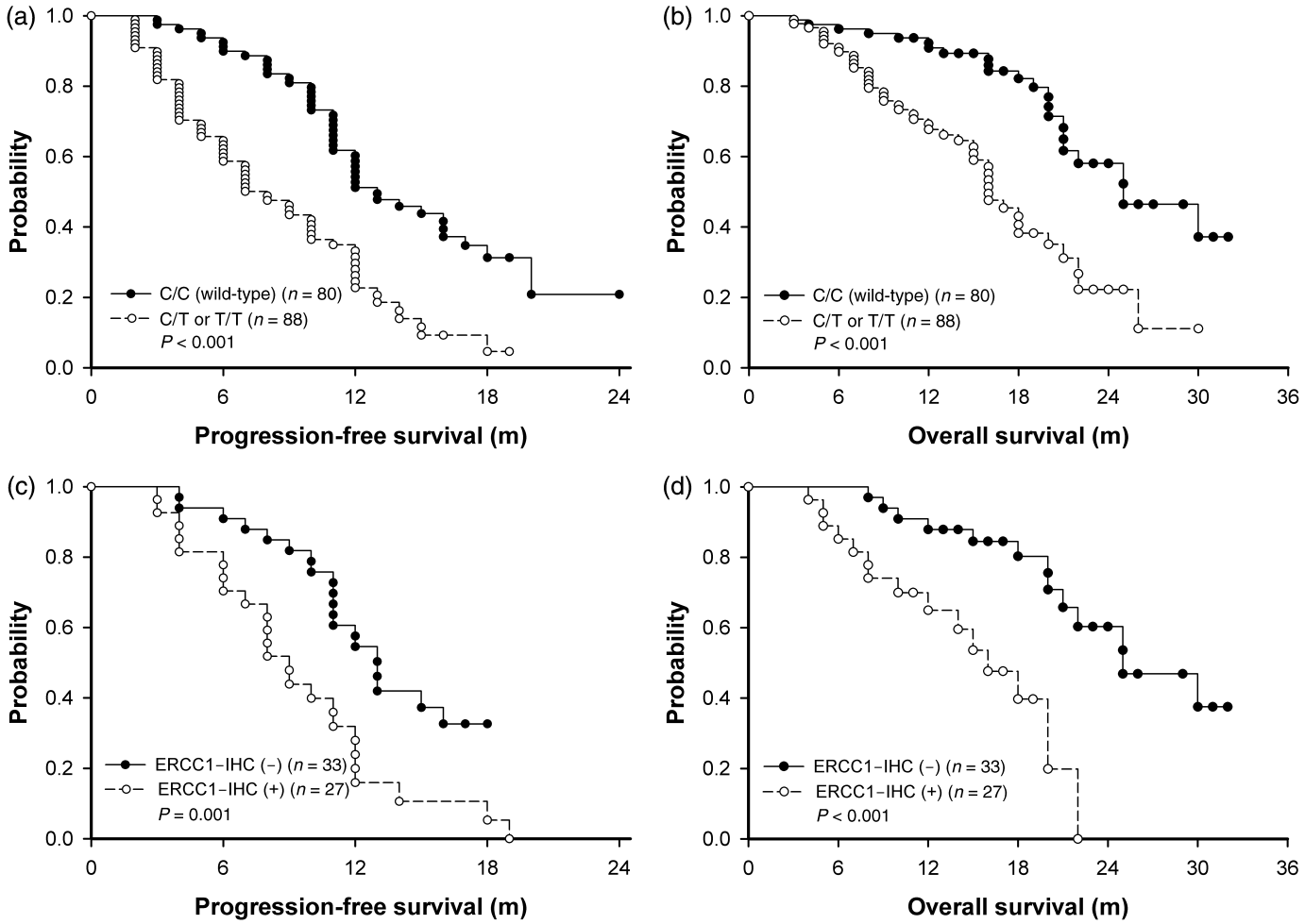


Fig. 2. Patients with codon 118 C→T polymorphism of the *ERCC1* gene or positive *ERCC1* immunohistochemical staining patterns have a shorter progression-free as well as overall survival time after being treated with FOLFOX-4. (a) Progression-free survival curves of 168 patients with *ERCC1*-118 C/C (filled circle) or C/T, T/T genotypes (open circle) have been plotted by Kaplan–Meier method ($P < 0.01$; log-rank test). (b) A similar method has been used to plot overall survival curves of patients with different *ERCC1* codon 118 genotypes ($P < 0.01$). (c) Progression-free survival time curves of 60 patients with negative (filled circle) or positive (open circle) *ERCC1* IHC staining patterns ($P < 0.01$). (d) A similar method has been used to plot overall survival curves of patients with different *ERCC1* IHC staining patterns ($P < 0.01$).

and 67.0%, respectively, observed for patients with the C/T or T/T genotypes ($P = 0.91$). Therefore, the influence of 5-FU on treatment response and survival in patients with or without *ERCC1* polymorphism was negligible.

Discussion

In 2001, Shirota *et al.* first reported that a higher intratumoral *ERCC1* mRNA level was associated with a poor prognosis in colorectal cancer patients treated with oxaliplatin-based chemotherapy.[9] The following year, Park *et al*. reported that in an experimental model, codon 118 T allele variant of *ERCC1* showed a trend towards higher *ERCC1* mRNA levels than those observed in the C allele.[14] Later on, a survival benefit was clearly demonstrated in colorectal cancer patients with the *ERCC1* codon 118 C/C genotype treated with 5-FU/oxaliplatin.[15] Concordantly, a prospective, multicenter trial further demonstrated that the codon 118 T/T genotype of *ERCC1* was independently associated with an adverse progression-free survival in colorectal cancer treated with first-line FOLFOX-4.[16] In the current study, a survival benefit was demonstrated in patients with the codon 118 C/C genotype (Fig. 2), which was compatible with previous findings. Interestingly, increased ERCC1 protein levels examined by IHC were clearly demonstrated in patients with the C/T or T/T genotypes (Fig. 1). This would account for a poor response and unfavorable prognosis to FOLFOX-4 treatment (Table 3 and Fig. 2).

