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Excision Repair Cross-Complementation group 1 (ERCC1) C118T SNP does not affect cellular response to oxaliplatin

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Abstract

**Aims:** ERCC1 is involved in the repair of oxaliplatin-induced DNA damage. Studies for the association of the C118T SNP with clinical response to treatment with platinum drugs have rendered inconsistent results. We investigated the ERCC1 C118T SNP with respect to overall and progression-free survival in patients with advanced colorectal cancer (ACC) treated with oxaliplatin and in vitro DNA repair capacity after oxaliplatin exposure. In addition we discuss discrepancies from other studies concerning ERCC1 C118T.

**Materials & methods:** Progression-free survival was determined in 145 ACC patients treated with oxaliplatin-based chemotherapy in a phase 3 trial. For the in vitro studies regarding ERCC1 functionality, we transfected an ERCC1 negative cell line with 118C or 118T ERCC1. Cellular sensitivity and DNA repair capacity after exposure to oxaliplatin was examined by Sulphorodamine B growth inhibition assay, COMET assay and Rad51 foci staining.

**Results:** We found no association between ERCC1 C118T and progression-free or overall survival. In addition, transfection of either 118C or 118T restores DNA-repair capacity of UV20 cells to the same level and chemosensitivity to oxaliplatin was similar in ERCC1 118C and 118T transfected cells.

**Conclusion:** This study shows that the ERCC1 C118T variants are not associated with survival in ACC patients treated with oxaliplatin or the in vitro sensitivity and DNA-repair capacity in 118C and 118T transfected cell lines. Therefore, ERCC1 C118T genotyping seems of no value in individualizing oxaliplatin based chemotherapy in ACC.
Introduction

The third-generation platinum analogue oxaliplatin is a widely used chemotherapeutic agent, especially in the treatment of colorectal cancer. The antitumor effect of oxaliplatin results from intercalation of diaminocyclohexane (DACH)-platinum (Pt) in the DNA helix, causing Pt-DNA cross-links, and ultimately leading to programmed cell death. However, several cellular DNA repair mechanisms are capable of repairing damage from Pt-DNA adducts, such as the Nucleotide Excision Repair (NER) system. Within the NER system, ERCC1 is involved in the excision of DNA adducts, which are then replaced by a new piece of DNA strand that is synthesized in situ. As a result, NER and ERCC1 function may influence cellular sensitivity towards platinum analogues.

Absolute ERCC1 defects in humans are rare and were found lethal in mice. Most information about ERCC1 protein function is therefore based on observations in cell lines with NER defects, such as the UV-light sensitive CHO mutant UV20 cell line. Removal of cisplatin-induced Pt-adducts in ERCC1 deficient UV20 CHO cells appeared to be low compared to its wild type counterpart AA8 cell line, stressing the importance of the NER system in cellular platinum sensitivity. The overall rate and efficiency of the NER process was found comparable for cisplatin and oxaliplatin, and lack of ERCC1 function is thought to influence oxaliplatin sensitivity as well.

A common single nucleotide polymorphism (SNP) in the ERCC1 gene (C118T, rs11615) changes the common AAC codon into the infrequently expressed AAT, both coding for asparagine (Asn). The consequences of this synonymous substitution are not fully understood, but there is evidence that the 118T allele may be associated with lower ERCC1 expression caused by a difference in translational efficiency for this codon. Differences in translation kinetics may also give rise to conformational changes of the ERCC1 protein, thereby causing a change in function. Consequently, it has been hypothesized that this SNP influences clinical response to oxaliplatin chemotherapy.

