Conference Report

Nucleotide excision repair pathway review I: Implications in ovarian cancer and platinum sensitivity

Abstract

Platinum-based chemotherapy has been the mainstay of treatment for advanced gynecological cancers following cytoreductive surgery and in radiation sensitization of cervical cancer. Despite its initial high overall clinical response rate, a significant number of patients develop resistance to platinum combination therapies. The precise mechanism of platinum-resistance is multifactorial and accumulation of multiple genetic changes may lead to the drug-resistant phenotype.

Platinum chemotherapy exerts its cytotoxic effect by forming DNA adducts and subsequently inhibiting DNA replication. It is now clear that the nucleotide excision repair (NER) pathway repairs platinum–DNA adducts in cellular DNA. Evaluation of genetic polymorphisms in cancer susceptibility as one etiology for platinum resistance may help us to understand the significance of these factors in the identification of individuals at higher risk of developing resistance to anti-cancer drug therapies. In this review, we summarized the relevant studies, both in vitro and in vivo, that pertain to NER in ovarian cancer and platinum resistance. It is evident also that there are a few limited studies in genetic polymorphisms of NER and ovarian cancer. These studies reviewed suggest that concurrent up-regulation of genes involved in NER may be important in clinical resistance to platinum-based chemotherapy in ovarian cancer. In the future, larger and well-designed population-based studies will be needed for a more complete understanding of relevant genetic factors that may result in improved strategies for determining both chemotherapy choice and efficacy in patients with advanced ovarian and cervical cancer. Review II will focus on the NER pathway in cervical cancer and platinum sensitivity.

Keywords: Nucleotide excision repair; DNA repair; Ovarian carcinoma; Platinum resistance; Polymorphisms

Introduction

Since its introduction in the mid-1970s, platinum (cisplatin or carboplatin) has been used as standard therapy for numerous cancers such ovarian, lung, colon, head and neck, and as a radiosensitizer for cervical cancer [1]. Platinum-based chemotherapy has been the mainstay of treatment for advanced epithelial ovarian cancer following aggressive cytoreductive surgery [2]. Initially, platinum-based combination chemotherapy is associated with a 60–70% clinical response rate in advanced ovarian cancer. However, the overall 5-year survival rate in these patients is still around 20–30% [3].

Although platinum-based regimens have produced higher overall response rates and an increase in median survival, a relevant clinical problem in the treatment of advanced ovarian cancer is the development of tumor resistance to platinum compounds [4,5]. Thus, overcoming drug resistance is the key to successful treatment of this disease. The mechanism of platinum-resistance is multifactorial and accumulation of multiple genetic changes may lead to the drug-resistant phenotype. However, we have yet to develop an assay to aide in individualized drug treatment. Ideally, identification of patients who are platinum resistant before therapy could lead to better selection of therapy and improve survival.

The antitumor effect of platinum compounds such as carboplatin or cisplatin has been correlated with binding to DNA and the production of intra- and inter-structural cross-links and formation of DNA adducts [6]. Bulky DNA adducts cause changes in DNA conformation that may affect DNA replication and inhibition of DNA synthesis [7]. Mechanisms by which tumor cells develop resistance to platinum agents are multifactorial but may include decreased drug accumulation, increased glutathione levels and metallothiones that sequester platinum, and enhanced DNA repair to remove cisplatin–DNA adducts [8]. For platinum-based therapy, DNA repair is an essential contributor to clinical drug resistance and any perturbation in this pathway may lead to suboptimal DNA repair capacity. Moreover, patients with persistent or recurrent tumor after primary therapy for ovarian cancer are occasionally candidates for secondary cytoreductive surgery or second-line chemotherapies [9]. Second-line therapies have been defined by whether patients responded to their initial platinum-based chemotherapy. In these cases, platinum sensitivity has been related to a disease progression-free interval of 12 to 24 months, and retreatment with platinum compounds may be considered [10,11].

doi:10.1016/j.ygyno.2007.07.043
Otherwise, platinum-resistant disease is best treated with non-cross resistant agents that have different anti-cancer mechanisms [12,13]. Therefore, an understanding of the molecular basis of drug resistance plays an important part in improving strategies in cancer chemotherapy.

A complex system of DNA repair enzymes play a central role in maintaining genomic integrity by counteracting insults from endogenous and exogenous damaging agents, including platinum compounds (see Fig. 1) [14]. There are at least four DNA repair pathways that operate on specific types of DNA damage: base-excision repair, mismatch repair, double-strand break repair, and nucleotide excision repair (NER) [15]. A large body of evidence now clearly shows that the NER pathway is the one that repairs platinum–DNA adducts in cellular DNA. As shown in Fig. 2 and summarized in Table 1, a number of critical genes or core factors participate in NER activity and any functional mutation in these genes will lead to NER abnormalities and increased susceptibility to cancer [16–18].

Nucleotide excision repair pathway (NER)

The NER genes recognize and repair bulky DNA damage caused by platinum compounds, environmental carcinogens and exposure to UV-light [19]. In humans at least three diseases have been linked to a hereditary defect in the NER pathway, xeroderma pigmentosum (XP), Cockayne syndrome (CS), and trichothiodystrophy (TTD) [20]. However, only patients with XP are predisposed to UV sunlight-induced skin carcinomas [21]. Thus, XP provides a unique model for the study of unrepaired DNA lesions and mutations caused by different carcinogens. Although CS and TTD do not show any skin cancer predisposition, various alterations of the XP NER genes or other repair genes give rise to these very different phenotypes.

The repair of damaged DNA involves at least 30 polypeptides within two different sub-pathways of NER known as transcription-coupled repair (TCR-NER) and global genome repair (GGR-NER) as illustrated in Fig. 2 [16–18]. TCR refers to the expedited repair of lesions located in the actively transcribed strand of genes by RNA polymerase II (RNAP II). Repair is much more efficient in actively transcribed genes than DNA damage in the overall genome, and the pathways differ only in the initial DNA damage recognition step. NER includes proteins encoded by seven XP complementation groups, XPA to XPG genes, the ERCC1 (excision repair cross-complementing group 1)–hHR23B (human homolog of yeast RAD23)–RPA (replication protein A) trimer, additional subunits of TFIIH (transcription factor with helicase activity), and Cockayne syndrome proteins CSA and CSB [22].

