

Platinum Resistance: The Role of DNA Repair Pathways

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Abstract Although platinum chemotherapeutic agents such as carboplatin, cisplatin, and oxaliplatin are used to treat a broad range of malignant diseases, their efficacy in most cancers is limited by the development of resistance. There are multiple factors that contribute to platinum resistance but alterations of DNA repair processes have been known for some time to be important in mediating resistance. Recently acquired knowledge has provided insight into the molecular mechanisms of DNA repair pathways and their effect on response to chemotherapy. This review will discuss the most important DNA repair pathways known to be involved in the platinum response, i.e., nucleotide excision repair (NER) and mismatch repair (MMR), and will briefly touch on the role of BRCA in DNA repair. The therapeutic implications of alterations in DNA repair which affect response to platinum in the treatment of patients with malignant disease, such as excision repair cross-complementation group 1 (ERCC1) deficiency and mismatch repair deficiency, will be reviewed.

Platinum chemotherapeutic agents have a broad range of activity in malignant disease and are used to treat many types of cancer. They are particularly active against germ cell tumors and epithelial ovarian cancer and play a primary role in the treatment of small cell and non-small cell lung cancer, cervical cancer, head and neck cancer, colorectal cancer, and bladder cancer (1). Unfortunately, resistance has limited the efficacy of these agents in most diseases. Resistance to platinum-based chemotherapy can be intrinsic or acquired and may be mediated by factors outside or within the cancer cell or at its cell membrane. Resistance to platinum chemotherapy is multifactorial (2–6). There are five recognized DNA repair pathways that protect cellular DNA from injury: nucleotide excision repair, mismatch repair (MMR), double-strand break repair, base excision repair, and direct repair. This review will focus on the first two mechanisms, which seem to play a key role in mediating platinum resistance in cancer treatment. They may also be important in assessing patient prognosis in the clinical setting (3, 7).

Cisplatin was the first platinum compound approved for the treatment of cancer, although its initial use was limited by gastrointestinal and renal toxicities. Subsequent efforts to develop an analogue with less toxicity resulted in carboplatin (1). Cisplatin and carboplatin work by binding to DNA and forming DNA adducts leading to intrastrand or interstrand cross-links which disrupt the structure of the DNA molecule, leading to steric changes in the helix (8). Alteration in the structure of the DNA molecule leads to cellular DNA damage recognition and repair which can result in the continued viability of the cell resulting in platinum resistance. It appears

that tumor cells can have intrinsic differences in DNA repair mechanisms when compared with their normal counterparts, although alterations may also be acquired.

The role that DNA repair pathways play in mediating platinum resistance has been studied for many years, first in the preclinical, and more recently, in the clinical setting, and this knowledge has recently been used prospectively in the clinical arena (9).

Molecular Mechanisms of DNA Repair

Nucleotide excision repair. Pathway nucleotide excision repair seems to be a key pathway involved in mediating resistance or sensitivity to platinum chemotherapeutic agents (8). Nucleotide excision repair is a highly conserved DNA repair pathway that repairs DNA lesions which alter the helical structure of the DNA molecule and interfere with DNA replication and transcription. Important steps in this pathway include the recognition of DNA damage and demarcation of the specific area affected, followed by the formation of a complex to unwind the damaged portion and excise it. Finally, the excised area is resynthesized and ligated to maintain the DNA molecule. The excision repair cross-complementation group 1 (ERCC1) protein plays a key role in nucleotide excision repair. ERCC1 dimerizes with xeroderma pigmentosum complementation group F, and this complex is required for the excision of the damaged DNA (Fig. 1).

One of the most impressive and important successes of cisplatin-based therapy is in its role in treating metastatic testicular cancer which has resulted in >90% of patients achieving cure (10). Studies of testicular carcinoma cell lines were notable for exquisite cisplatin sensitivity in comparison with cell lines derived from other cancers (11). Further *in vitro* study showed a deficiency in nucleotide excision repair in these cell lines, and in particular, reduced levels of ERCC1 and xeroderma pigmentosum complementation group F DNA repair proteins (11, 12). This finding has also been shown in multiple studies with human ovarian cancer cell lines,

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including cell lines with intrinsic cisplatin resistance which showed increased sensitivity to cisplatin after antisense RNA inhibition of ERCC1 (13). In addition, cell lines that developed resistance *in vitro* after exposure to cisplatin chemotherapy were found to have increased expression of ERCC1 (14).

