

Unlocking the Molecular Mechanisms of DNA Repair and Platinum Drug Resistance in Cancer Chemotherapy

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Abstract: This review article is devoted exclusively to DNA repair and acquired resistance to platinum compounds, with an emphasis on research needs and clinical application. It focuses on the role of genetic variants (gene mutations and SNPs) and epigenetic alterations (methylation and acetylation). Four major DNA repair pathways and one enzyme-correction mechanism are presented: Base Excision Repair, Nucleotide Excision Repair, DNA Double-Strand Break Repair, Mismatch Repair and Direct Damage Reversal. It is suggested that one cause of platinum resistance is more accurately described as *alterations of DNA repair system* rather than *activation of DNA repair mechanism*, as this cause of resistance is brought about by changes in genetic and epigenetic regulation. Given what is known, research on DNA repair and platinum resistance might best be directed at 1) transcriptional cis-elements (activators/repressors) within promoters of essential DNA repair genes, 2) effects of epigenetic alterations, and 3) connections between gene expression and DNA methylation or protein acetylation. Special attention should be directed at three interrelationships: between the different DNA repair pathways; between DNA methylation and protein acetylation; and between DNA repair pathways and DNA methylation or protein acetylation. Improved clinical outcomes may be achieved by restoring wild type p53 with small molecule drugs, the use of gene demethylation strategies, individual-targeted treatment of BRCA mutation carrier, and combining platinum compounds with molecularly-targeted drugs such as EGFR inhibitors.

Key Words: DNA repair, platinum resistance, genetic alteration, methylation, acetylation, epigenetic regulation.

1. INTRODUCTION

In recent years several excellent review articles have examined the multifactorial nature of platinum resistance in cancer chemotherapy [1-5]. These articles address the major causes of platinum resistance, including reduced membrane drug transport, increased cytoplasmic detoxification, and increased DNA repair leading to reduced apoptosis. Developmental milestones of platinum drugs, clinical applications, mechanism of action, and strategies to overcome resistance are also addressed.

Though these review articles include DNA repair, it is not discussed in detail. This review article is devoted solely to DNA repair and platinum resistance. A growing body of evidence suggests that acquired platinum resistance is more accurately described as caused by *alterations of DNA repair system* rather than *activation of DNA repair mechanism*, as the resistance is induced by changes in genetic and epigenetic regulation. This review classifies platinum resistance due to altered DNA repair system in terms of specific DNA repair pathway(s) and/or mechanisms involved.

Genomic and microsatellite instabilities, genetic defects of DNA repair genes, and epigenetic alterations such as DNA methylation and protein acetylation play an important role in cancers [6-11]. Genetic variants and defects in DNA

repair genes are associated with many human genetic syndromes or specific disorders [12-15]. Research findings suggest that intrinsic or acquired resistance to chemotherapeutic drugs, including platinum compounds, can be attributed at least in part to altered expression or mutation of DNA-repair genes, and alterations of DNA repair pathways [16-19].

2. THE STEPS OF PLATINUM THERAPY

A report on the 10th International Symposium on Platinum Coordination Compounds in Cancer Chemotherapy noted that diverse factors can lead to ineffective therapy with platinum compounds [20].

Successful platinum therapy requires first that the platinum drug stay in the bloodstream or peritoneal cavity long enough to reach malignant cells. Rapid drug clearance results in reduced quantities of drug reaching the tumor, while delayed clearance can lead to serious toxic effects. Second, the drug must traverse the cell membrane. This as a complex process involving both inward and outward transporters. Reduced transport can lead to insufficient intracellular platinum and treatment failure. Third, once inside cells, the platinum drug must bind DNA and cause sufficient damage. Finally, for cell death to occur, the damaged DNA must not be repaired. However, as discussed below, many genetic and epigenetic alterations in the DNA Repair System can influence or lead to platinum resistance [20].