Briefly, the pathway involves at least five steps (Fig. 2). In the first step, damage recognition (Fig. 2 steps 1a and 1b), the protein complex XPC–hHR23B together with XPE is involved in GGR-NER. A stalled RNAPII and Cockayne syndrome proteins, CSA and CSB, fulfill this function within the TCR–NER pathway at the site of the DNA lesion. The following steps in the GGR-NER and TCR-NER are the similar. The XPC–hHR23B complex then recruits TFIIH to the damaged site [23]. Similarly, the XPA–RPA complex, also essential in both sub-pathways, recruits other components of NER to the damaged site and may control NER substrate specificity [24]. Unwinding
of the DNA helix surrounding the lesion (Fig. 2 step 2) is accomplished by TFIIH and its two helicase subunits, XPB and XPD. Together with XPA–RPA, this complex opens the DNA structure forming a 30 base pair bubble around the lesion [25].

The rate-limiting dual incision step of the damaged DNA strand (Fig. 2 step 3) is then performed by the two endonucleases, XPG cutting 3′ to the lesion and XPF–ERCC1 cutting 5′ to the lesion; the latter requiring the structural presence of XPG and the XPA–RPA complex for the correct positioning of the two endonucleases [26]. Then excision and DNA synthesis of the single strand gap of 27 to 29 nucleotides is filled by DNA polymerases δ and/or ε (Fig. 2 step 4) [27]. DNA ligase I closes the 3′ nick in the final ligation step (Fig. 2 step 5). Study of the various NER proteins suggests a sequential assembly rather than a preassembled repairosome [28].

NER biology and ovarian cancer with implications in drug resistance

Substantial progress has been made in the last two decades defining specific mechanisms of resistance associated with alkylating agents and platinum compounds. Research in this area has been facilitated by the development of relevant experimental model systems in vivo and in vitro of human ovarian cancer. Further, all NER core factor genes have been cloned and expressed as recombinant proteins [29]. The following section summarizes the characteristics of each core factor with regards to ovarian cancer and platinum sensitivity. Relevant studies listed in Tables 2–8 were selected to outline the more salient features of each key core factor in the NER pathway with respect to platinum resistance and ovarian cancer.
ERCC1 is a 15-kb repair gene located on chromosome 19 and was the first human excision repair gene to be cloned [30]. ERCC1, a 33-kDa protein, forms a tight heterodimer endonuclease complex with XPF, that is responsible for the rate-limiting incision that cleaves the DNA strand at the phosphodiester bonds between 22 and 24 nucleotides 5′ to the damage [31].

Studies have demonstrated the functional importance of ERCC1 in the repair of cisplatin DNA adducts and in cisplatin sensitivity in human ovarian cancer cells in vitro. Using cisplatin-resistant human ovarian cancer cell lines, MCAS and A2780/CP70, Li et al. (Table 5, see Ref.[32]) showed that levels of ERCC1 mRNA expression, protein and gene transcription are increased and parallel cisplatin-induced DNA damage. Similar studies can be supported by introduction of antisense ERCC1 expression vectors. For example, Selvakumaran et al. (Table 5, see Ref.[33]) using antisense ERCC1 transfection of cisplatin resistant ovarian cell lines (OVCAR10, OVCAR4, CP70, C30, C200), demonstrated decreased DNA repair capacity (DRC) and increased cisplatin sensitivity both in an in vitro cisplatin cytotoxicity assay (MTT assay) and in vivo using SCID mice. When antisense ERCC1 transfectant cell lines were transplanted intra-peritoneal into SCID mice, cisplatin treated animals surviving longer than control mice (p = 0.002) (Table 6, see Ref.[33]). Similar studies using a panel of NER genes (ERCC1, XPA, XPB, XPC, XPD, XPG) also demonstrated that resistant cell lines treated with alkylating agents overexpressed these genes when compared to controls (Table 2, see Ref.[34] and Table 5, see Ref.[35]). These results indicate that cells defective in NER exhibit high sensitivity to platinum compounds when compared with NER-proficient cells.

Assays in ovarian cancer tissue support conclusions from studies in human ovarian cancer cell lines, however the results suggest that different mechanisms of regulation may act in different cell or tissue systems with regards to NER gene expression. In one study (Table 7, see Ref.[36]), mRNA levels of ERCC1 and XPA were assessed in mixed histology malignant ovarian cancer tissues from 28 patients. The authors found

### Table 1

<table>
<thead>
<tr>
<th>Human gene/protein</th>
<th>Chromosome location</th>
<th>Function in NER</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERCC1</td>
<td>19q 13.2-13.3</td>
<td>Damage recognition, 5’ DNA structure-specific endonuclease with XPF, no human mutants known</td>
</tr>
<tr>
<td>XPA</td>
<td>9q 22.3</td>
<td>Damage recognition, facilitates repair complex assembly, represented in human XP</td>
</tr>
<tr>
<td>RPA</td>
<td>17p 13.3</td>
<td>Damage recognition, endonuclease positioning, resynthesis</td>
</tr>
<tr>
<td>XPF/ERCC3</td>
<td>2q 14.3</td>
<td>Part of TFIH, essential for transcription initiation, 3′→5′ helicase, catalyzes open complex, represented in human XP/CS syndromes</td>
</tr>
<tr>
<td>XPC</td>
<td>3p 25</td>
<td>Damage recognition, molecular matchmaker, not required for TC-NER, represented in human XP</td>
</tr>
<tr>
<td>XPD/ERCC2</td>
<td>19q 13.2–13.3</td>
<td>Part of TFIH, essential for transcription initiation, 5′→3′ helicase, represented in human XP/CS/TTD syndromes</td>
</tr>
<tr>
<td>XPE/DDB1/2</td>
<td>11q 12–13</td>
<td>Chromatin repair</td>
</tr>
<tr>
<td>XPF/ERCC4</td>
<td>16p 13.3–13.11</td>
<td>5′ DNA structure-specific endonuclease with ERCC1, then with XPA to perform DNA damage recognition, represented in human XP</td>
</tr>
<tr>
<td>XPG/ERCC5</td>
<td>13q 32–33</td>
<td>3′ DNA structure-specific endonuclease, stabilizes the full open complex, represented in human XP/CS syndromes</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Gene</th>
<th>Reference</th>
<th>Cell line</th>
<th>N</th>
<th>Controls</th>
<th>Technique used</th>
<th>#runs</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERCC1, XPA, XPC, XPD, XPF, XPG</td>
<td>Damia et al., 1998 [34]</td>
<td>IGROV, OVCAR3, SKOV-3 (leukemia and colon cell lines also used)</td>
<td>8</td>
<td>Yes</td>
<td>MTT assay, HCR, Northern blot</td>
<td>3</td>
<td>Significant correlation between mRNA levels of XPA/XPC and ERCC1/XPC; no correlation between DDP and L-PAM sensitivities and mRNA expression; higher DRC in carcinoma cell lines vs. leukemic cell lines</td>
</tr>
<tr>
<td>ERCC1, XPA, XPD, XPG (CSB, p53)</td>
<td>Yu et al., 2000 [39]</td>
<td>A2780, A2780/CP70, SKOV3, MCAS, OvCar3, Caov4</td>
<td>6</td>
<td>Yes</td>
<td>Southern blot</td>
<td>2</td>
<td>Differences in mRNA expression is not due to allelic loss or gain in NER genes</td>
</tr>
</tbody>
</table>