The Mismatch Repair pathway. MMR is a highly conserved, strand-specific repair pathway which follows a stepwise process, initiated with the recognition of DNA damage (15, 16). MMR proteins, called Mut proteins, recognize mismatched or unmatched DNA base pairs or insertion-deletion loops and initiate the assembly of as yet unknown proteins which excise the affected area after which it is resynthesized by DNA polymerase (Fig. 2). When MMR is deficient, unrepaired areas of DNA accumulate, resulting in microsatellite instability (17). This accumulation occurs when unrepaired base pair mismatches are replicated during DNA synthesis, and due to slippage of the synthetic complex at these areas, repeats are formed. Defects in MMR may be inherited, as in the case of hereditary nonpolyposis colorectal carcinoma (18), or may occur through epigenetic silencing of an essential MMR gene (17, 19, 20). Epigenetic silencing of MMR seems to occur most often through Mut L homologue 1 (hMLH1) promoter hypermethylation, as has been shown in ovarian, endometrial, gastric, and colorectal carcinoma, among others (17, 21, 22).

A functional MMR system is required for the detection of damaged DNA created by cisplatin and carboplatin (23, 24). Platinum complexes interfere with normal MMR activity and prevent a repair from being completed. Inability to complete the repair of the DNA damage leads to apoptosis. When MMR is deficient, cells can continue to proliferate in spite of DNA damage caused by platinating agents, and are thus resistant. The *MLH1* and *MSH2* (Mut S homologue 2) genes seem to be particularly important in the normal function of the MMR system.

It is notable that MMR deficiency seems to confer resistance to cisplatin and carboplatin, and not to oxaliplatin (25), a newer platinum analogue. MMR proteins do not recognize the adducts formed by oxaliplatin, and the repair pathway is not triggered. Therefore, there is no failure of repair resulting in subsequent apoptosis. Oxaliplatin has activity in cells that are resistant to cisplatin and carboplatin (26).

Recent Advances and Translational Implications

Evaluation of ERCC1 mRNA levels in tumor samples taken from patients in small retrospective clinical trials of ovarian (27, 28), colorectal (7, 29), and non-small cell lung cancer (30) had shown an inverse correlation with either response to platinum therapy or survival.

More recently, tumor samples from a subgroup of 761 patients with metastatic lung cancer who participated in the International Adjuvant Lung Cancer trial were retrospectively evaluated by immunohistochemical analysis of ERCC1 (31). This study showed a statistically significant survival benefit in patients with low levels of ERCC1 who had received platinum-based chemotherapy, compared to patients with low levels of ERCC1 who did not receive chemotherapy and patients with high levels of ERCC1 who received cisplatin chemotherapy.

The results of the first prospective randomized trial using ERCC1 mRNA levels to assign chemotherapy in patients with non-small cell lung cancer were published recently (9). In this trial, 444 patients with stage IIIB (with malignant effusion) or