Ethnic differences regarding codon 118 C→T polymorphism of *ERCC1* has been observed between Caucasian and African populations, and this polymorphic variant is seen most commonly in Americans of European descent and is associated with altered NER function.[29] In the present study, a larger sample size ($n = 168$) of Asian patients was analyzed, and the percentage of C/C, C/T, and T/T genotypes were 47.6% ($n = 80$), 39.9% ($n = 67$), and 12.5% ($n = 21$), respectively. In comparison with Caucasian populations,[15,17] a significantly higher percentage (~50%) of the C/C genotype existed in Asian populations, which was very similar to previous reports,[15,26] and might account for an increased susceptibility and favorable outcomes for platinum-based chemotherapy.

Neurotoxicity, especially peripheral sensory neuropathy, is the principle and dose-limiting toxicity of oxaliplatin-based treatment.[2] Therefore, the feasibility of using genomic polymorphisms to predict the development of severe neuropathy in

Table 3. Analysis of factors affecting patient survival with metastatic colorectal carcinoma receiving FOLFOX-4 treatment (n = 168)

Characteristics	*P*-values (univariate)	*P*-values (multivariate)
Age (years)		
<50 *versus* ≥50	0.51	0.43
Gender		
Male *versus* female	0.86	0.82
Performance status		
0 *versus* 1, 2	0.46	0.08
Primary tumor		
Colon *versus* rectum	0.78	0.64
Histological differentiation		
Well-moderate *versus* poorly	0.72	0.60
Invasive extent†		
T1-2 *versus* T3-4	0.16	0.06
Nodal status†		
Negative *versus* positive	0.03	0.04
Metastasis at diagnosis†		
No *versus* Yes	0.04	0.03
Serum CEA level (ng/mL)		
≤6 *versus* >6	0.51	0.36
TSER 28-bp polymorphism		
2R/2R *versus* 2R/3R or 3R/3R	0.13	0.28
ERCC1 codon 118 polymorphism		
C/C (wild-type) *versus* C/T or T/T	0.01	0.02