Although many authors have studied the association between ERCC1 C118T genotype and treatment outcome of oxaliplatin-based chemotherapy in colorectal cancer, only one previous study tried to determine if this SNP is causally involved in oxaliplatin resistance. It was shown that the polymorphism does not alter cellular sensitivity to platinum treatment, but no attempt was made to clarify its influence on DNA repair capacity. Because of the contradictory results of clinical association studies for this SNP, we investigated the potential association of ERCC1 C118T with overall and progression free survival in advanced colorectal cancer (ACC) patients treated with oxaliplatin based chemotherapy in the phase 3 CAIRO trial of the Dutch Colorectal Cancer Group (DCCG). In addition, we performed functional in vitro tests using ERCC1 transfected cells, to study the involvement of these genetic variants not only in oxaliplatin sensitivity, but also on cellular DNA repair mechanisms.
Patients and methods

Patients
Patient DNA for the clinical association study was isolated from venous EDTA blood, collected as part of the CAIRO study (previously described in detail). The study flowchart and number of patients available for analysis are shown in Figure 1. As this pharmacogenetic substudy was initiated later than the CAIRO clinical trial and not all study centers participated, the number of patients included in the pharmacogenetic analyses is limited to a total of 268 patients. Baseline characteristics and stratification parameters were not different between our subset of patients and the total CAIRO population (data not shown). Tumor response to treatment was assessed every 9 weeks by computed tomography (CT) scanning using Response Evaluation Criteria In Solid Tumors (RECIST 1.0). All included patients gave written informed consent before inclusion for the main study and the pharmacogenetic side study.

Genotyping ERCC1 C118T
DNA was isolated from whole blood with the total MagnaPure Total Nucleic Acid Isolation Kit I on the MagnaPure LC (Roche Diagnostics, Mannheim, Germany). Chromosomal DNA was quantified using the Nanodrop (Isogen, IJsselstein, The Netherlands) and diluted to a concentration of 10 ng/μl. TaqMan assay was purchased from Applied Biosystems (Nieuwerkerk...
aan den IJssel, The Netherlands). The ERCC1 C118T SNP was determined on Realtime PCR system 7500 (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands) according to their instructions. PCR primer sequences are available on request.

Cell lines/plasmids
Wildtype CHO (AA8) and ERCC1 negative CHO (UV20) cells were used for in vitro transfection experiments. All cells were grown in DMEM supplemented with 5% FCS and G418 when required, at 37°C and 5% CO2 conditions. All reagents were obtained from Invitrogen (Breda, The Netherlands).

Transfection of ERCC1 C118T in UV20-cells
Bicistronic plasmids expressing the open reading frame of wild type or mutant ERCC1 allele at codon 118, and expressing Green Fluorescent Protein (GFP) for selection of transfected cells, were created by Gateway technology (Invitrogen, Breda, The Netherlands). Briefly, the ERCC1 gene was PCR amplified from human cDNA, which was genotyped as heterozygous for ERCC1 C118T, with attB-flanked primers p121 and p122. The PCR product was recombined in pDONR201. The PCR product in the resulted entry vector was sequenced to confirm genotype. Second, the entry vector was recombined with destination vector pExp-IRES-GFP (Clontech, Oxford, UK) that had been made gateway compatible using the Gateway Conversion system (Invitrogen, Breda, The Netherlands). The resulting plasmids were designated pTS401 and pTS402 and were used to transfect UV20 cells with GeneJuice (VWR, Amsterdam, The Netherlands). Transfected cells were selected by cellsorting, and plasmid expression was maintained by growth under selection by G418 (Sigma-Aldrich, Zwijndrecht, The Netherlands). The derived cell lines were designated UV20+118C (transfected with pTS401) and UV20+118T (transfected with pTS402). UV20 cells were also transfected with blank plasmid, and designated UV20-GFP.

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Characteristics</th>
<th>Origin/Reference</th>
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<tr>
<td>AA8</td>
<td>wildtype CHO-cells</td>
<td>ATCC</td>
</tr>
<tr>
<td>UV20</td>
<td>ERCC1 deficient CHO- cells</td>
<td>Thompson (1980)</td>
</tr>
<tr>
<td>UV20+118C</td>
<td>UV20 expressing ERCC1-118C and GFP</td>
<td>this study</td>
</tr>
<tr>
<td>UV20+118T</td>
<td>UV20 expressing ERCC1-118T and GFP</td>
<td>this study</td>
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<tr>
<td>UV20-GFP</td>
<td>UV20 expressing GFP</td>
<td>this study</td>
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<th>Plasmids</th>
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<tr>
<td>pDONR201</td>
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<tr>
<td>pEXP-IRES-GFP</td>
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<td>TS401</td>
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<td>TS402</td>
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Table 1. Plasmids and cell lines used in the ERCC1 transfection experiment.
Sulphorodamine B growth inhibition assay

