DNA repair and cisplatin resistance in non-small-cell lung cancer

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Abstract

The results of cisplatin-based chemotherapy seem to have reached a plateau, and empirical approaches are targeting the inclusion of novel biological agents with different mechanisms of action, but their clinical benefit is still unknown. In preparing this review of cisplatin resistance, we posed two questions: Who are we writing for and why? We believe that medical oncologists should be involved in the reality of the growing list of genetic mechanisms of cancer and chemoresistance. Only by becoming familiar with these mechanisms will we be able to circumvent them. In this review, we provide some insight into DNA repair defects involved in non-small-cell lung cancer (NSCLC) and cisplatin effect. Some DNA repair genes, like ERCC1, have been shown to be crucial in predicting cisplatin resistance and can be used for tailoring cisplatin-based chemotherapy.

Keywords: DNA repair; Cisplatin resistance; Lung cancer

1. Introduction

Cell repair capacity is stored in the linear sequence of approximately \(3 \times 10^9\) copies of the four bases guanine, cytosine, adenine and thymine, aligned in the DNA. A growing list of reports identify DNA damage with the regulation of DNA repair gene transcription and the control of cell cycle progression and apoptosis via DNA damage checkpoints. Different pathways of DNA repair are polymorphic and vary interindividually and with age. These features influence the chemosensitivity of tumor cells toward DNA-reactive cytotoxic drugs [1]. DNA repair is a counteragent in carcinogenesis and an accomplice in cancer therapy resistance [1]. There are several major DNA repair pathways. Excision repair, including nucleotide excision repair (NER) has been strongly linked to cisplatin resistance. Base excision repair (BER) also plays an important role in chemotherapy resistance. Finally, the repair of double-strand breaks, induced by cytotoxic agents, radiotherapy, and reactive oxygen species, is carried out by homologous recombination and non-homologous DNA end joining. Other pathways are mismatch repair (MMR) and one-step repair (OSR), meaning the direct reversal of DNA damage. The repair protein O\(^6\)-alkylguanine-DNA alkyltransferase, also known as O\(^6\)-methylguanine-DNA methyltransferase (MGMT), intervenes in OSR through removal of an alkyl group from the O\(^6\)-atom of guanine in the DNA of cells exposed to alkylating agents. With increasing size of the alkyl group, the relative contribution of MGMT to the repair of O\(^6\)-alkylguanines in DNA decreases and excision repair becomes more relevant (reviewed in [1]). As an example of OSR, treatment with chloroethylnitrosoureas (BCNU) correlates with MGMT activity; in the process of cytotoxic interstrand cross-links in target cell DNA, BCNU initially alkylate the O\(^6\)-atom of guanine. Intriguingly, MGMT levels vary greatly between tumors, which has been used in pharmacogenomic interpretation. Hypermethylation of MGMT (abrogating OSR) was observed in 40% of brain tumors treated with BCNU and was related to significantly better survival [2]. Interestingly, the activity of temozolomide has been linked to tumor MGMT. However, when temozolomide was combined with CPT-11, this mechanism of resistance was circum-

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Keywords: DNA repair; Cisplatin resistance; Lung cancer
vented in tumor cells that were either MGMT proficient or MMR deficient [3].

The possibility of individualizing DNA repair profiles is becoming a central issue in the search for improved chemotherapy results. However, while gene expression profiling with cDNA arrays is on the cutting edge of technology, it has no immediate application in the clinical setting. Cancer cells accumulate multiple genetic abnormalities in signal transduction pathways during carcinogenesis and cancer progression. One Achilles’ heel of tumor cells is defects in DNA repair, which can be responsible for lung oncogenesis while simultaneously conferring a chemotherapy advantage, especially with cisplatin, the drug that has been studied to the greatest extent. Cisplatin is the paradigm of cytotoxic drugs used in the treatment of non-small-cell lung cancer (NSCLC), and like many DNA alkylators, acts as a cross-linker, inhibiting DNA replication, which is the critical target in cancer chemotherapy. Cross-links between guanine bases are induced by cisplatin, carboplatin and oxaliplatin. Cisplatin and carboplatin form an identical cross-link, while the oxaliplatin cross-link is structurally very different due to the bulky 1,2-diaminocylohexane group in the adduct (reviewed in [4]).