Platinum compounds such as cisplatin, carboplatin and oxaliplatin are frequently used in clinical settings. These nonclassic alkylating agents are used alone or in combination with other drugs in the treatment of cancer of the ovary, tes-

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tis, bladder, head and neck, cervix, lung, bone and nervous system [21].

3. PLATINUM DRUG RESISTANCE

In clinical practice, drug resistance refers to progressive disease appearing in patients who are receiving chemotherapeutic drugs with manageable toxicity. Minor changes in drug sensitivity in tumor cells may result in clinical resistance due to the low therapeutic index [22,23].

In vitro findings indicate that increased DNA repair activity/capacity is often an important factor in acquired platinum resistance in cancer cells [24,25]. Scanlon and colleagues found that repeated cisplatin challenges resulted in a cell line with stable resistance and elevated levels of expression of DNA repair genes [26]. Other studies demonstrate that removal of platinum adducts from DNA occurs more effectively in platinum-resistant cells compared to sensitive parental cells. Hypersensitivity to platinum-containing drugs often results from a reduced DNA repair capacity in response to platinum-DNA adducts [27,28].

Nucleotide excision repair (NER) is a major pathway for platinum-induced DNA adduct repair, with ERCC1 (the critical enzyme within the NER pathway) contributing to platinum resistance through promoter-mediated transcriptional regulation [28-31].

Conversely, the observation that testicular cancer cells have low constitutive expression for NER enzymes (XPA, ERCC1, and XPF) may help explain why testis tumors respond well to platinum chemotherapy [32-35]. In non-small cell lung cancer ERCC1 expression is predictive of shorter survival; undetectable level of ERCC1 in such tumors is associated with significant survival [36,37]. A phase III clinical trial with cisplatin-based intervention directed at quantitative mRNA expression of *ERCC1* gene is now underway with non-small cell lung cancer patients [38].

More recently, investigators report that cancer stem cells appear to contribute to cisplatin resistance in a breast cancer mouse model [39].

4. THE DNA REPAIR SYSTEM

The cellular genome is under constant attack by exogenous mutagens (cytotoxic and genotoxic chemicals and drugs, ultra-violet light, and ionizing radiation) and endogenous mutagens (from normal cell metabolism or biochemical processes such as deamination, depurination and oxidation) [40-42]. In addition, DNA replication errors occur following inadvertent mismatches or as a consequence of insertions/deletions [43,44]. The damage, modification, and replication errors change gene expression and result in the loss or malfunction of a gene-encoded protein.

Fortunately, an elaborate DNA repair system consisting of several repair pathways and mechanisms has evolved in mammalian cells to minimize genetic damage and to monitor the integrity of the genome. Cells can rely on a wide variety of enzymes and repair processes to restore their DNA. At present, about 150 genes are known to be directly involved in DNA repair, including genes that encode DNA repair enzymes and genes that respond in other ways to DNA damage

[45]. These gene products operate to repair DNA and to modulate the cellular response to chemotherapy. Other genes affect DNA repair indirectly by regulating cell cycle control [45-48].

In the case of platinum resistance, the DNA repair system is diverted to the repair of platinum-induced damage intended to kill tumor cells. Platinum adducts are repaired by a process of ‘cutting, removing, and replacing’ that involves many proteins in the nucleotide excision repair pathway [20,30,49,50].

DNA damage is repaired by one or more of the four major DNA repair pathways (BER, NER, DSBR and MMR) or by a simple enzyme-correction mechanism (DDR). The type of repair depends on the type of damage (Fig. 1). Alterations in any of the pathways or mechanism can lead to platinum resistance.