Oxaliplatin cytotoxicity was assessed using the Sulphorodamine B (SRB) growth inhibition assay described by Skehan et al. A total of $1.2 \times 10^3$ cells (AA8, UV20, UV20-GFP, UV20+118C, UV20+118T) were seeded into 96-well plates in a volume of 150 µl and incubated at 37°C overnight. Oxaliplatin concentrations were prepared in culture medium immediately before use. To the cells, 50 µl of drug-medium mixture was added. Triplicates were used for each drug concentration. Plates were incubated for 24 h at 37°C. Following drug treatment, the medium was replaced with 200 µl of fresh complete medium, and the plates were incubated for 3 days at 37°C. The growth medium in the wells was removed, and 50 µl of ice-cold 50% (w/v) trichloroacetic acid was added to fix the cells for 1 h at 4°C. Then, cells were washed six times with water, and stained with 50 µl of 0.4% (w/v) SRB-1% acetic acid for 20 min at room temperature. Unbound dye was removed by washing six times with 1% acetic acid, and plates were dried. The dye was solubilized by the addition of 150 µl of 10 mM Tris-base into each well. Plates were left at room temperature for 20 min, and the optical density (OD) at 570 nm was measured.

Clonogenic assay

The clonogenic assay was performed as described by Franken et al. Briefly, a total of 50 exponentially growing cells (AA8, UV20, and transfected cells UV20+118C, UV20+118T and UV20-GFP) were plated in triplicate in 6-wells plates and incubated for 24 h to allow for cellular attachment. Attached single cells were treated with different concentrations of oxaliplatin (195 nM, 781 nM and 3125 nM) for 24 h at 37°C in medium. Cells were washed and incubated for 7–9 days until visible colonies of more than 50 cells were obtained. Cells were then stained with 1% Giemsa and the number of colonies in each dish was counted.

Modified COMET assay

DNA damage was studied by the modified COMET assay. Exponentially growing cells were exposed to oxaliplatin 195 nM or 3125 nM for 1 h. The cells were washed and incubated overnight at 37°C and 5% CO₂. Next, the cells were trypsinized and resuspended in 1 ml culture medium. To induce DNA damage, the cells were treated with 30 M H₂O₂ (peroxide) for 10 min at 4°C. After spinning down, the degraded DNA was resuspended in 1% low melting point agarose (Promega, Leiden, The Netherlands) on a microscope slide. The slides were soaked in lysisbuffer (100 mM EDTA, 10 mM Tris, 2.5 M NaCl, 1% Triton and 10% DMSO) for at least 90 min. In order to denature the DNA, the slides were air-dried and incubated in electrophoresis buffer (1 M EDTA, 300 mM NaOH pH 13) for 40 min. Next, the DNA was electrophoresed at 25 V and 250 mA for 30 min. After electrophoresis, the slides were washed at least three times with 0.4 M Tris pH 7.5 prior to staining with ethidiumbromide (20 g/l) (Promega, Leiden, The Netherlands) for 5 min.

Comet tails were visualized using FITC filtered fluorescent microscopy and visually scored as described elsewhere. In these experiments, cells with high amounts of oxaliplatin-
induced cross-links have shorter tails compared to cells in which cross-links have been repaired effectively. This is explained by the fact that unrepaired platinum-induced DNA cross-links will stay together after DNA degradation by H$_2$O$_2$, resulting in larger DNA fragments. Mean difference in tail olives moment (and tail areas) between treated and untreated cells indicates the presence of DNA damage due to exposure to oxaliplatin. The damage level is expressed according to the formula: (unexposed cells – oxaliplatin exposed cells) tail olives moment/tail olives moment of unexposed cells.