MTT assay = 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyltetrazolium bromide assay. PCR = polymerase chain reaction. DDP = cisplatin. L-PAM = melphalan.
higher levels of ERCC1 ($p=0.059$) and XPA ($p=0.011$) mRNA in platinum resistant tumor tissue when compared to tumor tissues clinically sensitive to platinum-based therapy. Further, Reed et al. (Table 4, see Ref. [37]) showed that median values of mRNA expression for ERCC1 and XPB were more than twofold higher in ovarian clear cell tumors than other comparative histologies (serous, mucinous, endometrioid, poorly differentiated). These findings are consistent with the de novo resistance to platinum-based chemotherapy clinically characteristic of clear cell carcinoma. Another study (Table 4, see Ref. [38]), reported loss of heterozygosity (LOH) on chromosome 19q in a cohort of 20 varied histology pre-treatment ovarian tumor samples. Fifty-three percent of these tumors showed LOH in this region where ERCC1 and XPD/ERCC2 repair genes lie, suggesting a pathway of carcinogenesis in sporadic ovarian cancers. However, a report by Yu et al. (Table 2, see Ref. [39]) examined the genomic DNA from 22 ovarian cancer patient tissue specimens. They found no evidence for allelic loss and/or allelic gain for ERCC1 and XPD to explain differences in platinum sensitivity among the varied human ovarian cancers. However, they did find one platinum-resistant ovarian tumor that showed allelic gain for the two genes. They concluded that other mechanisms must be invoked to explain the marked variability in mRNA expression for these genes.

In contrast to other reports, a study by Codegoni et al. (Table 4, see Ref. [40]) examined the expression of different DNA repair genes in a varied tissue sample of 33 ovarian cancer patients pre-treatment. Univariate analysis showed a negative correlation ($p=0.037$) between the expression of ERCC1 and mortality. That is, patients who showed higher tumor expression of ERCC1 had a higher probability of response to therapy and of longer survival. Although counterintuitive to most of the literature, there may be several explanations. One may be that the levels of ERCC1 mRNA in this study could not adequately reflect the levels of the functional ERCC1 protein. For example, Dabholkar et al. (Table 7, see Ref. [36]) reported that in ovarian cancer a large proportion of ERCC1 mRNA may exist as alternatively spliced species without exon 8, which is essential for the functional ability of ERCC1 protein to make the 5′ DNA incision.

Based on these studies, there exists a positive association between levels of ERCC1 expression and clinical resistance to platinum-based chemotherapy. Thus, ERCC1 may prove a useful predictive marker for pharmacological approaches such as customized chemotherapy.

**XPA**

XPA represents the most common XP gene complementation group located on chromosome 9q [41]. The XPA gene codes for a 31 kDa protein that binds to damaged DNA more than to undamaged DNA. The minimal DNA binding region is located at the C terminus that is further organized into two subdomains: a zinc-finger core important for protein–protein interactions and a loop-rich domain essential for DNA binding [42,43]. In addition, cooperative binding DNA has been observed for XPA interactions with RPA, XPF–ERCC1, and TFIIH, and therefore, plays a central role in correctly positioning the repair complex around the DNA lesion [44].

Xeroderma pigmentosum patients with a defect in the NER gene XPA exhibit the most severe form of the disease. This is supported by studies on XPA knock-out mice by homologous recombination of target constructs in embryonic stem cells (Table 3, see Ref. [45]). To permit the functional analysis of the XPA gene in vivo, the authors showed that XPA −/− mice had a higher susceptibility to UV-B induced eye and skin tumors and to 7,12-dimethylbenz[a]anthracene (DMBA) induced skin tumors than controls. Paradoxically, Stevens et al. (Table 4, see Ref. [46]) found XPA protein expression associated with better chemotherapy response and predicted better progression free survival (PFS) and overall survival (OS) in a cohort of patients with metastatic ovarian cancer. Using metastatic peritoneal and pleural effusion specimens, they found strongest expression of XPA from patients with a complete response to chemotherapy when compared to those with partial or no response ($p=0.03$). Patients with recurrent disease that had a better PFS ($p<0.001$) and OS ($p=0.04$) expressed more XPA in tumor cells than those with a worse prognosis. Similar to the previous study with ERCC1 (Table 4, see Ref. [40]), this study counters the “double-edged sword” hypothesis of NER gene activation, that is, cancer cell expression of certain NER gene
Table 4
Human tissue - nucleotide excision repair pathway in ovarian tissue - biology