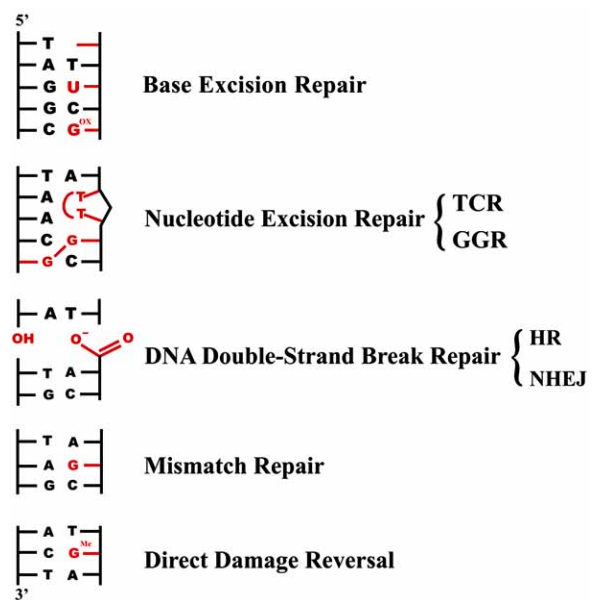


Fig. (1). The DNA Repair System.

4.1. Base Excision Repair (BER)

As the name implies, base excision repair (BER) removes modified bases from DNA damaged by normal cellular metabolism, such as reactive oxygen species generated during oxidative metabolism. Deamination or alkylation can chemically mutate single bases in DNA resulting in incorrect base pairing and, consequently, mutations in the DNA [51,52].

BER is brought about by DNA glycosylases, specialized enzymes, that cleave the bond linking a modified base to the deoxyribose sugar. There are numerous DNA N-glycosylases that hydrolyze the N-glycosylic bond between the base and the deoxyribose. In other words, specific DNA glycosylases recognize different damaged bases [51-54]. For example, an incorrect uracil base (that appears in DNA as a consequence of the spontaneous deamination of cytosine) is removed by the enzyme Uracil DNA N-glycosylase [55,56]. Glycosylase cleaves the β -N glycosylic bond creating an apurinic/apyrimidinic (AP) site that is recognized by an AP endonuclease. The latter nicks the damaged DNA creating a free 3'-OH

terminus. The 3'-OH terminus is extended by DNA Polymerase (Pol I) and accompanied by excision of the AP site. The sealing of the new DNA strand is achieved by DNA Ligase [57,58]. Generally, BER is an efficient repair pathway; however, because DNA polymerase β (responsible for replacing the removed nucleotide) lacks proofreading capability, error-prone DNA replication often occurs [58,59].

4.2. Nucleotide Excision Repair (NER)

Nucleotide excision repair (NER) enzymes remove nucleotides after recognizing bulky distortions in the shape of the DNA double helix that result from transcription-blocking lesions caused by endogenous and environmental insults, including platinum-induced DNA adducts [60-62]. Unlike the BER pathway that operates by removing either 1 or 2-6 damaged nucleotides 3' to a damaged base, NER incises DNA both on 5' and 3' sides of the lesion, and releases the damage in an oligonucleotide 24-32 residues long (single-stranded DNA segment) and its associated deoxyribose, creating a single-strand gap in the DNA subsequently filled in by DNA polymerases using the undamaged strand as a template. Thus, the steps in the NER pathway contain damage recognition, dual-incision, removal of incised nucleotides and deoxyribose, and gap fill-in synthesis [30, 63-65] (Fig. 2).