**Rad51 foci staining**

Rad51 foci, indicative of DNA damage, were shown as follows.$^{21}$ Exponentially growing cells (AA8, UV20, and transfected cells UV20+118C, UV20+118T and UV20-GFP) were exposed to oxaliplatin and incubated as described in section 2.5. Next day, cells were washed with PBS and fixated with 2% formaldehyde for 15 min at room temperature. To enhance permeability, cells were incubated in 0.1% Triton for 10 min. To prevent non-specific binding of Rad51 antibody, the cells were washed with PBS+ (PBS containing 0.15% glycine (Biorad, Veenendaal, The Netherlands) and 0.5% BSA (Sigma–Aldrich, MO, USA)). Rabbit-anti-Rad51 antibody (Sigma–Aldrich, MO, USA) diluted in PBS+ was added to the cells and incubated at room temperature for 90 min under dark and humid conditions. Alexa488 conjugated goat-anti-rabbit antibody (Molecular Probes, Leiden, The Netherlands) was used to visualize Rad51 foci using FITC filtered fluorescent microscopy. Rad51 expression was quantified by counting the number of cells that have more than 5 foci. A representative image of the Rad51 foci staining used for analysis is shown in Figure 2.

**Statistical methods**

DNA from venous blood and tumor samples was obtained from a subset of patients participating in the previously described CAIRO study.$^{13}$

One-way ANOVA with LSD-t test was used to compare the results of the SRB assay. A Cox proportional hazard model was used to investigate the association between ERCC1 status and death and/or progression. PFS for second-line treatment was defined as the time from randomization until first progression reported after the start of second-line treatment and, if a patient did not start second-line treatment, the date of first progression reported after randomization or death or last follow-up. Likewise, PFS for third-line treatment was defined as the time from randomization until first progression, death, or last follow-up after the start of third-line treatment. OS was calculated as the interval from the date of randomization until death from any cause or until the date of last follow-up. Overall and progression-free survival curves were estimated with the Kaplan–Meier method and compared with the Log-Rank test. Association between survival and ERCC1 status were analyzed by a univariate Cox proportional hazards model.

All tests were two-sided and p values of less than 0.05 were considered statistically significant. All data received before February 2008, with a median follow-up of 40.6 months
(range 0.3; 53 months), are included in this report.

![Figure 2. Representative picture used for assessment of Rad51 foci of oxaliplatin treated cells.](image)

All cells have been incubated with 3125nM oxaliplatin, during 24 hours.
A: AA8 (wildtype CHO cells); B: UV20 (ERCC1-/- CHO cells); C: UV20+118C (UV20 cells transfected with ERCC1-118C allele); D: UV20+118T (UV20 cells transfected with ERCC1-118T allele).

**Results**

**ERCC1 C118T is not associated with survival in ACC patients treated with oxaliplatin**

Genotyping was successful in 251 out of 268 patients, and genotype frequencies were 118TT 92 (37%), 118TC 129 (51%), 118CC 30(12%). The genotype frequencies were in Hardy–Weinberg equilibrium (HWE) and comparable with those reported in Caucasians.22,23

A total of 145 patients treated with capecitabine and oxaliplatin were evaluated for analysis of PFS and OS. The C118T genotype was not correlated with PFS (p = 0.145 and p = 0.614 for second-and third-line treatment, respectively) or with OS (second-line therapy: p = 0.121; third-line therapy: p = 0.331). Similar results were obtained when patients of both regimens receiving second-and third line treatment were analyzed together. (Table 2)
Table 2. ERCC1 C118T genotypes and clinical outcome after treatment with CAPOX
CAPOX, capecitabine-oxaliplatin; CI, confidence interval; ERCC1, Excision Repair Cross-Complementation group 1 (ERCC1); OS, overall survival; PFS, progression free survival.