<table>
<thead>
<tr>
<th>Gene</th>
<th>Reference</th>
<th>Ovarian tissue</th>
<th>Fixation</th>
<th>Treatment</th>
<th>Technique used</th>
<th>Study Type</th>
<th>Controls</th>
<th>Ntissue</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERCC1-XPB</td>
<td>Reed et al., 2003 [37]</td>
<td>Varied: serous, mucinous, clear cell, endometrioid, poorly differentiated</td>
<td>Flash-frozen</td>
<td>Pre-tx</td>
<td>RT-PCR, Southern Hybridization</td>
<td>cohort</td>
<td>Yes</td>
<td>No</td>
<td>ERCC1-XPB mRNA expression higher in clear cell than the other histo-types</td>
</tr>
<tr>
<td>ERCC1</td>
<td>Codegoni et al., 1997 [40]</td>
<td>Varied: serous, mucinous, endometrioid, undifferentiated</td>
<td>Frozen</td>
<td>Pre-tx</td>
<td>Northern blot</td>
<td>cohort</td>
<td>Yes</td>
<td>No</td>
<td>Higher expression of ERCC1 mRNA—higher response and survival (opposite of intuitive findings)</td>
</tr>
<tr>
<td>ERCC1, ERCC2</td>
<td>Bicher et al., 1997 [38]</td>
<td>Varied: serous, mixed, endometrioid, clear cell, transitional, MMMT, mature teratoma</td>
<td>Flash-Frozen</td>
<td>Pre-tx</td>
<td>LOH/MSI-PCR analysis</td>
<td>cohort</td>
<td>Yes</td>
<td>No</td>
<td>LOH on chrom 19q (ERCC1, ERCC2 locus) seen in 53% of ovarian tumors—suggests pathway of carcinogenesis in sporadic ovarian cancers</td>
</tr>
<tr>
<td>ERCC1, XPD</td>
<td>Yu et al., 2000 [39]</td>
<td>n=12 responders, n=10 non-responders specimens</td>
<td>Flash-frozen</td>
<td>Pre-tx</td>
<td>Southern blot</td>
<td>cohort</td>
<td>No</td>
<td>No</td>
<td>Differences in mRNA expression are not due to allelic loss or gain in NER genes; 1 platinum-resistant tumor showed allelic gain for ERCC1 and XPD</td>
</tr>
<tr>
<td>XPA</td>
<td>Stevens et al., 2005 [46]</td>
<td>Metastatic peritoneal &amp; pleural effusion specimens</td>
<td>Paraffin</td>
<td>Pre-tx</td>
<td>Immunocytochemistry staining</td>
<td>cohort</td>
<td>Yes</td>
<td>No</td>
<td>XPA protein expression associated with better chemotherapy response and predicted better PFS and OS</td>
</tr>
<tr>
<td>XPA</td>
<td>States et al., 1996 [47]</td>
<td>n=3 cisplatin resistant, n=3 cisplatin sensitive specimens</td>
<td>Frozen</td>
<td>Pre-tx</td>
<td>Single strand conformation polymorphism (SSCP) + PCR, multiplex PCR</td>
<td>cohort</td>
<td>No</td>
<td>No</td>
<td>XPA mRNA overexpression is not associated with XPA promoter mutations or gene amplification</td>
</tr>
<tr>
<td>XPA, XPB, XPC, XPD, XPE, XPFG</td>
<td>Takebayashi et al., 2001 [48]</td>
<td>Varied: serous, mucinous, endometrioid</td>
<td>Paraffin</td>
<td>Pre-tx</td>
<td>LOH/MSI-PCR analysis</td>
<td>cohort</td>
<td>Yes</td>
<td>Yes (adjc)</td>
<td>LOH seen in XP factors could be crucial in carcinogenesis of human sporadic carcinomas</td>
</tr>
<tr>
<td>ERCC1, XPA, XPB, CSB</td>
<td>Reed et al., 2000 [87]</td>
<td>Mixed samples-advanced stage</td>
<td>Flash-frozen</td>
<td>Pre-tx</td>
<td>RT-PCR</td>
<td>cohort</td>
<td>Yes</td>
<td>No</td>
<td>Evidence for order of NER genes: ERCC1, XPB, XPA, CSB, independent of cisplatin sensitivity or resistance</td>
</tr>
</tbody>
</table>

RT-PCR = reverse transcription polymer chain reaction.
LOH = loss of heterozygosity.
MSI = microsatellite instability.
species should be greater in more advanced-staged or aggressive tumor, and in those with disease recurrence because activation of NER pathway protects tumor cells from the effects of chemotherapy. This would correlate with a shorter PFS and OS, indicating drug resistance. These data argue against a significant role for XPA abnormalities could be a crucial step in the carcinogenesis of human sporadic carcinomas.

**RPA**

The RPA gene is located on chromosome 17p and codes for a heterotrimeric single-stranded binding protein essential for replication, recombination and repair [49]. RPA3s three subunits, p70, p32, and p14, play a role in damage recognition and in positioning of the dual incision steps of excision repair through cooperative DNA binding with XPF–ERCC1 and XPG, such that the two nucleases incise only the damaged strand [50]. RPA also interacts with and modulates the enzymatic activity of DNA and RNA polymerases, helicases, and nucleases.

Analysis of DNA repair gene expression profiles in cisplatin-resistant human ovarian cancer cell lines and tissues with regards to RPA supports the current literature. Utilizing cDNA microarray technology, one study (Table 5, see Ref. [51]) revealed that DNA repair genes XPG and RPA were overexpressed 2.1 fold greater in cisplatin-resistant cell lines, compared to cisplatin-sensitive cell lines, KFr as compared to KF cells. A similar correlation was not found using clinical ovarian cancer tissue for these repair genes. Interestingly, RPA

| Table 5 |
|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| **Gene** | **Reference** | **Cell line** | **N Controls** | **Technique used** | **# runs** | **Results** |
| ERCC1 | Li et al., 2000 [32] | Cisplatin resistant-MCAS, A2780/CP70 | 2 Yes | cDNA probe, Northern & Western blot, nuclear run analysis | 8 | Levels of ERCC1 mRNA expression, protein and gene transcription are ↑ and parallel cisplatin-induced DNA damage |
| ERCC1-XPF | Selvakumaran et al., 2003 [33] | Cisplatin sensitive-A2780 cisplatin resistant-OVCAR10, OVCAR4, CP70, C30, C200 | 6 Yes | Antisense ERCC1 transfection MTT assay, RT-PCR, HCR, Western/Northern blot | 3 | Cell lines expressing antisense ERCC1 showed ↓ DRC and ↑ cisplatin sensitivity |
| ERCC1-XPF, XPA, XPB, XPC, XPD, XPG | Ferry et al., 2000 [35] | Cisplatin sensitive-A2780 cisplatin resistant-CP70, C30, C200 | 4 Yes | MTT assay, Northern blot single lesion excision assay | 2 | NER genes overexpressed in most resistant cell lines compared with parental cell line A2780; ERCC1 XPF determinant of ↑ NER in cisplatin resistance model C200 |
| XPG, RPA (XRCC5, XRCC6) | Sakamoto et al., 2001 [51] | Cisplatin sensitive-KF cisplatin resistant-KFr 3-fold, KFrP200 30-fold | 3 Yes | cDNA microarray | 2 | KFr cells had overexpression of DNA repair enzyme associated genes compared to KF cells |
| CSB | Lu et al., 2001 [86] | Cisplatin resistant-A2780/CP70 | 3 Yes | Phosphorothioate antisense oligonucleotides (AO’s), RT-PCR, cell proliferation assays | 3 | Cells with AO’s to CSB had a 50% reduced proliferation rate and more sensitive to cisplatin, oxaliplatin, ionizing radiation in cell culture |