After more than two decades of clinical trials, we have reached a therapeutic plateau that is often interpreted nihilistically by non-experts in lung cancer treatment, who argue that nothing is better than the archaic cyclophosphamide or vindesine, respectively, resulted in a 10% improvement in 1-year survival [5]. Pooled data from eight cisplatin trials (778 patients) showed a hazard ratio of 0.73, with an absolute improvement of 10% at 1 year and a 1–1/2-month increase in median survival time [6]. An almost identical number of patients (797) were randomized in a British trial comparing MIC (mitomycin/ifosfamide/cisplatin), radiotherapy alone and palliative care [7]; the magnitude of the benefit was similar to that reported in the meta-analysis [6]. In recent years, new cisplatin combinations have been tested, including vinorelbine [8]. The Le Chevalier et al. study [8] showed a better median survival time for vinorelbine/cisplatin than for vindesine/cisplatin or vinorelbine alone. More recently, a Southwest Oncology Group (SWOG) study [9] found that the median survival time with vinorelbine/cisplatin was similar to that obtained with paclitaxel/carboplatin (8.1 and 8.6 months, respectively). When looking at clinical parameters as prognostic factors in multivariate analyses, cisplatin and female gender emerge as independent variables in addition to performance status, as demonstrated in a review of SWOG trials including a total of 2531 patients [10]. The European Lung Cancer Working Party trial also identified female gender as an independent prognostic variable [11]. Deficient DNA repair capacity enhances cisplatin activity and females have less DNA repair capacity than males. Finally, the yardstick in chemotherapy, the four-arm Eastern Cooperative Oncology Group (ECOG) trial [12], included 1155 eligible patients and observed an overall 19% response rate, 7.9-month median survival time, 33% 1-year survival, and 11% 2-

Table 1 Outcomes in NSCLC trials

<table>
<thead>
<tr>
<th>Response(%)</th>
<th>Survival</th>
<th>Median time to progression (month)</th>
<th>Reference in text</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median (month)</td>
<td>1 year (%)</td>
</tr>
<tr>
<td>CAP</td>
<td>15</td>
<td>6</td>
<td>21</td>
</tr>
<tr>
<td>VdP</td>
<td>25</td>
<td>8</td>
<td>22</td>
</tr>
<tr>
<td>BSC</td>
<td>–</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>MIC 2</td>
<td>32</td>
<td>6.7</td>
<td>25</td>
</tr>
<tr>
<td>BSC</td>
<td>–</td>
<td>4.8</td>
<td>17</td>
</tr>
<tr>
<td>VC</td>
<td>30</td>
<td>10</td>
<td>≈ 40*</td>
</tr>
<tr>
<td>VdC</td>
<td>19</td>
<td>8</td>
<td>≈ 30*</td>
</tr>
<tr>
<td>V</td>
<td>14</td>
<td>7.7</td>
<td>≈ 30*</td>
</tr>
<tr>
<td>VD</td>
<td>28</td>
<td>8.1</td>
<td>36</td>
</tr>
<tr>
<td>Paclitaxel/ carbo</td>
<td>25</td>
<td>8.6</td>
<td>38</td>
</tr>
<tr>
<td>Paclitaxel/C</td>
<td>21</td>
<td>7.8</td>
<td>31</td>
</tr>
<tr>
<td>Gemcitabine/C</td>
<td>22</td>
<td>8.1</td>
<td>36</td>
</tr>
<tr>
<td>Docetaxel/C</td>
<td>17</td>
<td>7.4</td>
<td>31</td>
</tr>
<tr>
<td>Paclitaxel/carbo</td>
<td>17</td>
<td>8.1</td>
<td>34</td>
</tr>
</tbody>
</table>

CAP, cyclophosphamide/doxorubicin/cisplatin; VdP, vindesine/cisplatin; BSC, best supportive care; MIC2, mitomycin/ifosfamide/cisplatin in patients with extensive disease; VC, vinorelbine/cisplatin; V, vinorelbine; C, cisplatin; carbo, carboplatin.

a Survival was extrapolated from reported Kaplan–Meier survival curves.
b Significant difference in time to progression between the paclitaxel/cisplatin control arm and the gemcitabine/cisplatin arm.
year survival. No differences were found between paclitaxel/cisplatin and the three experimental arms. However, median time to progression was significantly better in the cisplatin/gemcitabine arm than in the cisplatin/paclitaxel arm (\(P = 0.001\)). These findings seem to indicate that cisplatin resistance is linked to relative levels of excision repair cross-complementing 1 (ERCC1) mRNA expression. ERCC1 is one of the master genes involved in the NER pathway and is required for synergism between cisplatin and gemcitabine, indicating that the threshold of ERCC1 expression leading to cisplatin failure may be higher when cisplatin is combined with gemcitabine.