Table 3. Sulphorodamine B (SRB) assay determined oxaliplatin sensitivity in parental AA8, UV20 (ERCC1-/-), and transfected cells UV20+118C, UV20+118T and UV20-GFP.
Growth inhibition was determined using the SRB assay and the percentage of surviving cells was calculated as described. All results are the mean of at least three independent experiments and error bars show the standard deviation of the mean.
ERCC1, but not C118T genotype, influences cellular sensitivity to oxaliplatin in vitro

To test whether C118T genetic variants are causally related to cellular sensitivity to oxaliplatin, we conducted transfection experiments with ERCC1 negative CHO cells. Figure 3 shows the sensitivity to oxaliplatin of AA8 (wildtype CHO), ERCC1 defective CHO mutant UV20 and the three transfected cell lines (UV20+118C, UV20+118T and UV20-GFP). The drug concentrations to inhibit cell growth by 50% (IC\textsubscript{50} values) in the ERCC1-defective cells (UV20 and UV20-GFP) are approximately 16-fold lower than for their parental cell line (AA8). UV20 and UV20-GFP showed similar IC\textsubscript{50} values, indicating that the transfection of GFP had no effect on cell growth. In contrast, the ERCC1 overexpressing cells, UV20+118C and UV20+118T, show substantial resistance to oxaliplatin. IC\textsubscript{50} values for these cells were approximately 32-fold higher compared to the UV20 cell line (p-value < 0.005, paired Student's t-test). No difference was found in IC\textsubscript{50} values between UV20+118C and UV20+118T. These results indicate that ERCC1 influences cellular sensitivity to oxaliplatin, and that chemosensitivity is not different between ERCC1 118C and 118T expressing variants.

We repeated these experiments using the clonogenic assay. Consistent with the results from the SRB assay, all ERCC1 expressing cells (AA8, UV20+118C and UV20+118T) showed significant numbers of surviving colonies after treatment with oxaliplatin. However, ERCC1 negative UV20 cells and cells transfected with the empty vector (UV20-GFP) showed no colony formation after treatment with oxaliplatin. A schematic representation of these results is shown in Figure 4.

Figure 4. Clonogenic assay determined oxaliplatin sensitivity in parental AA8, UV20 (ERCC1-/-), and transfected cells UV20+118C, UV20+118T and UV20-GFP.
Number of visible colonies of more than 50 exponentially growing cells, after 24h treatment with different concentrations of oxaliplatin (195nM, 781nM and 3125nM) and subsequent incubation during 7-9 days
ERCC1, regardless of C118T genotype, is essential for oxaliplatin-induced DNA-damage in vitro

To validate whether the high susceptibility of UV20 cells to oxaliplatin is caused by a defect in DNA repair, we evaluated oxaliplatin-induced DNA damage using the COMET assay, in wild-type AA8 cells, ERCC1 defective UV20, and the transfected variants UV20+118C and UV20+118T. As expected, tails of the ERCC1 deficient UV20 cell line are smaller than AA8 tails. The comet tails in UV20+118C and UV20+118T are comparable with AA8. These results indicate that DNA repair activity in ERCC1 deficient UV20 is fully restored by introducing ERCC1 and that this is equally the case for ERCC1 118C and 118T transfected cells. (Figure 5)

A representative image of the modified COMET assay used for data analysis is shown in Figure 6.

![Figure 5](image_url)

**Figure 5.** Difference in comet tail length after exposure to oxaliplatin of parental AA8, UV20 (ERCC1-/-), and transfected cells UV20+118C and UV20+118T.

A large decrease in comet-tail olives moment is indicative of higher amounts of residual DNA-cross links caused by oxaliplatin. Results are the mean of 100 comets and error bars show the standard deviation of the mean.