†According to the international tumor-node-metastasis (TNM) staging system for colorectal carcinoma.

patients treated with oxaliplatin makes sense. It has been shown that the 105Val allele variant of the glutathione *S*-transferase *P1* gene confers a significantly decreased risk of developing severe oxaliplatin-related cumulative neuropathy.[30] Although the codon 118 C→T polymorphism of *ERCC1* affects treatment response to oxaliplatin, the influence of this polymorphism on the incidence of severe (grade 3 or 4) oxaliplatin-related neuropathy remains unclear. As the codon 118 C→T polymorphism of *ERCC1* led to a higher ERCC1 protein expression and consequential excision repair, we supposed that this polymorphism would also be associated with a lower incidence of severe neuropathy after oxaliplatin treatment. However, the negative result in the present study might indicate different *ERCC1* expression and oxaliplatin metabolism between normal peripheral-nerve tissues and tumor tissues, which warrants further studies to confirm the mechanism (Table 2).

In the current study, a significant improvement in the response rate ($P = 0.01$), and progression-free ($P < 0.01$), as well as overall ($P < 0.01$) survival time, was observed in patients with the *ERCC1* codon 118 C/C genotype (Table 3 and Fig. 2) as in previous studies.[15,16] However, contrary results existed in the studies; for example, patients with the T/T genotype have higher objective response rates to oxaliplatin/5-FU treatment and better clinical outcomes than patients with the C/T or C/C genotypes.[17,18] In some cases, these results should be interpreted with caution because of methodological pitfalls. For example, some studies were analyzed on a retrospective basis with a very limited sample size. Furthermore, physiologic factors, concurrent medications, and environmental factors may also play important roles in interindividual variability in responses to chemotherapy. Therefore, prospective studies with larger sample sizes, accruing a homogenous population of patients treated with the same regimens, and conducting follow ups with the same intensity are warranted.

Interestingly, discrepancies in the association between *ERCC1* codon 118 genotypes and survival have existed in patients with different types of malignant diseases. For example, in non-small cell lung cancer patients treated with platinum-based chemotherapy, the median survival time for patients with the C/C genotype is not superior to those with C/T or T/T genotypes.[31] Furthermore, in advanced ovarian as well as gastric cancer patients, there is no significant correlation between *ERCC1* codon 118 genotypes and the response or survival outcomes for platinum-based chemotherapy.[32,33] One possible explanation is that, in addition to codon 118 polymorphism, different genetic–epigenetic interactions as well as transcriptional regulations of *ERCC1* may play a role in regulating its expression in different cell types.[10] Another explanation for these discrepancies is that *ERCC1* has to cooperate with other members of the NER pathway to repair DNA-platinum lesions.[34] These effects are sure to override an alteration of *ERCC1* gene expression induced by the codon 118 polymorphism.

In summary, we found that Asian populations have a high percentage (~50%) of the C/C genotype in codon 118 of *ERCC1*. The codon 118 C→T polymorphism of *ERCC1* is able to increase ERCC1 protein expression and consequential excision repair of platinum-DNA lesions. Further, this might be a key determinant for decreased response to FOLFOX-4 treatment and unfavorable outcomes for patients with metastatic colorectal carcinoma.

Acknowledgments

National Science Council of the Republic of China grant NSC 94-2320-B-010-050 supported this study. We thank Mr Chih-Tung Ho for excellent technical assistance.