DRC = DNA repair capacity.
AO = antisense oligonucleotides.
MTT assay = 3-(4,5-dimethylthiazo-2yl)-2,5-diphenyltetrazolium bromide assay.
specific binding is not observed when single-stranded DNA contains cisplatin adduct as occurs when the damage is UV-induced, a characteristic of the damage-specific zinc-finger domain of the p70 subunit [52].

XPB/ERCC3

The XPB gene located on chromosome 2q codes for a DNA-dependent ATPase and helicase [53]. XPB (ERCC3) and XPD (ERCC2) are subunits of TFIIH, a multisubunit repair complex first identified as a general transcription factor for RNA polymerase II. The XPB and XPD proteins are helicases of opposite polarities (undwinding the DNA in a 3′→5′ and 5′→3′, respectively) that function in repair to open the helix in the vicinity of the DNA damage.

As has been shown previously, tumor tissue samples from human ovarian cancer patients were assessed for relative expression of XPB and CSB mRNA between clinically resistant patients and patients responding to platinum-based chemotherapy (Table 7, see Ref. [54]). In a cohort of pre-treatment patients, XPB mRNA expression averaged five times higher in platinum-resistant tumors (p=0.001); those for CSB mRNA levels averaged six times higher (p=0.033), as compared to platinum-sensitive tumors. As noted for ERCC1, the study by Reed et al. (Table 4, see Ref. [37]) showed that mRNA expression levels in both XPB and ERCC1 were more than two-fold higher in clear cell ovarian tumors when compared to other less aggressive histologies. XPB helicase functions to link DNA repair with DNA transcription, thus these studies suggest a coordinated mRNA expression of XPB and ERCC1, as well as with other genes in the NER repairosome complex.

XPC

The 33-kb XPC gene located on chromosome 3p codes for a 125-kDa XPC protein involved in DNA damage recognition [55]. XPA has a preference for single-stranded, damaged, or bent DNA, and is the sole XP factor not essential for TCR-NER but only for GGR-NER [56,57]. XPC forms a complex with hHR23B, a 58-kDa protein homolog of the yeast NER factor Rad23 who functions in excision repair is to stabilize XPC [58].

Table 7
Human tissue - nucleotide excision repair pathway in ovarian tissue - platinum resistance

<table>
<thead>
<tr>
<th>Gene</th>
<th>Reference</th>
<th>Ovarian tissue</th>
<th>Fixation</th>
<th>Treatment</th>
<th>Technique used</th>
<th>Study Type</th>
<th>Controls</th>
<th>Ni tissue</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERCC1, XPA</td>
<td>Dabholkar et al., 1994 [36]</td>
<td>Mixed samples-advanced stage</td>
<td>Frozen</td>
<td>Pre-tx</td>
<td>RT-PCR, Southern and Northern blot</td>
<td>Cohort (n=28)</td>
<td>Yes</td>
<td>No</td>
<td>Higher levels of ERCC1 and XPA mRNA detected in platinum resistant tumor tissues; upregulation of ERCC1 occurs before XPA gene expression</td>
</tr>
<tr>
<td>XPB, CSB</td>
<td>Dabholkar et al., 2000 [54]</td>
<td>Mixed samples-no prior platinum therapy</td>
<td>Frozen</td>
<td>Pre-tx</td>
<td>RT-PCR</td>
<td>Cohort (n=27)</td>
<td>Yes</td>
<td>No</td>
<td>5-fold higher XPB and 6-fold higher CSB mRNA expression in tissue resistant to platinum (non-responders); assessed mRNA in concurrent NER genes</td>
</tr>
<tr>
<td>ERCC1, XPD</td>
<td>Dabholkar et al., 1992 [61]</td>
<td>Mixed samples-ovarian cancer</td>
<td>Frozen</td>
<td>Pre-tx</td>
<td>Slot blot analysis, PCR</td>
<td>Cohort (n=26)</td>
<td>Yes</td>
<td>No</td>
<td>ERCC1 RNA expression 2.6-fold higher in clinically resistant (non-responders) patients vs. responders; XPB/ERCC2 no difference between the two groups</td>
</tr>
</tbody>
</table>

RT-PCR = reverse transcription polymerase chain reaction.

Table 8
Polymorphism studies - nucleotide excision repair pathway in ovarian tissue

<table>
<thead>
<tr>
<th>SNP</th>
<th>Reference</th>
<th>Tissue type</th>
<th>N</th>
<th>Controls</th>
<th>Technique used</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERCC1</td>
<td>Yu et al., 2000 [101]</td>
<td>Cell lines: cisplatin sensitive-A2780 cisplatin resistant-A2780/CP70 MCAS (cisplatin resistant)</td>
<td>3</td>
<td>Yes</td>
<td>MTT assay, RT-PCR, Southern blot, PCR-SSCP</td>
<td>MCAS cells + polymorphism codon 118 → 50% reduction in codon usage → reduced cisplatin-DNA adduct repair compared to A2780/CP70 and ↓ ERCC1 mRNA expression</td>
</tr>
<tr>
<td>XPA, XPC, XPD exon10, XPD exon 23, XPG</td>
<td>Adewole et al., 2006 [105]</td>
<td>Lymphocytes: from advanced epithelial ovarian cancer</td>
<td>146</td>
<td>No</td>
<td>RFLP-PCR genotyping</td>
<td>Heterozygous XPA had shorter survival and time to recurrence vs homozygous wt XPA; homozygous XPG had shorter survival vs homozygous wt XPG; XPC, XPD (exon 10 and 23) associated with ↓ risk of recurrence and death (n.s)</td>
</tr>
</tbody>
</table>