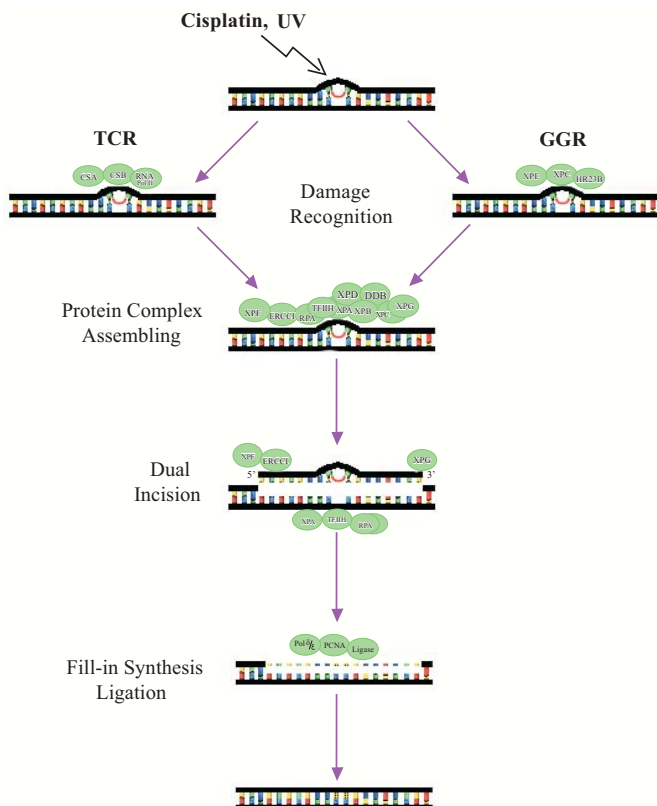


Fig. (2). Nucleotide Excision Repair (NER) Pathway.

The NER pathway can be divided into two subtypes: transcription-coupled repair (TCR) and global genome repair (GGR). TCR focuses specifically on the template strand, actively transcribing genes and removing lesions that block RNA elongation. Experiments demonstrate that damage within the transcribed strands of genes is usually repaired more quickly than damage in non-transcribed strands or in

non-gene regions [62,66,67]. TCR is coupled to the actions of RNA polymerase II, as it transcribes template strands during transcription. When RNA polymerase II encounters a helix distortion that stalls transcription, it recruits nucleotide excision repair proteins, giving more attention to actively transcribed genes. This is critical. If transcription stalls, RNA polymerase II can lead to arrest of the cell cycle or apoptosis [68-71]. By comparison, GGR addresses the remainder of genome, including the nontemplate strand of transcribed genes and the nontranscribed regions of the genome. GGR employs heterodimeric complexes XPC-hHR23B for damage-recognition and DDB (DNA-damage binding) to initiate repair of the damaged DNA [72,73].

NER is accomplished by a large multiprotein complex of nearly 2-dozen enzymes [74,75]. It is suggested that low levels of NER factors render tumors more susceptible to platinum drugs [32,76,77]. Among the NER enzymes, excision repair cross-complementing 1 (ERCC1) plays a pivotal role in the removal of bulky platinum-lesions [36,77-79]. ERCC1/XPF heterodimer with XPG are responsible for the dual incision process (5' side by ERCC1/XPF and 3' side by XPG) [49,64,65]. As predicted, ERCC1 expression is a useful marker for resistance to platinum compounds; high level of ERCC1 mRNA is associated with clinical resistance to platinum-based chemotherapy in patients with several cancers [77,79].

4.3. DNA Double-Strand Break Repair (DSBR)

Double-strand breaks (DSBs) occur when both strands in the double helix are severed by radioactive decay (ionizing radiation) or other stresses to the DNA. DSBs-damage is especially toxic to cells because it can lead to genome rearrangements [80-84]. Two principal mechanisms repair DSBs: homologous recombination (HR) and non-homologous end joining (NHEJ).

HR requires an identical or nearly identical sequence as template and typically uses a sister chromatid or a homologous chromosome. HR occurs primarily during the late S phase and G₂ phase of the cell cycle when the double helix in a sister chromatid can provide the sequence information for proper repair of breaks. It is also used in the event of inter-strand covalent cross-links within a double helix [80-85]. This error-free process, however, has a potential risk of leading to loss of heterozygosity for recessive mutations.

Worth noting, two important proteins in the HR process, BRCA1 and BRCA2, appear to play a role in DNA damage response, cell cycle checkpoints, and DNA repair in the maintenance of genomic integrity. They perform this function by cooperating with other proteins involved in homologous recombination, most notably Rad51, MSH2/MSH6 and MLH2 in response to DNA damage. By allowing repair of platinum damage, HR helps cells tolerate platinum damage. Consequently, ovarian carcinomas with mutated BRCA1 or BRCA2 are sensitive to platinum compounds. The corollary of these observations is that genetic reversion of BRCA alleles with truncation mutations may lead to the acquisition of platinum resistance, evidence for which has been noted in recent *in vitro* and clinical studies [85-90].