2. Pharmacogenomic profiling to predict chemotherapy response

Since the Human Genome Project was completed, a wealth of data has surfaced on genetic polymorphisms and their influence on therapeutic drugs. Pharmacogenomics describes the complex interaction of genes across the genome; it can be used to predict drug response and clinical outcomes, reduce adverse events, and select dosing of drugs. One primary goal of pharmacogenomics is to customize drugs for defined sub-populations of patients (reviewed in [13]). Pharmacogenomic research will change regulatory legislation [14] and the standard clinical approach in the design of phase I, II and III trials. With the influence of pharmacogenomics, important features of future clinical trials will include not only sample size but also allele frequency and gene effect. Table 2 summarizes the potential benefits of the pharmacogenomic approach to clinical trials.

Multiple examples of the relationship between alleles and drug response are evident when single nucleotide polymorphisms (SNPs) are screened. A SNP is the DNA sequence variation that occurs when a single nucleotide (A, T, C or G) in the genome sequence is altered [13]. For instance, a polymorphism of the \(\beta_2\)-adrenergic receptor may influence airway responses to inhaled albuterol in asthma [15]. Another example is the effect of a polymorphism in the gene for insulin-like growth factor-1, which is related to low birthweight and susceptibility to diabetes and cardiovascular disease [16]. Recently, a polymorphism in glutathione S-transferase P1 (GSTP1) was shown to correlate with MGMT methylation in sputum in individuals at high risk for lung cancer [17]. The GSTP1 plays an important role in protecting DNA against damage. A polymorphic site at codon 105 of the GSTP1 (an A-to-G substitution) leads to an isoleucine-to-valine change in the hydrophobic binding region of the protein. Lung cancer patients also had a higher frequency of the GG phenotype than controls [17]. The NADPH quinone oxidoreductase (NQO1) is also involved in antioxidant defenses through metabolism of quinone antioxidants. A polymorphic variant of the gene (C-to-T transition at bp 609 of exon 6) is associated with 3-fold decrease in NQO1 activity. The variant NQO1 allele confers an increased risk of p16 suppression through hypermethylation. This data provides the first link between these polymorphisms and aberrant promoter methylation of the p16 and MGMT genes in respiratory epithelium of subjects at high risk for lung cancer [17].

3. Predictive markers of response

Thymidylate synthase (TS) is a folate-dependent enzyme that is essential for DNA replication and repair. Polymerase chain reaction (PCR) quantification showed that intratumoral TS mRNA levels were related to response and survival in gastric cancer patients treated with fluorouracil (FU) and cisplatin. Patients with high TS mRNA levels (> 4.6) had a median survival of 6 months, while in those with lower levels (< 4.6), median survival was not yet reached at 43 months of follow-up [18]. In colorectal tumors, the TS expression cutoff value for non-response to FU was > 4.1 [19]. Increased TS expression is an important mechanism of resistance to pemetrexed (MTA, multitargeted antifolate), while other drugs like CPT-11, oxaliplatin and paclitaxel remain highly cytotoxic in cells that overexpress TS [20]. The TS gene has a polymorphic tandem repeat sequence. TS genes with a three-repeat sequence have greater expression than those with a two-repeat sequence, indicating the value of TS polymorphism as a predictor of the efficacy of TS-directed chemotherapy. Recent evidence indicates that TS mRNA with a three-repeat sequence has greater translation efficiency than that with a two-repeat sequence [21]. TS genotyping and quantification of TS mRNA are useful in predicting patient response to FU-based chemotherapy (Table 3), and the reverse transcription (RT)-PCR is the most sensitive method for the detection of low-abundance mRNA, often obtained from limited tissue samples (reviewed in [22]). Intriguingly, interindividual variation in response to methotrexate has also been related to TS mRNA levels. The three-repeat TS polymorphism was correlated with significantly lower survival in metho-

| Table 2 | Potential benefits of the pharmacogenomic approach to clinical trials |
|-----------------------------------------------|
| (1) Predicting patient chemotherapy response |
| (2) Developing customized chemotherapy combinations |
| (3) Limiting severe side effects |
| (4) Improving efficacy and patient compliance |
| (5) Improving the accuracy of delivering adequate chemotherapy doses |
| (6) Improving drug development and legal approval of new drugs |
The diphosphate form of gemcitabine inhibits ribonucleotide reductase (RR), which is responsible for creating the deoxyribonucleoside triphosphate building blocks of DNA. Overexpression of RR has been linked to gemcitabine resistance in the human KB cancer cell line [24]. Recent studies have demonstrated that cells with wild-type p53 are more sensitive to the cytotoxic effects of gemcitabine [25,26]. Flavopiridol, a cyclin-dependent kinase inhibitor, enhanced gemcitabine cytotoxicity in several cell lines by down-regulating RR subunit M2 mRNA [27]. Up-regulation of other transcripts are also related to chemotherapy resistance. For example, X-ray repair cross-complementing gene 1 (XRCC1) confers resistance to CPT-11 [28] (Table 3).