Because ERCC1 is also considered to be involved in homologous recombinational repair (HRR), we stained for Rad51 foci in oxaliplatin treated cells. Rad51 is important for recognition of DNA damage in HRR and impaired ERCC1 function leads to constant expression of Rad51 at the site of the lesion. Results for these analyses are shown in Figure 7. The data shown are the means of at least three experiments. After 24 h, the amount of foci per cell was more abundant in UV20 compared to AA8 (Figures 2B and A, respectively) or ERCC1 complemented cells. (Figures 2C and D) In UV20 ERCC1 negative cells, on average 61% of cells showed more than five Rad51 foci, versus only 27% for AA8 wildtype cells. In the ERCC1 overexpressing cells (UV20+118C, UV20+118T) very few Rad51 foci positive cells were present, irrespective of their codon 118 genotype (13% versus 11% for 118C and 118T, respectively). These results indicate that HRR pathway is less active in the ERCC1 deficient cell line and can be restored to wildtype activity by ectopic expression of ERCC1 118C, as well as 118T.
Figure 6. Representative image of the modified COMET assay used for interpretation of remaining DNA damage after treatment with oxaliplatin.

A: AAV8, not treated with oxaliplatin; B: AAV8 treated with oxaliplatin 3125nM; C: UV20, not treated with oxaliplatin; D: UV20, treated with oxaliplatin 3125nM; E: UV20-118C, not treated with oxaliplatin; F: UV20-118C, treated with oxaliplatin 3125nM; G: UV20-118T, not treated with oxaliplatin; H: UV20-118T, treated with oxaliplatin 3125nM.

Figure 7. Change is Rad51 foci staining after treatment with increasing oxaliplatin concentrations, for parental AA8, UV20 (ERCC1-/-), and transfected cells UV20+118C and UV20+118T.

Results are means of at least three experiments.

Discussion

Despite numerous publications in recent years, there has been ongoing debate on the role of ERCC1 and the common SNP at codon 118 of this gene regarding chemosensitivity to oxaliplatin in colorectal carcinoma. This is the most comprehensive study of ERCC1 C118T functionality in the repair of oxaliplatin-induced DNA-damage to date. We investigated the association of ERCC1 genotype with PFS and OS after oxaliplatin-containing chemotherapy in ACC and found no effect of the C118T SNP. We did, however, confirm ERCC1 functionality in the repair of oxaliplatin-induced DNA-damage, using a modified COMET assay and staining of Rad51. Most importantly, we excluded an effect of the ERCC1 C118T SNP on cellular sensitivity to oxaliplatin and on DNA-repair efficacy in oxaliplatin-induced DNA damage, by using cellular transfection experiments.
Publications regarding the effect of \textit{ERCC1} C118T in platinum therapy have been both extensive and contradictory. Consistent with our results, several authors reported no effect of the SNP on clinical efficacy of oxaliplatin-based chemotherapy in colorectal carcinoma patients.\cite{23,27-30} Other studies, however, found shorter survival times for the \textit{ERCC1} 118T allele in ACC patients treated with oxaliplatin-containing schedules.\cite{10,11,31-33} Contrarily, a smaller number of authors found an adverse effect of the C allele.\cite{34-36} It should be noted that our study population, as most other clinical pharmacogenetic association studies, lacks statistical power to definitively rule out an effect of the \textit{ERCC1} C118T polymorphism on oxaliplatin efficacy. However, our results concur with those from a meta-analysis published in 2011, showing that PFS following oxaliplatin-based chemotherapy for colorectal cancer patients carrying the \textit{ERCC1} 118C allele was not influenced by rs11615 genotype in Caucasian populations.\cite{37}

Based on the results of our clinical association study, we questioned the functional relevance of the \textit{ERCC1} SNP C118T. By in vitro experiments, transfecting UV20 cells with either 118C or 118T, we showed that the SNP does not have functional consequences for either in vitro sensitivity to oxaliplatin, DNA-repair capacity, or HRR. Previously, Gao et al. followed a similar approach and also found no differences in viability upon platinum treatment between \textit{ERCC1} 118C and 118T expressing cells.\cite{8} Seetharam et al. found that \textit{ERCC1} mRNA and protein expression were upregulated upon oxaliplatin in resistant CRC cell lines, but not in oxaliplatin sensitive cell lines. In addition, silencing of \textit{ERCC1} by siRNA led to an increase in chemosensitivity in previously oxaliplatin-resistant cell lines.\cite{38}