NHEJ repairs damage mostly in the G₁ phase of the cell cycle when sister chromatids are not available for the HR process. In NHEJ, DNA ligase IV forms a complex with XRCC4 to directly join the two ends. Fidelity in NHEJ is provided by short homologous sequences called microhomologies that are present on the single-stranded tails of the DNA ends that are to be joined*. If these overhangs are compatible, repair is usually accurate. However, if there is loss of nucleotides at the site of the DSB, deletions can occur or the NHEJ machinery may join termini that are not matching, thus leading in some cases to translocations. NHEJ is especially important because it occurs before the cell has replicated its DNA and because of its widespread use. Indeed, it is often referred to as the caretaker of the genome. It is also important in joining the DSBs induced during V(D)J recombination, the process that generates immunoglobulin diversity in B-cells and receptor diversity in T-cells. However, due to no template available for this repair, it is considered an error-prone process and often results in small insertions/deletions [90-94].

4.4. Mismatch Repair (MMR)

Any mutation that disrupts the super-helical structure of DNA can compromise a cell's genetic stability. The mismatch repair (MMR) pathway is a final line of cell defense that primarily corrects post-replication errors caused by DNA polymerase slippage during DNA replication and recombination, as well as some forms of DNA damage caused by endogenous and exogenous insults. This pathway recognizes and repairs mismatches of the normal base pairing (A•T, C•G) and erroneous insertion and deletion of bases that arise during DNA replication and recombination. In the case of MMR deficiency, unrepaired damaged-DNA accumulates, resulting in microsatellite instability, and can replicate with the mismatched base pair [95-99].

Fojo points out that mismatch repair is strand-specific*. To explain, errors arise and are repaired on a daughter strand during DNA synthesis. This selectivity is possible because mismatch repair relies on cues that help it to distinguish the newly synthesized daughter strand from the template or parental strand. The latter allows the mismatch repair machinery to detect the mismatch in the newly synthesized DNA, to determine which of the mispaired bases is incorrect, and to repair the error by excising it and then repairing it.

The mismatch repair process is highly conserved in evolution and has been most extensively studied in prokaryotes. Eleven human homologues of prokaryotic proteins have been identified [45]. Three proteins in prokaryotes - MutS, MutH, and MutL - are essential in detecting a mismatch and directing repair machinery to it. The human homologues of these include the MutS homologue hMSH2 (a mismatch and loop recognition protein), the MutL homologues hMLH1 (a protein that excises mismatched bases) and hPMS2 (a protein that acts as coordinator between MMR proteins and DNA polymerase δ or ϵ , and can complement hMLH1 function) [100-102].

MMR proteins can bind to DNA-adducts created by pharmacologic agents such as platinum drugs and are used to

repair some forms of DNA damage [103]. Numerous reports indicate that MMR genes can be regulated by epigenetic mechanisms (DNA methylation), affecting promoter regions of genes such as *hMLH1* and *hMSH6*, leading to transcriptional silencing (MMR defects). In studies of non-familial tumors MMR genes were rendered defective by somatic mutation or promoter methylation. MMR-deficient cells that have lost MMR function or that lack expression of *hMLH1* or *hMSH2* become resistant to DNA-damaging platinum compounds due to futile repair [104-107]. Platinum-resistant ovarian cancer cell lines with an acquired loss of *hMLH1* and *hPMS2* expression have also been reported [108-110].

Of note, the last step of fill-in synthesis within DNA repair pathways BER, NER and MMR is carried out by DNA polymerases β or δ or ϵ (Pol $\beta/\delta/\epsilon$) [45, 51, 75, 96, 111].