### Table 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Potential marker</th>
<th>Association with better response</th>
<th>Degree of evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>FU</td>
<td>TS</td>
<td>Low RNA expression; homozygosity for gene promoter 2R</td>
<td>I</td>
</tr>
<tr>
<td>Methotrexate</td>
<td></td>
<td></td>
<td>II</td>
</tr>
<tr>
<td>Pemetrexed</td>
<td></td>
<td></td>
<td>III</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>ERCC1 XPD polymorphisms</td>
<td>Low RNA expression; XPD polymorphism at codons 751, 312</td>
<td>II</td>
</tr>
<tr>
<td>Gemcitabine</td>
<td>RR</td>
<td>Low RNA expression</td>
<td>III</td>
</tr>
<tr>
<td>Irinotecan</td>
<td>XRCC1</td>
<td>Low RNA expression</td>
<td>II/III</td>
</tr>
</tbody>
</table>

I, accumulated evidence; II, some evidence; III, not yet reported.

trexate-treated children with acute lymphoblastic leuke-

mia [23].

D. DNA Repair Capacity, Lung Cancer Risk and Chemoresistance

Many cancer chemotherapeutic agents, including cisplatin, cause interstrand cross-links, which accounts for their therapeutic cytotoxic properties. Similarly, many carcinogens are bi-functional, causing both mono-

adducts and intra- or interstrand cross-links in DNA. DNA repair capacity is genetically determined; it modulates lung cancer susceptibility and treatment response (reviewed in [29]). DNA repair capacity has been assessed in peripheral blood lymphocytes by the host-cell reactivation assay, which measured cellular reactivation of a reporter gene damaged by exposure to 75 μM benzo(a)pyrene diol epoxide (BPDE) [30]. With this functional assay, the mean level of DNA repair capacity was significantly lower in lung cancer patients than in controls. Younger cases (<65 years) and smokers were more likely than controls to have reduced DNA repair capacity [30]. BPDE-induced DNA adducts are repaired by the NER pathway, in which ERCC1 plays a pivotal role, raising the hypothesis that lung cancer patients with lower ERCC1 levels-and thus lower DNA repair capacity-may have enhanced response and survival with cisplatin-based chemotherapy. In an experimental model, elevated DNA repair capacity was associated with resistance to cisplatin in lung cancer cell lines. In this case, the overall DNA repair capacity was estimated based on the ability of cells to reactivate the pRSV-CAT (chloramphenicol acetyltransferase) plasmid damaged by cisplatin. The pRSV-CAT plasmid contains the bacterial gene for CAT under the control of the RSV long-terminal repeat promoter. Platination of the pRSV-CAT plasmid will diminish or abolish CAT gene expression as a consequence of DNA damage after transfection into cells. Repair of these lesions will restore CAT gene expression and provide information about the overall repair capacity of a given cell population. NSCLC cells were found to be significantly more resistant to cisplatin than small-cell cancer cell lines isolated from untreated patients [31].

The epidemiology of DNA repair capacity and of its effect on cancer susceptibility has been fully developed. In 1998, 64 reports addressed the association of cancer susceptibility with defects in DNA repair capacity [32]. Several assays of DNA repair capacity have been used. With the host cell reactivation assay, DNA repair capacity has been measured in peripheral blood lymphocytes with the host cell reactivation assay and calculated as the percentage of residual CAT gene expression after the repair of ultraviolet radiation- or cisplatin-damaged plasmid DNA divided by that in undamaged plasmid DNA. The host cell reactivation assay measuring the activity of the CAT gene has been used in cells transfected with BPDE-treated plasmid. As a single unrepaired DNA adduct can effectively block CAT transcription, any CAT activity will reflect the ability of the transfected cells to remove BPDE-induced adducts from the plasmids. The most susceptible sub-group of cigarette smokers on the basis of their low DNA repair capacity were case patients who were young (<60 years), female, or light smokers, or who reported a family history of cancer. In contrast, in a study comparing 316 newly diagnosed lung cancer patients and 316 cancer-free control subjects, heavy smokers among both case patients and control subjects tended to have more proficient DNA repair capacity than lighter smokers, suggesting that cigarette smoking may stimulate DNA repair capacity in response to the DNA
damage caused by tobacco carcinogens [33]. This is discussed in more detail below in connection with XPD polymorphisms. Similarly, female smokers with the GSTM1 null genotype, which results in diminished glutathione S-transferase (GST) activity, had the greatest lung cancer risk compared with other groups of females and males with different GSTM1 genotypes. The absence of detoxifying GST activity may result in an excess of internal exposure to tobacco carcinogens, leading to a higher level of DNA damage or adduct formation (reviewed in [33]).