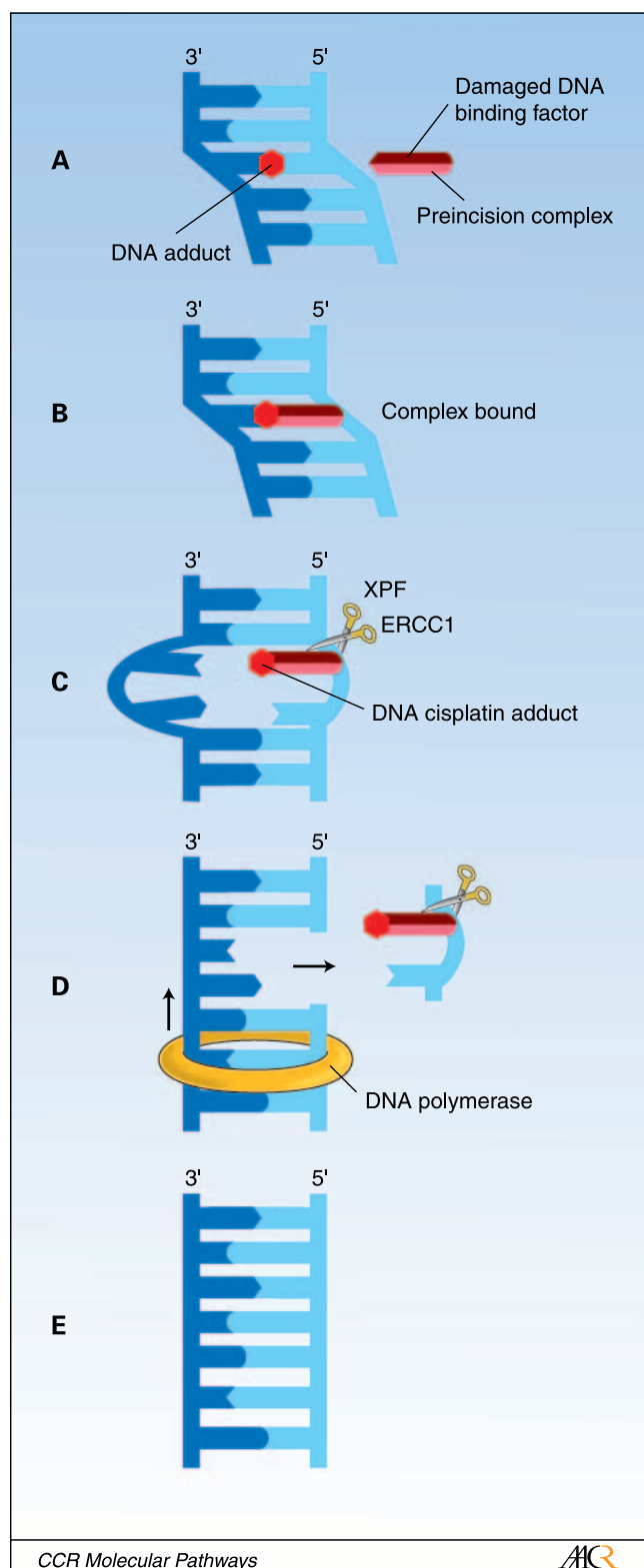
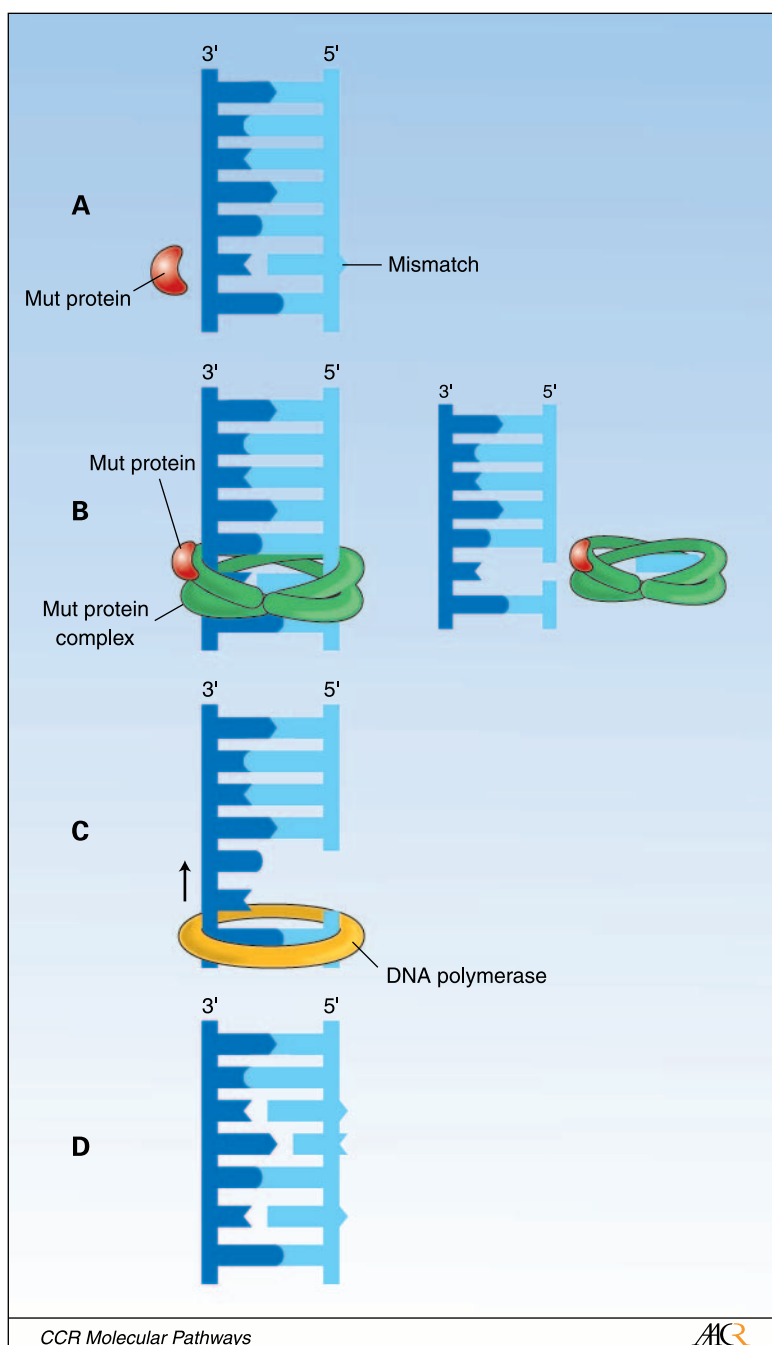