MBO = mixed backbone oligonucleotide.
RT-PCR = reverse transcription polymerase chain reaction.
PCR = polymerase chain reaction.
SSCP = single strand conformation polymorphism.
RFLP = restriction fragment length polymorphism.
Models of defective NER in XPC have been studied. Using XPC knock-out mice by homologous recombination in embryonic stem cells, Cheo et al. (Table 3, see Ref. [59]) showed that XPC −/− mice and fibroblasts had greater susceptibility to UV-B-induced DNA damage and thus, highly prone to skin cancer than the XPC +/+ wild-type. As noted previously in the study by Damia et al. (Table 2, see Ref. [34]) of mRNA expression of a panel of different NER genes in eight different human cancer lines, there was a statistically significant correlation between the relative expression of XPA/XPC (p<0.05) and ERCC1/XPC (p<0.05) mRNAs. However, the same was not true for overall DNA repair capacity using a host cell reactivation assay of a damaged plasmid by cisplatin and melphanal, suggesting that other factors may play a role in regulating the cellular sensitivity/resistance to various antitumor drugs. This is one of the few studies that established that biological responses to similar DNA damage can vary in different cells depending on cell-cycle checkpoints or cell death regulatory factors.

**XPD/ERCC2**

The XPD gene is located on chromosome 19q and codes for an evolutionarily conserved 5′→3′ helicase, a subunit of the TFIIH repair complex essential for transcription initiation [60]. Another subunit of TFIIH, p44, interacts with XPD to stimulate its helicase activity.

Studies mentioned above using a panel of NER genes (ERCC1, XPA, XBP, XPC, XPD, XPG) have demonstrated that resistant cell lines treated with alkylating agents overexpressed these genes when compared to controls (Table 2, see Ref. [34] and Table 5, Ref. [35]). However, an earlier study by Dabhulkar et al. (Table 7, see Ref. [61]) suggested the differences in expression between responders and non-responders. Their results gave a clear indication for different roles of NER genes; the assertion that ERCC1 serves as an excision nuclease, whereas XPD/ERCC2 serves as a helicase.

**XPE/DDB1/2**

The XPE protein is coded for by its gene located on chromosome 11q and is involved in GGR-NER [62]. A damaged DNA-binding (DDB) protein is a p127 (DDB1)–p48(DDB2) heterodimer encoded by the XPE gene involved in the recognition of a wide spectrum of DNA lesions [63]. This protein is presumed to participate in DNA damage recognition and binding so as to mediate DNA excision repair. However, no direct role has been demonstrated for the protein in NER in vitro. Some, but not all, XPE patients lack functional DDB factor, and thus, prone to UV light-damage skin abnormalities and reduced DNA repair capacity. It is not exactly clear why the activity is missing from only some XPE strains. Purified DDB protein microinjection into XPE cells restores DNA repair synthesis to normal levels in strains lacking DDB activity, but does not stimulate repair in XPE cells with DDB activity or in cells from other XP groups [64]. It is possible that XPE strains containing DDB activity, DDB is altered so as to affect DNA repair events other than DNA binding. Studies in human ovarian cancer cell and tissue models have not been described to our knowledge.

**XPB/ERCC2**

XPB, a 112-kDa protein, was one of the last repair genes to be cloned and located to chromosome 16p [65]. XPB forms a heterodimer complex with ERCC1 with interacting domains mapped to the carboxyl-terminus of both proteins [66]. The XPB–ERCC1 complex has four helix–hairpin–helix motifs thought to mediate 5′ DNA structure-specific endonuclease activity at the damage site. Depending on the structure and sequence context of DNA damage, this complex also has functional interaction with RPA and XPA [44,50].

As noted in the study by Ferry et al. (Table 5, see Ref. [35]), the relative constitutive mRNA levels of several nucleotide excision repair genes (ERCC1–XPF, XPA, XBP, XPC, XPD, XPG) were evaluated by northern blot analysis. NER genes were overexpressed in most cisplatin resistant ovarian cancer cell lines (CP70, C30, C200) compared with the cisplatin sensitive parental cell line (A2780). However, ERCC1–XPF endonuclease was the determinant of increased NER in the cisplatin resistance model C200. Similarly, the ERCC1–XPF complex was evaluated in vitro and in vivo using an antisense ERCC1 transfection model as noted above for ERCC1 (Table 5, see Ref. [33] and Table 6, see Ref. [33]). Cell lines expressing the antisense ERCC1 construct showed decreased DRC and increase cisplatin sensitivity. Cisplatin-treated SCID mice with antisense ERCC1 transfectants survived longer than controls. These studies support that the ERCC1–XPF complex is required to repair DNA crosslinks through recombinational repair; thus deletion of XPF/ERCC4 or ERCC1 may be lethal.

**XPG/ERCC5**

The gene encoding for the 135-kDa 3′ DNA structure-specific XPG (ERCC5) endonuclease is located on chromosome 13q [67]. XPG belongs to the flap-endonuclease 1 (FEN-1) family of structure-specific endonucleases and preferentially binds to and cleaves bubble, splayed arms, and stem loops adjacent to 5′ single-stranded DNA [68]. The 3′ incision to damaged DNA mediated by XPG precedes the 5′ incision made by the ERCC1–XPF complex [69]. XPG also interacts with XPA, RPA, XPC–HR23B, and TFIIH as part of the functional assembly of the NER repair complex. Additionally, under some conditions XPG copurifies with TFIIH; therefore, like TFIIH, is essential in coupling various repair processes to DNA transcription [70].

Following the same line as the other DNA repair gene expression profiles in cisplatin-resistant human ovarian cancer
cell lines and tissues, XPG supports current studies. As already shown for RPA, the study by Sakamoto et al. (Table 5, see Ref. [51]) using cDNA microarray technology, revealed that DNA repair genes XPG and RPA were overexpressed 2.1 times greater in cisplatin-resistant cell lines, KFr as compared to cisplatin-sensitive cell line, KF. Similarly, Ferry et al. (Table 5, see Ref. [35]) showed that several NER genes, including XPG, were constitutively overexpressed in the most resistant cell line C200, suggesting that increased platinum–DNA adduct removal is associated with cisplatin resistance in human ovarian cancer model systems.