4.5. Direct Damage Reversal (DDR)

DDR is an enzyme-catalyzed process used to correct the most frequent cause of point mutations in humans. For example, the spontaneous addition of a methyl group (CH₃-) to a C (cysteine), followed by deamination of a T (Thymine). Most of these errors are repaired by enzymes called DNA glycosylases that remove the mismatched T and restore the correct C [112,113].

To illustrate, alkylating agents induce a major mutagenic/cytotoxic DNA lesion, alkylguanine (O⁶-MeG), which is repaired by a protein encoded by the *MGMT* gene. However, the enzyme MGMT or alkyltransferase (O⁶-methylguanine-DNA methyltransferase) can perform its job only once. Upon completion of DDR, MGMT is inactivated; thus, it is a suicide enzyme [114].

A DNA lesion O⁶-MeG not repaired by MGMT leads to a mispairing with Thymine during DNA replication, resulting in G•C to A•T point mutations. Unrepaired O⁶-MeG lesions enter the S-phase of the cell cycle and mispairings with Thymine are replicated. At that point, the errors are subject to the mismatch repair pathway [115,116].

The higher the level of MGMT, the more effective the removal of the alkyl groups, meaning that MGMT overexpression renders the host cell resistant to the effects of therapeutic agents. Therefore, MGMT is a major determinant of resistance in cells exposed to alkylating cytostatic drugs. Several reports indicate that DNA methylation leading to transcriptional silencing of *MGMT* in the DDR process can modulate drug sensitivity [116-119].

5. GENETIC AND EPIGENETIC ALTERATION OF DNA REPAIR SYSTEM

Genetic and epigenetic alterations in the DNA repair system can influence the degree of sensitivity of tumor cells to chemotherapeutic drugs.

5.1. Genetic Alteration of DNA Repair Genes can Modulate Gene Expression and Drug Sensitivity

In 1997 the author of this article discovered a significant single nucleotide polymorphism (SNP) in the *ERCC1* gene, an important component of NER (GenBank Acc# AF001925). This C/T SNP at codon 118 of *ERCC1* gene converts a common codon usage to an infrequent codon usage, reducing

* Antonio Tito Fojo, personal communication, April 20, 2008.

frequency of use two-fold, prompting the prediction that this polymorphism would be associated with reduced *ERCC1* translation and improved response to platinum chemotherapy [120]. Researchers have shown that the *ERCC1* (codon 118) SNP is associated with platinum sensitivity and predicts better overall survival of colorectal and non-small cell lung cancer patients treated with platinum combination chemotherapy [121-125].

The *p53* gene is an important DNA repair component in the DNA Repair System and a key regulator of cell cycle checkpoints and apoptosis. *p53* is the most common mutated gene in human cancers. Loss of the gene is a critical event in carcinogenesis, as cells lacking *p53* function are deficient in platinum-induced DNA damage repair. It modulates platinum sensitivity *via* ATM/ATR-dependent DNA-damage-response pathways [13-15,18,19,126,127]. A recent report suggests that *p53* influences cisplatin sensitivity through loss of MMR in human colon cancer cells [18]. Furthermore, *p53* acetylation plays a critical role in *p53*-dependant stress response, leading cells to apoptosis [128,129].

Clinicians have long known that different subtypes of ovarian cancer respond differently to treatment and have different prognoses. High-grade serous ovarian cancers typically harbor mutations in *p53*. These cancers also have mutations of *BRCA1* or *BRCA2*, as well as defective homologous recombination (HR), the preferred mechanism of the DNA double-strand break repair pathway [87,130].

The degree of sensitivity to platinum compounds depends on the interplay between the various components involved in DNA repair. For example, in ovarian and breast cancers, mutations in *BRCA1* or *BRCA2* that result in a loss of *BRCA1/2* activity and impaired homologous recombination (HR) confer sensitivity to platinum compounds [88-90,131,132]. In contrast, retention of proficient mismatch repair (MMR) is needed to retain sensitivity to platinum compounds [18,105,108].