Fig. 1. A, NER-nucleotide excision repair begins when a DNA adduct is formed and causes a change in the shape of the DNA helix. B, damaged DNA binding factor binds to preincision complex and this protein complex localizes to the damaged area of DNA. C, the DNA helix is unwound and the damaged portion is excised by ERCC1/XPF. D, DNA polymerase then resynthesizes the absent portion of DNA. E, when NER-nucleotide excision repair is complete, the DNA is repaired and resumes its normal helical shape. In the setting of ERCC1 deficiency, the DNA cannot be repaired, and the altered DNA is unable to replicate, or perform its normal function, leading to cell death.

Fig. 2. *A*, MMR occurs when the Mut protein recognizes a mismatch or insertion/deletion loop. *B*, the Mut protein orders the assembly of a protein complex which localizes to the affected area on the DNA molecule and excises it. *C*, DNA polymerase then resynthesizes the missing portion of DNA. *D*, accumulation of insertion deletion loops on a strand of DNA in the setting of MMR deficiency.



stage IV non-small cell lung cancer were enrolled from August 2001 to October 2005 and randomized to a control arm in which they received standard cisplatin/docetaxel chemotherapy versus a genotypic arm in which patients with high levels of ERCC1 received a non-platinum-containing regimen of gemcitabine/docetaxel and patients with low ERCC1 expression received cisplatin/docetaxel. Patients in the experimental (genotypically assigned) arm had a statistically significant improvement in response rate compared with the patients of the control arm, although the progression-free survival and overall survival rates were not significantly different. Of note, 18% of patients in the experimental arm were withdrawn due to the lack of sufficient tumor specimens for analysis.

Additionally, the patients in the control arm did not have specimen analysis for ERCC1 levels, as was done in the International Adjuvant Lung Cancer-Bio study (9).

Data from *in vitro* systems have shown that suppression of ERCC1 expression enhances or restores platinum sensitivity. This finding has significant therapeutic implications, as the addition of agents targeting ERCC1 may enhance platinum activity and/or reverse resistance, although this has yet to be tested in patients. Of interest, clear cell ovarian cancer has been found to have higher levels of ERCC1 (32). In the treatment of ovarian carcinoma, it is known that patients with clear cell histology tend to have platinum-resistant disease and have a poorer prognosis compared with their counterparts with other

histologies. Additionally, a subset of clear cell ovarian cancers has been shown to have a high level of MMR deficiency (33). This may be a plausible group in which to evaluate alternative chemotherapeutic regimens in comparison with standard carboplatin-based chemotherapy in a prospective, randomized fashion.