DNA repair capacity has been shown to be significantly higher in medical students during examination periods than after vacation, suggesting a positive association between subject stress levels and DNA repair capacity [34]. In short, defective DNA repair capacity is one of the major factors responsible for carcinogenesis, and at the same time, like a double-edged sword, it can confer a favorable cytotoxic effect. Preliminary hints as to the therapeutic benefit of deficient DNA repair capacity stem from molecular epidemiology studies assessing the DNA repair capacity by the host cell reactivation assay in lymphocytes, which measures the NER capacity.

5. NER capacity and cisplatin effect

It is a common belief that cisplatin exerts its cytotoxic effect by disrupting the DNA macromolecule, mainly through the formation of intrastrand adducts and interstrand cross-links that are repaired through the NER pathway. It is also postulated that tumors that are defective in MMR become more resistant to cisplatin than their MMR-proficient counterparts. The NER pathway consists of several steps: damage recognition, dual incision/ excision, repair synthesis, and ligation. Around 30 proteins participate in this repair process; above all, ERCC1 has a crucial role in the incision process, which is the rate-limiting step of the pathway. ERCC1 is a 15-kb repair gene located on human chromosome 19. ERCC1 forms a heterodimer with XPF, and the ERCC1/XPF complex is responsible for the incision to cleave the damaged strand at the phosphodiester bonds between 22 and 24 nucleotides 5’ to the lesion. A functional ERCC1 is important in the repair of cisplatin DNA adducts and in cisplatin sensitivity in intact cells (reviewed in [35]). ERCC1 mRNA levels, measured by quantitative PCR, were examined in gastric cancer patients treated with cisplatin/FU. cDNA was obtained from primary gastric tumors before chemotherapy, and ERCC1 mRNA levels were expressed as the ratio of the PCR product of the ERCC1 gene and the β-actin housekeeping gene. Primers used for the PCR amplification of the ERCC1 gene were modified from those described originally by Dabholkar [36]. The ERCC1 mRNA level for the 17 responders was 4.9, while the median ERCC1 mRNA level for the 16 resistant patients was eight. The difference between responders and non-responders was statistically significant [35]. The median survival for patients with ERCC1 mRNA levels < 5.8 was not reached at the time the report was published, while median survival for those with levels > 5.8 was only 5.4 months. The difference was highly significant, disclosing for the first time that intratumoral levels of ERCC1 mRNA influence the outcome of gastric cancer patients treated with cisplatin/FU [35]. This study gave no conclusive results on whether ERCC1 mRNA levels could be an independent predictive marker for cisplatin benefit. Originally, ERCC1 mRNA levels were assessed in ovarian cancer tissue from 28 patients harvested before treatment with carboplatin- or cisplatin-based chemotherapy. RT-PCR-based assay was used to determine the level of expression of ERCC1 and β-actin, as well as human xeroderma pigmentosum group A correcting gene (XPAC). Autoradiographs were generated by RT-PCR-amplified RNA after hybridization of Southern blots with radiolabeled probes. Numerical values for the expression of the ERCC1 and XPAC genes in the ovarian tumor tissue samples were obtained using the densitometric readout of the autoradiographic signal generated by the 32P-labeled ERCC1 or XPAC probe divided by the densitometric reading for β-actin. In this case, the numerical values were different from those reported in the literature using quantitative PCR. Thirteen non-responders showed greater levels of ERCC1 mRNA than 15 responders [36].

The relationship between ERCC1 expression and the repair of cisplatin-induced DNA adducts has also been analyzed in human ovarian cancer cells in vitro. A 1-h exposure of MCAS cells (human ovarian cancer cells derived from a 60-year-old Japanese female with mucinous cystadenocarcinoma) yielded a 2-fold increment in ERCC1 mRNA levels and in ERCC1 protein, measured by Northern and Western blottings, respectively, [37].