References

1 Rixe O, Ortuzar W, Alvarez M *et al*. Oxaliplatin, tetraplatin, cisplatin, and carboplatin: spectrum of activity in drug-resistant cell lines and in the cell lines of the National Cancer Institute's Anticancer Drug Screen panel. *Biochem Pharmacol* 1996; **52**: 1855–65.
2 de Gramont A, Figer A, Seymour M *et al*. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. *J Clin Oncol* 2000; **18**: 2938–47.
3 Raymond E, Faivre S, Woynarowski JM, Chaney SG. Oxaliplatin: mechanism of action and antineoplastic activity. *Semin Oncol* 1998; **25**: 4–12.
4 Altaha R, Liang X, Yu JJ, Reed E. Excision repair cross complementing-group 1: gene expression and platinum resistance. *Int J Mol Med* 2004; **14**: 959–70.
5 Kwon HC, Roh MS, Oh SY *et al*. Prognostic value of expression of *ERCC1*, thymidylate synthase, and glutathione *S*-transferase P1 for 5-fluorouracil/oxaliplatin chemotherapy in advanced gastric cancer. *Ann Oncol* 2007; **18**: 504–9.
6 Smith S, Su D, Longrais IR *et al*. *ERCC1* genotype and phenotype in epithelial ovarian cancer identify patients likely to benefit from paclitaxel treatment in addition to platinum-based therapy. *J Clin Oncol* 2007; **25**: 5172–9.
7 Luca AH, Hernandez J, Mountzios G *et al*. Excision repair cross complementation group1 immunohistochemical expression predicts objective response and cancer-specific survival in patients treated by cisplatin-based induction chemotherapy for locally advanced head and neck squamous cell carcinoma. *Clin Cancer Res* 2007; **13**: 3855–9.
8 Olaussen KA, Dunant A, Fouret P *et al*. DNA repair by *ERCC1* in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. *N Engl J Med* 2006; **355**: 983–91.
9 Shirota Y, Stoehlmacher 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* 2001; **19**: 4298–304.
10 Li Q, Gardner K, Zhang L, Tsang B, Bostick-Bruton F, Reed E. Cisplatin induction of *ERCC1* mRNA expression in A2780/CP70 human ovarian cancer cells. *J Biol Chem* 1998; **273**: 23 419–25.
11 Yu JJ, Mu C, Dabholkar M, Bostick-Bruton F, Reed E. Alternative splicing of *ERCC1* and cisplatin-DNA adduct repair in human tumor cell lines. *Int J Mol Med* 1998; **1**: 617–20.