In a series of samples taken from 24 patients with ovarian carcinoma who underwent biopsies before and after treatment with five to six cycles of cisplatin-based chemotherapy, 15 specimens showed microsatellite stability before undergoing treatment (34). All of these converted to microsatellite instability in the posttreatment specimens, and 11 of the 15 showed down-regulation of hMLH1 expression. Independently, Fink and co-investigators showed increased MMR deficiency and down-regulation of hMLH1 expression after treatment with cisplatin in paired tumor samples taken from patients before and after treatment (35). More recently, it has been shown that ovarian carcinomas which show hMLH1 hypermethylation are platinum resistant (36), although microsatellite instability and hMLH1 hypermethylation are rare in ovarian carcinoma. Cameron and colleagues have shown success in the reversal of acquired MMR deficiency through demethylation of the CpG island of the *MLH1* promoter region using 5-azacytidine (37).

Additionally, it has been shown that a combination of p53 inactivation and MMR deficiency results in cisplatin resistance (38, 39). Lin and Howell evaluated human colon carcinoma cells exposed to sequential cycles of cisplatin and found that treatment with cisplatin led to the selection of resistant lines and that, although the loss of either p53 or MMR function resulted in platinum resistance, resistance occurred more rapidly in cells in which both p53 and MMR were defective.

A small retrospective analysis reviewed the outcome of 93 patients with advanced non-small cell lung cancer treated with gemcitabine and oxaliplatin versus gemcitabine and cisplatin (40). A statistically significant difference in the response rate was seen in patients with hMSH2 deficiency (38% gemcitabine/oxaliplatin versus 0% gemcitabine/cisplatin), suggesting that oxaliplatin is active in cells with MMR deficiency.

Gifford and associates evaluated 138 blood samples taken from patients with ovarian or primary peritoneal carcinoma who participated in SCOTROC1 (41). This trial included patients with stage IC to IV disease; patients were randomized to receive carboplatin with either paclitaxel or docetaxel, and blood samples were collected before chemotherapy and at the time of disease relapse. Plasma DNA was analyzed for the

methylation of *hMLH1*. Twenty-five percent of samples taken at relapse were positive for methylation of *hMLH1* that was not positive at diagnosis. Additionally, this methylation correlated with poor overall survival independent of the disease-free interval.

Knowledge of the differential effects of oxaliplatin and cisplatin/carboplatin in the treatment of cancers with high incidence of MMR deficiency may indicate that studies should be done in other disease sites in a fashion similar to those done in patients with lung cancer based on ERCC1 expression. However, before such trials can be done, a reproducible method for evaluating MMR deficiency must be developed which is efficient enough to be used in real time for patients awaiting treatment for their cancer. Challenges remain, as not all of the steps in the pathway and the molecules involved in MMR are completely elucidated.

On a final note, the evolving story of the role of the tumor suppressor genes, *BRCA-1* and *BRCA-2*, in DNA repair suggests that they have roles in mediating the response to chemotherapeutic treatment in some cancers (42). Their importance in homologous recombination during the repair of double-stranded breaks has been purported as a possible explanation of the increase in cancer risk in carriers of germ line mutations of *BRCA-1* or *BRCA-2* (43). More recently, it has been shown that *BRCA-1* plays an integral role in BASC (*BRCA1*-associated genome surveillance complex), a group of proteins which includes multiple DNA repair proteins, including those involved in MMR (44). The *BRCA* pathway can also be disrupted in sporadic cancers (45). *In vitro*, it has been shown that inhibition of the Fanconi anemia/*BRCA* pathway can enhance sensitivity to cisplatin in cancer cell lines and that repair of defects in this pathway can induce cisplatin resistance (46). One strategy that has been pursued is to inhibit the base excision repair pathway by targeting the enzyme poly-ADP ribose polymerase (PARP) in *BRCA-1* and *BRCA-2* mutant cells. This was done with RNA interference as well as through treatment of *BRCA-1* or *BRCA-2*-deficient cells with small molecule inhibitors of PARP-1 and PARP-2 (47, 48). In both cases, PARP inhibition resulted in enhanced cell death in *BRCA*-deficient cells. PARP inhibitors are currently being evaluated for safety and efficacy in early phase trials in combination with various chemotherapeutic agents.

As the capacity to evaluate tumors at the molecular level expands, such tools are likely to predict response to treatment, and ultimately lead to more individualized, targeted, and more effective therapies.

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