Intriguingly, ERCC1 antisense RNA abrogates the gemcitabine-mediated cytotoxic synergism with cisplatin in human colon tumor cells that were proficient in NER. Experimental results indicate that stable expression of ERCC1 antisense mRNA down-regulates the level of mRNA and repair activity. The down-regulation of the repair activity significantly correlated with the reduction of the cytotoxic synergism between gemcitabine and cisplatin [38]. These findings raise the hypothesis that the cutoff for ERCC1 mRNA levels used to predict cisplatin response could be higher when cisplatin is combined with gemcitabine. In fact, in the ECOG trial [12] (Table 1), the only significant difference was observed in time to progression when paclitaxel/cisplatin was compared with gemcitabine/cisplatin. Time to progression was longer in the gemcitabine/cisplatin
arm. This could give a slight advantage in patients harboring relatively high ERCC1 mRNA levels.

Along the same lines, ERCC1 mRNA levels have been correlated with oxaliplatin resistance in colorectal cancer patients. Median survival for patients with ERCC1 expression <4.9 was 10 months, while for patients with ERCC1 expression >4.9, it was 1.9 months [39]. These findings indicate that intratumoral ERCC1 mRNA may be an independent predictive marker for oxaliplatin combination chemotherapy. Both this study and the original one in gastric cancer [35] were carried out by investigators from the University of Southern California (USC)/Norris Comprehensive Cancer Center and Response Genetics.

We have analyzed the role of ERCC1 expression in NSCLC patients treated with gemcitabine/cisplatin. The median ERCC1 expression in 56 patients analyzed, relative to the expression of the control β-actin, was 6.7. Patients with ERCC1 expression >6.7 had a median survival of 5 months, in contrast with those with lower levels, where the median survival was 15 months. This difference was statistically significant, and importantly, in a Cox multivariable analysis, ERCC1 levels surfaced as an independent predictive variable. The fact that the cutoff was higher than previously described indicates that a certain level of ERCC1 is required for synergism between gemcitabine and cisplatin [40].

The potential role of the 5′-UTR in ERCC1 mRNA expression has also been examined. RT-PCR was carried out with primers targeting the 5′-UTR region to amplify a fragment containing exon I (UTR) and exon II (containing the initiation codon) of the ERCC1 gene in 121 ovarian cancer samples. Interestingly, two PCR amplimers from the same sample for the target segment within the UTR region appeared in some samples. The prevalence of the two amplimers occurred in the group of patients with high ERCC1 mRNA levels (48%). In contrast, only 5% of patients with a single amplimer showed high ERCC1 mRNA levels. Direct DNA sequencing of the cDNA from each of the 121 ovarian cancer tumor samples confirmed that tumors with two amplimers contained two distinct sequences. The longer sequences included the complete target sequence, 261-bp (wild type), and the shorter sequences demonstrated a 42-bp deletion [41]. This 42-bp deletion seems to be associated with high ERCC1 mRNA levels.

Overall, ERCC1 stands out as a potential predictive marker for cisplatin-based chemotherapy and it could be the basis for customized chemotherapy.

7-Hydroxystaurosporine (UCN-01) is an inhibitor of cyclin-dependent kinases [42], but interestingly it enhances cisplatin cytotoxicity by interfering with ERCC1 [43]. A host-cell reactivation assay has been used to quantify the effect of UCN-01 on DNA repair activity. The results indicated that UCN-01 enhanced DNA platination in A549 cells up to 71% when compared with the untreated controls. The increase could not be explained by an enhanced cellular uptake of cisplatin because the intracellular platinum level was not affected by cisplatin treatment. The effects of UCN-01 on DNA repair activity were also analyzed by an in vitro repair synthesis assay. In the extracts from UCN-01-treated A549 cells, the repair activity was significantly reduced when compared with that of their untreated counterparts [43]. This kind of G2 checkpoint inhibitor warrants further investigation in association with cisplatin in those tumors with high ERCC1 mRNA levels. The loss of G2 cell cycle checkpoint can increase chemosensitivity. After DNA cytotoxic damage, cells are usually arrested at the G2 checkpoint. However, G2 checkpoint inhibitors can prevent tumor cells from stopping at this checkpoint, leading to cell death in the mitotic cell division. This phenomenon has been termed ‘mitotic catastrophe’ (reviewed in [1,42]) (Fig. 1). The Chk1 kinase and the Cdc25C pathway are potential targets of UCN-01 and flavopiridol in the abrogation of G2 checkpoint [44].