Of special interest, two recent reports indicate that secondary *BRCA1* and *BRCA2* mutations in *BRCA1/2*-mutated ovarian cancers can restore the wild-type and may play a critical role in acquired resistance to platinum chemotherapy [133,134].

Mamenta *et al.* suggest that increased replicative-bypass and NER activity together account for most of the enhanced tolerance of platinum-DNA adducts in platinum-resistant cell lines [135]. Cummings and colleagues reported that *ERCC1* plays a role in homologous recombination repair. Using siRNA to *ERCC1* in cancer cell lines enhanced cells to cisplatin, and siRNA to *XPA* and *ERCC1* in MMR-deficient prostate cancer cell lines sensitized cells to cisplatin and mitomycin C [136]. These findings prompt two questions. Do alterations of MMR affect NER (or *vice versa*) and then influence drug resistance? Are there interrelationships between other DNA repair pathways?

5.2. Epigenetic Alterations such as DNA Methylation and Histone Acetylation can Silence Transcription

In studies of platinum resistance caused by altered expression and functional mutations of DNA repair genes, DNA

methylation is emerging as an important mechanism of protein regulation. For example, hypermethylation of the *BRCA1* promoter with transcriptional silencing and aberrant expression has been described as a possible mechanism of increased platinum sensitivity [137]. This mechanism has been extensively investigated in DNA repair MMR pathway.

Early studies found that cell lines *deficient* in MMR displayed cisplatin resistance and that *proficient* mismatch repair (MMR) is needed for cisplatin to induce cytotoxicity. Later reports note that MMR deficiency or reduced expression of MMR proteins (*hMLH1* or *hMSH2*), secondary to promoter hypermethylation, leads to increased resistance to cisplatin and carboplatin but not to oxaliplatin (as the enzymes do not recognize oxaliplatin-induced DNA-adducts), resulting in poor survival of patients with ovarian, breast and colorectal cancers [105,108-110,138-140].

Of interest, *in vivo* investigations using the demethylating agent DAC (2'-deoxy-5-azacytidine, decitabine) to treat *hMLH1*-negative platinum-resistant ovarian and colon tumors resulted in re-expression of *hMLH1* and enhanced sensitivity to cisplatin and carboplatin [141]. This finding and other research have led to a strategy of reactivating transcription of the epigenetically-silenced MMR gene, *hMLH1*, to increase efficacy of chemotherapy for patients whose tumors lack *hMLH1* expression due to *hMLH1* promoter methylation [141-143].

Histone/protein acetylation can also silence genes and influence drug sensitivity [144-148]. Histone acetylation, DNA methylation, and RNA interference are interdependent mechanisms for gene silencing [149]. The possibility that histone/protein acetylation might modulate platinum sensitivity is an area that deserves further investigation.

6. DNA POLYMERASE IN PLATINUM DRUG RESISTANCE

Eukaryotes have at least 15 DNA polymerases. These can be divided into seven families based on sequence homology: A, B, C, D, X, Y, and RT. Some of the DNA polymerases are involved in error correction; many play a role in DNA repair pathways [45,150-152].

For example, the A-family contains both replicative and repair polymerases; the X-family includes eukaryotic polymerases (*pol β*, *pol σ*, *pol λ*, and *pol μ*). *Pol β* is required for the base excision repair (BER) pathway; *Pol λ* and *Pol μ* are involved in NHEJ mechanism of DNA double-strand breaks repair pathway. *Pol η*, *ι*, *κ*, and *Rev1* of the Y-family and *Pol ζ* in B-family play a role in the bypass of DNA damage. Furthermore, the proofreading mechanism employed during cell cycle progression utilizes DNA polymerases such as *pol δ* to detect copy errors. *Pol η* is used when the regular *Pol δ* is unable to copy unrepaired DNA lesions, thus is an error-prone DNA polymerase [150-154].