Given our results and those by Gao and co-workers, it remains striking that many authors found an effect of the \textit{ERCC1} C118T polymorphism on oxaliplatin efficacy. Several explanations can be offered for this discrepancy. Firstly, it should be noted that many of the previous pharmacogenetic association studies did not correct adequately for multiple testing and positive results may therefore have arisen by chance. Secondly, differences in genotype frequencies between populations may have caused accidental statistical associations, especially in populations with mixed ethnicities. Whereas the 118C allele is the minor allele in Caucasians, it is the common variant found in people from Asian descent.\cite{22} Notably, a positive effect of the \textit{ERCC1} 118C allele was pre-dominantly found in Asian populations.\cite{11,31,32} An alternative hypothesis is that the C118T SNP is indirectly functional due to its linkage disequilibrium with another SNP in the \textit{ERCC1} gene. The C118T SNP was found to be in a tight haplotype block with other SNPs in both coding and non-coding regions of the \textit{ERCC1} gene.\cite{27,34,39,40} Consistent with this hypothesis, a haplotype containing both \textit{ERCC1} C118T and a SNP in the 3’-UTR of \textit{ERCC1} (C8092A, rs3212986) was recently shown to associate with decreased DNA damage repair capacity for patients carrying \textit{ERCC1} 8092A.\cite{20,41} In addition, with the redefinition of the gene concept, cis- or trans-acting elements and genetic variations could also be located distantly in the genome.\cite{42,43}

It can be argued that our analyses did not include other loci with putative influence on oxaliplatin efficacy. Although some authors found an effect of glucuronyl-S-transferase pi (\textit{GSTP1}) on oxaliplatin toxicity\cite{44,45}, we previously showed that this polymorphism did not
influence chemosensitivity in our CAIRO population. However, it cannot be excluded that results for the ERCC1 polymorphism were confounded by the effect of another coinciding polymorphism or haplotype.

Although the use of in vitro data and transfection experiments offers important information on the molecular physiology of oxaliplatin DNA damage repair, it is subject to limitations. Protein and mRNA expression in transfected cells are plasmid-derived and are no longer regulated by epigenetic systems, leading to supernatural levels of protein. This limits functional analyses in these transfected cells in general and impairs extrapolation of in vitro results to epidemiologic populations. However, in the case of ERCC1, protein functionality and, consequentially, the level of DNA repair are dependent on the concomitant expression of ERCC4. Higher ERCC1 protein levels due to unregulated plasmid derived transcription will therefore not lead to more effective DNA repair. Therefore, we believe our transfection model provides a valid proxy for the analysis of the in vivo effect of the ERCC1 C118T polymorphism on DNA repair after treatment with oxaliplatin.

Overall, our results show that ERCC1 activity is essential in response or resistance to oxaliplatin in colorectal cancer. However, it remains unclear if inter-individual variations in ERCC1 activity influence chemosensitivity. More importantly, it is not known how ERCC1 activity can reliably be measured in a clinical setting. Many studies have used ERCC1 staining in correlation to oxaliplatin efficacy. However, a recently published article highly questions the validity of ERCC1 staining, stressing that only one ERCC1 isoform is functionally active, whereas staining methods cannot differentiate between functional and non-functional iso-forms. Others have used ERCC1 mRNA expression, but results have been equally diverse. Nonetheless, pretreatment indicators of ERCC1 activity are warranted, before its use as a biomarker is possible.

Conclusion

In summary, we showed that ERCC1 function is essential in repair of oxaliplatin-induced DNA damage and thereby influences oxaliplatin cytotoxicity in vitro. However, we found no evidence for functional differences of ERCC1 C118T variants in the cellular response to oxaliplatin exposure. This coincides with our findings that this genetic variant is not associated with clinical outcome in ACC patients treated with oxaliplatin. Further research should focus on elucidating the optimal method for assessing ERCC1 activity and the search for genetic variation in other loci of ERCC1, or epigenetic regulation affecting the gene, since it is evident that ERCC1 expression is indispensable for DNA repair in response to platinum treatment.
Funding

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