**CSA/CKN1**

Cockayne syndrome (CS) is a very rare autosomal recessive disorder characterized primarily by postnatal growth failure, diverse developmental abnormalities, and progressive neurological degeneration [71]. Most CS patients have increased skin photosensitivity but do not develop skin tumors, unlike patients with xeroderma pigmentosum. Conventional measurements of NER after UV radiation, indicate no defects in CS cells, however in contrast with normal human cells, CS cells do fail to show a kinetic difference and are considered to be defective in strand-specific repair of transcriptionally active genes [72]. Thus, CS cells are deficient in TCR but proficient in GGR. Complementation analysis in heterodikaryons, derived from fused cells of CS patients which show delayed recovery of bulk DNA and RNA synthesis following exposure to UV radiation, has defined two distinct complementation groups, CSA and CSB [73,74]. XPB patients and certain patients belonging to XPD or XPG systems.

The CSA gene maps to chromosome 5q and encodes a 44-kDa polypeptide with signature features of a WD repeat protein [75]. WD repeat proteins are associated with diverse cellular functions and also coordinate multi-protein complex assemblies, where the repeating units serve as a rigid scaffold for protein interactions [76]. Studies have shown that CSA protein interacts with CSB protein and the p44 protein, a subunit of the human RNA polymerase II TFIH, XPA, XPG, and CSA [81]. Although in NER related assays, there is no evidence for a CSA or CSB related difference, de Waard et al. [81] has shown minor variance in function in the cellular response to genotoxic stress (oxidative DNA damage) between CSB and CSA cells. Moreover, a CSB deficiency rather than a CSA deficiency might cause a metaphase fragility for genes encoding specific highly structured transcripts [82].

TCR of UV-induced lesions, as well as recovery of RNA synthesis after UV-B treatment, has been shown to depend on both CSA and CSB as mentioned above [72]. With regards to CSA, the study by Dabholkar et al. (Table 7, see Ref. [54]) in ovarian cancer tissues of a cohort of 27 patients, those that were resistant to platinum-based therapy had a six-fold higher mean level of expression of the CSB gene (p = 0.033) when compared to responders. Interestingly, these same platinum-resistant tumors did not show significantly higher mRNA levels of the multidrug-resistance gene (MDR1) or of the metallothionein-II (MT-II) gene; alternate molecular mechanisms of cisplatin-resistance in cell lines and ovarian cancer in some studies [83,84].

Cisplatin adducts can induce stalling of RNAPII and the CSB gene product can help clear the stalled RNAPII and promote transcriptional recovery after DNA damage [85]. In a study by Lu et al. (Table 5, see Ref. [86]), phosphorothioate backbone antisense oligodeoxynucleotides (AO’s), which inhibit expression of specific gene products, were characterized to target CSB mRNA in A2780/CP70 cisplatin-resistant human ovarian carcinoma cell lines. The AO’s reduced the proliferation rate of the A2780/CP70 cells by 50% and rendered them more sensitive to cisplatin, oxaliplatin, H2O2, and ionizing radiation in cell culture. Further, the authors were able to potentiate the anti-tumor effects of cisplatin against A2780/CP70 tumor xenografts implanted in athymic nude mice by chemically modified “mixed backbone” AO’s (MBO’s) targeting CSB (Table 6, see Ref. [86]). The MBO’s enabled a non-toxic (3 mg/kg) dose of cisplatin to have the same degree of anti-tumor efficacy as a more toxic (5 mg/kg) cisplatin dose; a dose whose adverse side effects were demonstrated by a reduction in weight gain. The study concluded that the CSB gene product may be viewed as an anti-cancer target.
NER gene coordinated expression

The concept of coordinated mRNA expression of NER genes through which cisplatin–DNA intrastrand adducts are repaired has been extensively studied by Reed et al. [8,87]. In order to address whether NER genes are coordinately expressed, up- or down-regulated simultaneously or within some defined order in the pathway, Reed et al. (Table 4, see Ref. [87]) examined the patterns of expression in human ovarian cancer tissues. A cohort of 28 tumor specimens was assessed for mRNA expression in ERCC1, XPA, XPB, and CSB/ERCC6 using RT-PCR. Deduction of the order of appearance of the mRNAs of the different genes, i.e. the expression of one preceding another, independently or jointly, resulted in the following obligate order in human ovarian cancer tissues: ERCC1 (DNA damage recognition and excision) occurs before, XPB (linkage of DNA repair and transcription) then, XPA (fine tunes DNA damage recognition), and followed by CSB/ERCC6 (essential for gene specific repair). This biological order suggested in this study is in line with the current functions of specific NER proteins and the order appears to be independent of cisplatin sensitivity or resistance. Further, it has been suggested by other investigators that coordinated expression of these genes may show disruption in malignant tissues. Dabholkar et al. [88] have shown that as cells move from a state of normalcy to higher grades of malignancy, there is increasing disorder in the NER process. Thus, the variability of induction for each NER gene suggests that regulation may not be coordinated tightly in tumor cells. However, understanding the NER process is important because of the potential use of anti-sense gene therapy against specifics in the repair pathway and a possible mechanism of reversal of drug resistance in this disease.

NER pathway and platinum resistance

Review of the current literature strongly supports that tissue culture studies are consistent with clinical studies, suggesting that DNA repair is of major importance in determining clinical outcome and clinical resistance to platinum-based chemotherapy in human ovarian cancer. However, other wide range of metabolic or structural properties within tumors may lead to drug resistance [89]. These include: decreased drug uptake (P-glycoprotein), increased detoxification (glutathione-S-transferase GST), evasion of apoptosis (mismatch repair deficiency, dysfunctional p53), and increased DNA repair. Further, it is well known that at low levels of resistance (10–15-fold over baseline), DNA repair predominates as the primary mechanism of cellular resistance to platinum compounds [8,54]. At higher levels of resistance (>40-fold over baseline), glutathione metabolism appears to be the important pathway in human ovarian cancer cell lines [90].