Some researchers report transient expression of *pol β* to protect cellular DNA in response to cisplatin [153,155,156]. Other studies indicate that DNA polymerases (*pol β*, *pol η*, *pol μ*) bypassed cisplatin lesions by translesion synthesis. While the tolerance mechanism involves enhanced replicative bypass, certain DNA polymerases (*pol β* & *pol η*) can

bypass cisplatin-DNA adducts by translesion synthesis, with polymerase η shown to play a role in cellular tolerance to cisplatin and carboplatin [157-159]. A recent study suggests that the DNA repair gene *POLH*, encoding DNA polymerase η (pol η), is an important determinant of cellular response to cisplatin, which could have implications for acquired or intrinsic resistance [160].

Finally, it is worth noting that platinum resistance due to enhanced NER can be reversed by giving aphidicolin, which inhibits DNA polymerase α and δ , indicating a potential strategy to circumvent platinum resistance [161,162].

7. RESEARCH AND CLINICAL IMPLICATIONS

Given the research findings to date, unlocking the molecular mechanisms of DNA repair in platinum resistance might best be pursued through studies directed at 1) transcriptional cis-elements (activator/repressor) within promoters of critical DNA repair genes, 2) effects of epigenetic alterations, and 3) links between gene expression and DNA methylation or protein acetylation. Special attention should be directed at three interrelationships: between the different DNA repair pathways; between DNA methylation and protein acetylation; and between DNA repair pathway(s) and DNA methylation or protein acetylation.

The frequent occurrence of p53 mutations in human cancers supports strategies to restore wild type p53 to reverse the malignant phenotype and enhance drug sensitivity. Platinum therapy combined with small molecule drugs that restore wild type function to mutated p53 may be an effective therapeutic program for some patients [4].

Platinum therapy combined with epigenetic interventions, including demethylation as a strategy to modulate transcriptional gene expression, may produce better outcomes for cancer patients. A phase II trial in Brazil tested this hypothesis by using decitabine (DAC) in combination with cisplatin as first-line therapy in the treatment of patients with advanced squamous cell carcinoma of the cervix. A positive outcome has led to a phase III multicenter study of 264 women with measurable stage IVB, recurrent or persistent cervical cancer [163]. In Poland and the United States DAC is being used to treat acute myelogenous leukemia (AML) and myelodysplastic syndromes (MDS) [164-169].

Hereditary breast cancer accounts for 5% to 10% of all breast cancers; BRCA1/2 mutations are carried in half of these cases [170]. Ten percent to 15% of all epithelial ovarian cancers are associated with germline BRCA1/2 mutations [171]. These BRCA1/2-deficient cancers are sensitive to platinum chemotherapy. This suggests that a selective, individual-targeted treatment with platinum compounds may be more effective for those patients [172-174]. An international study of BRCA mutation-carrier breast cancer patients treated with carboplatin is now underway [175].

Finally, platinum compounds combined with molecularly-targeted drugs, such as EGFR-inhibitors, appear to increase chemosensitivity and induce apoptosis *in vitro*. As Kelland notes, cancer drug discovery and development efforts are now giving more attention to specific molecular abnormalities characteristic of a cancer. Specifically, EGFR-

inhibitors (imatinib/Gleevec, trastuzumab/Herceptin, bevacizumab/Avastin, erlotinib/Tarceva, gefitinib/Iressa, sunitinib/Sutent and sorafenib/Nexavar) might not be highly effective alone but appear to achieve an optimal effectiveness when combined with cytotoxic drugs, including platinum-compounds [2].

ACKNOWLEDGEMENTS

The author would like to thank Dr. Antonio Tito Fojo of the National Cancer Institute for his thoughtful review of drafts and suggestions; Dr. Lloyd R. Kelland of University College London for his helpful comments; and Dr. Michael M. Gottesman of the National Institutes of Health for his encouragement and support. The author also thanks Dr. Weixin Wang for useful discussions in 2002 regarding cell cycle checkpoint and DNA damage response, and Michael D. Mueller for editorial assistance.

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Received: May 01, 2008

Revised: July 21, 2008

Accepted: September 01, 2008