doi: 10.1111/j.1349-7006.2008.01031.x

12 Yu JJ, Thornton K, Guo Y, Kotz H, Reed E. An *ERCC1* splicing variant involving the 5′UTR of the mRNA may have a transcriptional modulatory function. *Oncogene* 2001; **20**: 7694–8.
13 Yu JJ, Mu C, Lee KB *et al*. A nucleotide polymorphism in *ERCC1* in human ovarian cancer cell lines and tumor tissues. *Mutat Res* 1997; **382**: 13–20.
14 Park DJ, Stoehlmacher J, Zhang W, Tsao-Wei D, Groshen S, Lenz H-J. *ERCC1* polymorphism is associated with differential *ERCC1* gene expression. In: *American Association for Cancer Research*. San Francisco, CA: 2002 ASCO Proceedings.
15 Stoehlmacher J, Park DJ, Zhang W *et al*. A multivariate analysis of genomic polymorphisms: Prediction of clinical outcome to 5-FU/oxaliplatin combination chemotherapy in refractory colorectal cancer. *Br J Cancer* 2004; **91**: 344–54.
16 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* 2007; **25**: 1247–54.
17 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* 2005; **11**: 6212–17.
18 Martinez-Balibrea E, Abad A, Aranda E *et al*. Pharmacogenetic approach for capecitabine or 5-fluorouracil selection to be combined with oxaliplatin as first-line chemotherapy in advanced colorectal cancer. *Eur J Cancer* 2008; **44**: 1229–37.
19 Chen P, Wiencke J, Aldape K *et al*. Association of an *ERCC1* polymorphism with adult-onset glioma. *Cancer Epidemiol Biomarkers Prev* 2000; **9**: 843–7.
20 Quintela-Fandino M, Hitt R, Medina PP *et al*. DNA-repair gene polymorphisms predict favorable clinical outcome among patients with advanced squamous cell carcinoma of the head and neck treated with cisplatin-based induction chemotherapy. *J Clin Oncol* 2006; **24**: 4333–9.
21 Zhou W, Gurubhagavatula S, Liu G *et al*. Excision repair cross-complementation group 1 polymorphism predicts overall survival in advanced nonsmall cell lung cancer patients treated with platinum-based chemotherapy. *Clin Cancer Res* 2004; **10**: 4939–43.
22 Thomas CK, Kathleen MD, Chunqiao T *et al*. Relationship between *ERCC1* polymorphisms, disease progression, and survival in the gynecologic oncology group phase III trial of intraperitoneal versus intravenous cisplatin and paclitaxel for stage III epithelial ovarian cancer. *J Clin Oncol* 2008; **26**: 3598–606.
23 Marsh S, Collie-Duguid ES, Li T, Liu X, Mcleod HL. Ethnic variation in the thymidylate synthase enhancer region polymorphism among Caucasian and Asian populations. *Genomics* 1999; **58**: 310–12.
24 Liu CY, Chen PM, Chiou TJ *et al*. UGT1A1*28 polymorphism predicts irinotecan-induced severe toxicities without affecting treatment outcome and survival in patients with metastatic colorectal carcinoma. *Cancer* 2008; **112**: 1932–40.
25 Jada SR, Lim R, Wong CI *et al*. Role of UGT1A1*6, UGT1A1*28 and ABCG2 c.421C>A polymorphisms in irinotecan-induced neutropenia in Asian cancer patients. *Cancer Sci* 2007; **98**: 1461–7.
26 Kamikozuru H, Kuramochi H, Hayashi K *et al*. *ERCC1* codon 118 polymorphism is a useful prognostic marker in patients with pancreatic cancer treated with platinum-based chemotherapy. *Int J Oncol* 2008; **32**: 1091–6.
27 Marsh S, McKay JA, Cassidy J, McLeod HL. Polymorphism in the thymidylate synthase promoter enhancer region in colorectal cancer. *Int J Oncol* 2001; **19**: 383–6.
28 Horie N, Aiba H, Oguro K, Hojo H, Takeishi K. Functional analysis and DNA polymorphism of the tandemly repeated sequences in the 5′-terminal regulatory region of the human gene for thymidylate synthase. *Cell Struct Funct* 1995; **20**: 191–7.
29 Gao R, Price DK, Sissung T *et al*. Ethnic disparities in Americans of European descent versus Americans of African descent related to polymorphic *ERCC1*, ERCC2, XRCC1, and PARP1. *Mol Cancer Ther* 2008; **7**: 1246–50.
30 Lecomte T, Landi B, Beaune P, Laurent-Puig P, Loriot MA. Glutathione S-transferase P1 polymorphism (Ile105Val) predicts cumulative neuropathy in patients receiving oxaliplatin-based chemotherapy. *Clin Cancer Res* 2006; **12**: 3050–6.
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* 2004; **10**: 4939–43.
32 Marsh S, Paul J, King CR, Gifford G, Mcleod HL, Brown R. Pharmacogenetic assessment of toxicity and outcome after platinum plus taxane chemotherapy in ovarian cancer: the Scottish Randomised Trial in Ovarian Cancer. *J Clin Oncol* 2007; **25**: 4528–35.
33 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* 2006; **24**: 1883–91.
34 Tripsianes K, Folkers GE, Zheng C *et al*. Analysis of the XPA and ssDNA-binding surfaces on the central domain of human *ERCC1* reveals evidence for subfunctionalization. *Nucl Acids Res* 2007; **35**: 5789–98.