Chk1 mediates phosphorylation of Cdc25C, leading to a p53-induced specific isoform of the 14-3-3 protein, which renders Cdc25C inactive and prevents progression into mitosis until DNA is repaired [45] (Fig. 1). Recently, a model of the DNA damage checkpoint that integrates BRCA1 has been proposed, showing that BRCA1 could be a Chk1 activator [46]. The isoform 14-3-3δ plays a crucial role in the G2 checkpoint by sequestering Cdc2-cyclin B1 in the cytoplasm. The DNA methylation status and expression level of the 14-3-3δ gene was recently examined in 37 lung cancer cell lines and 30 primary lung tumor specimens. Small-cell lung cancer cell lines showed frequent DNA hypermethylation (69%) with silencing of the 14-3-3δ gene. Among NSCLCs, large cell lung cancer cell lines showed frequent methylation (57%). These findings suggest that the 14-3-3δ gene is involved in lung cancer in a histological type-specific manner [47].

Understanding the role of cell-cycle checkpoints in DNA repair could be extremely important in designing novel therapeutic approaches in NSCLC [48]. Fig. 1 describes possible novel chemotherapy approaches based on the strengths and weaknesses of tumor cells with defects in DNA repair capacity and the activity of cytotoxic drugs at various checkpoints of the cell cycle. Docetaxel/cisplatin can enhance mitotic catastrophe when combined with G2 inhibitors that abrogate the G2 checkpoint, and selective cyclooxygenase (COX) inhibitors like celecoxib could inhibit COX-2 and prostaglandin (PGE2). Moreover, the antiproliferative effects of celecoxib have been observed in a similar magnitude in COX-2-negative epithelial cell lines [49]. The antiproliferative effects of these drugs could be mediated by peroxisome proliferator-activated receptor-
The PPARγ is a member of the nuclear hormone receptor superfamily that regulates transcription of target genes in a ligand-dependent manner. PPARγ mRNA proteins are expressed in NSCLC cell lines, with higher levels in adenocarcinoma. PPARγ protein was also expressed by immunohistochemistry in 50% of primary lung cancers. In addition, ligands of PPARγ induced differentiation and apoptosis in NSCLC [50]. PPARγ is also highly expressed in adipocytes and functions as a master regulator of adipogenesis to promote adipocyte differentiation and maturation.

Synthetic thiazolidinediones (TZDs) have been identified as specific activators of PPARγ, and troglitazone has been used for treatment of diabetic patients by enhancing insulin sensitivity. TZDs have been demonstrated to inhibit growth of several carcinoma cell lines that express endogenous PPARγ. In elegant experiments, troglitazone induced cellular growth arrest and apoptosis in NSCLC cell lines. Troglitazone stimulates expression of the growth arrest and DNA-damage inducible (GADD)153 gene. However, it did not stimulate GADD153 mRNA levels in undifferentiated cells lacking PPARγ expression [51]. On these grounds, it has been suggested that the combination of differentiation therapy with histone deacetylases (HDAC) inhibitors and ligands of the PPARγ could cause a potent growth-inhibitory and differentiation-inducing effect, mainly in lung adenocarcinomas [52]. HDAC inhibitors are a growing family of enzymes involved in the general control of the acetylation of histones and in the accessibility of DNA to the transcriptional machinery. Some HDACs are recruited by transcription repressors to silence specific genes. HDAC inhibitors can counterbalance gene differentiation markers and enhance the effect of PPARγ. In addition, HDAC inhibitors can not only relieve gene silencing but also inhibit VEGF expression, acting as antiangiogenic agents [53]. PPARγ activation has been shown to induce differentiation and growth inhibition in liposarcoma patients treated with troglitazone [54].

Interestingly, microtubule-damaging agents like paclitaxel and docetaxel induce COX-2 [55], which leads us to speculate that celecoxib may attenuate COX overexpression and enhance the therapeutic effect of taxanes.

Fig. 2 shows a theoretical approach that envisages the novel management of NSCLC in different disease stages. This approach is based mainly on the outcome plateau that seems to have been reached with cisplatin doublets [12]. For instance, the therapeutic effect of docetaxel/cisplatin can be enhanced by adding flavopir-
idol, as described above. The effect of flavopiridol on ERCC1 is not known, but it merits investigation in prospective trials. Other agents can be added to this combination of genotoxic drugs (cisplatin, docetaxel) with G2 checkpoint inhibitors (flavopiridol). For example, celecoxib can be used because of its anti-angiogenic properties and its function as a COX-2 inhibitor. In addition to this model, HDAC inhibitors and a PPARγ agonist can be included, opening the gates to maintenance treatment.

6. DNA repair polymorphisms

Table 4 summarizes the three principal DNA repair pathways and lists several genes in which polymorphisms have been described. XRCC3, involved in the homologous double-stranded repair pathway, prevents chromosomal fragmentation, translocations and deletions. XRCC3 is structurally related to Rad51, which is a key component of recombination repair. An association has been observed between the exon 7 T/C polymorphism at position 18067 of the XRCC3 gene and the risk of developing melanoma [56].

![Diagram](https://example.com/diagram.png)

Fig. 2. Theoretical model illustrating the combination of different agents and their synergistic effect. The model can be used in different stages of NSCLC.

One of the most important polymorphisms is that of the XPD gene, which is involved in the NER pathway. The disease xeroderma pigmentosum was characterized by deficient repair of damaged DNA containing ultraviolet-induced pyrimidine dimers [57]. XPD, also known as excision repair cross-complementing group 2 (ERCC2), is involved in NER and basal transcription. Two important polymorphisms have been identified at Lys751Gln and Asp312Asn, which have been associated with altered DNA repair capacity and, hence, with lung oncogenesis. In an epidemiological study matching 341 lung cancer cases with 360 smoker control subjects, a host-cell reactivation assay measuring the activity of the CAT gene was used in cells transfected with BPDE-treated plasmids. DNA repair capacity was lower in the lung cancer patients than in the controls. The variants Gln751Gln and Asn312Asn had suboptimal DNA repair capacity, with a significant increase in the hazard ratio, in contrast with the wild-type genotypes both in cases and controls, which exhibited the most proficient DNA repair capacity [58]. The frequency of homozygous variants is 10% for either codon. In an intermediate group with heterozygous polymorphisms, the frequency was 40% at either codon. This leads us to speculate that this group could have an intermediate outcome. The clinical interest of these findings lies in their potential usefulness in identifying in constitutional DNA from lymphocytes the polymorphisms associated with suboptimal DNA repair capacity and their potential role in identifying patients with better response to cisplatin chemotherapy.

Table 4

<table>
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<th>DNA repair pathways</th>
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<tr>
<td>NER pathway ERCC1, XPD, XPF</td>
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<td>Base excision pathway (BER) XRCC1</td>
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<td>Homologous double-stranded repair pathway XRCC3</td>
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A recent study has added some complexity to the clinical interpretation of these polymorphisms, since the relevance of the XPD variants decreases significantly in function of the patient’s pack-year history. This study analyzed XPD polymorphisms in 1092 lung cancer patients and 1240 controls and observed a stronger influence of Asn312Asn than of the Gln751Gln. However, when comparing individuals with Asn312Asn plus Gln751Gln versus individuals with wild-type genotypes, the hazard ratio was 2.56 in non-smokers and 0.69 in heavy smokers (80 pack-years). A stronger gene-smoking interaction was noted for the 312 than for the 751 polymorphism [59]. In a clinical interpretation of these findings, smoking history may be essential for correlating the polymorphisms with potential response to cisplatin-based chemotherapy.

Ethnicity can also influence the frequency and the clinical usefulness of polymorphisms. A recent report from Korea shows that the assessment of XPD polymorphisms may not be useful in Korean patients, since the frequency of the Gln751Gln genotype was observed in 0.4% of a total of 250 male lung cancer patients. In addition, the frequency of the heterozygous Lys751Gln was only 11% [60].

The only study correlating cisplatin response with XPD polymorphisms was performed at the USC/Norris Comprehensive Cancer Center in relapsing colorectal cancer patients treated with oxaliplatin/FU. Responders were higher in the Lys751Lys genotype group, and median survival for this group was 17.4 months, compared with 12.8 months for those with Lys751Gln, and only 3.3 months for those with Gln751Gln (P = 0.002). The polymorphism Asp312Asn had no effect on survival or response. This study is significant, as it hints at three potentially different survival outcomes according to the polymorphism pattern [61].

Other polymorphisms are involved in the BER, of which the most studied is the XRCC1 Arg399Gln. This polymorphism has been associated with reduced DNA repair capacity and with the incidence of seven different tumors (head and neck, breast, bladder, lung, stomach, colorectal, and non-melanoma skin cancer). The XRCC1 homozygous variant Gln399Gln has been linked to reduced risk of skin cancer [62]. USC investigators have also correlated this polymorphism with lower response and survival in colorectal cancer patients.

Finally, the homologous double-stranded repair pathway could play a decisive role in chemoresistance. This has been elegantly reviewed elsewhere [63].

In conclusion, we are on the verge of being able to use our considerable knowledge on genetic abnormalities in a simple way, translating them from the bench to the bed. Analysis of ERCC1 mRNA, obtained from paraffin-embedded samples, can enable investigators to follow a pharmacogenomic approach in tailoring cisplatin-based chemotherapy. Further clinical research is needed to clarify whether XPD polymorphisms may be a good alternative for ERCC1 mRNA levels in designing designing cisplatin chemotherapy.

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References