The precise molecular mechanisms of platinum (cisplatin, carboplatin) anti-tumor activity have been extensively studied. These agents exert their cytotoxic effects by forming unstable alkyl groups that react with nucleophilic (electron-rich) sites on organic compounds such as DNA [6]. Cisplatin forms many di-adducts in DNA, reacting mostly with guanines [91]. Cisplatin–DNA adducts include 65% (GpG) intrastrand cross-links, 25% (ApG) intrastrand cross-links and 5–8% interstrand crosslinks between the guanines in the sequence (GpC). Intrastrand adducts can cause changes in DNA conformation that may affect DNA replication and an inhibition of DNA synthesis [7]. These adducts are responsible for the cytotoxicity of the drug, and clinical response seem to be correlated with the level of platinum–DNA in the circulation [92]. These studies have shown that patients who exhibit a clinical complete response (remission) tend to have greater leukocyte DNA adduct levels (platinum lesions per kb of cellular DNA) than those patients that do not respond (except for testicular cancer). These DNA damage measurements were taken in nucleated human peripheral blood cells, and data suggests that these levels are in the range of that observed in tumor tissues taken at autopsy [92]. Thus, it would seem obvious that there exists a rationale for using surrogate tissue for DNA repair assays. In a functional assay to measure inherited repair capacity, it is not optimal to use tumor tissue because of its specificity in carcinogen–interactive affinity and altered DNA repair machinery. Therefore, using peripheral blood cells as surrogate tissue is a viable window to examine NER activities and evaluate genetic traits similar to those in target tissues. Although molecular predictors of treatment response to platinum have been studied, translational assays have not yet been developed for use in individualized chemotherapy. However, the emerging field of pharmacogenetics employing single nucleotide polymorphism (SNP) technology to examine constitutional DNA to determine efficacy and response to chemotherapy shows some promise [93].

NER gene polymorphisms in ovarian cancer

Single nucleotide polymorphisms (SNPs) in drug metabolism, drug transport, drug target, and DNA repair have been implicated in interpatient variability in response to many chemotherapy agents [93]. SNPs are the most common DNA sequence variations that occur when a single nucleotide in the genome sequence is altered and may confer individual variability. Most of the NER genes studied are polymorphic. Although the significance is largely unknown, the implication is that these SNPs may affect the function of these proteins and therefore the efficiency of DNA repair. SNPs that do not cause an amino acid change may also affect DNA repair by causing mRNA instability and therefore may affect risk of environmentally induced cancer [94]. Thus, individuals carrying NER genotypes associated with inefficient NER capacity will result in reduced removal of carcinogen-induced adducts resulting in increased cancer risk. On the other hand, inefficient NER genotypes will result in reduced removal of chemotherapy-induced DNA adducts, therefore enhancing chemotherapy drug efficacy and improving clinical outcome.

Recently, several reviews have examined the role of the NER polymorphisms with clinical cancer outcomes and response to chemotherapy [95,96]. More specifically, some studies have assessed SNPs in the XP complementation groups as predictors of chemotherapy response and prognosis in several cancers such
as colorectal, lung, head and neck, and bladder [97–100]. Studies evaluating the prognostic significance of NER gene member polymorphisms in human ovarian cancer are few to date and only those that are clinically relevant are described below.

**ERCC1**

None of the eight known polymorphisms of the ERCC1 gene result in an amino acid change [94]. In human ovarian carcinoma cell lines, a silent C → T transition at codon 118 has been described by Yu et al. (Table 8, see Ref. [101]). This polymorphism resulted in a triplet code for the same amino acid, asparagine, however, the TT genotype resulted in a 50% reduction in codon usage in MCAS (cisplatin-resistant mucinous cystadenocarcinoma) cell lines when compared to A2780/CP70 (also cisplatin-resistant) ovarian cancer cell lines. This reduction translated into decreased ERCC1 mRNA expression and reduced cisplatin-DNA adduct repair. A more recent study in lung cancer, found that patients with the wild-type ERCC1 genotype of the same SNP demonstrated a better survival than individuals with the variant genotypes [102].

**XPA**

Two polymorphisms in this gene, an A → G change in nucleotide 23 (A23G) in the 5′ region, and a G → A change in nucleotide 709 (G709A) in exon 6 that results in an amino acid change of arginine to glycine in codon 228 (Arg228Gln), have been identified [103]. We have already mentioned that enhanced XPA mRNA levels in cisplatin-resistant human ovarian cancer are not associated with XPA mutations or gene amplification; the more likely causes are alterations in regulatory factors [47].

Wu et al. [104] have previously reported on the XPA SNP, that the G variant allele seems to have a protective effect in lung cancer patients with at least one copy of the G allele; those individuals had a more efficient DNA repair capacity than individuals with the homozygous A allele. In the only study to our knowledge, in patients with advanced ovarian cancer treated primarily with platinum-based chemotherapy, the same authors found that individuals with a heterozygous variant XPA allele had a shorter median survival (21.5 months, \( p = 0.03 \)) and shorter median time to recurrence (11.3 months, \( p = 0.05 \)) than individuals with the homozygous wild-type allele (37.9 and 13.9 months, respectively) (Table 8, Ref. [105]). These findings are consistent with the hypothesis that an efficient DNA repair genotype is associated with a reduced cancer risk but poorer clinical outcome.

**XPB/ERCC3**

Two polymorphisms in this gene have also been reported by Butkiewicz et al. [103], an A → G change in nucleotide 445 in exon 3 (A445G), and a G → T change in nucleotide 1299 (G1299T) in exon 8. Although both cause an amino acid change, the frequencies of these two variants are rare, both 1.4%, that large studies would be needed to investigate any association with cancer outcomes.

**XPC**

A common biallelic polymorphism (PAT) in intron 9 consisting of an 83 base poly(AT) insertion and a 5-base deletion within intron 9 in linkage disequilibrium with a single nucleotide polymorphism in exon 15 of the XPC gene (A2920C, Lys939Gln), have been reported [106]. One study investigated the role of the variant allele of XPC (XPC–PAT+) in a case control study of squamous cell carcinoma of the head and neck [99]. The authors found that the frequency of the XPC–PAT+ allele was higher in the cases than in controls supporting their hypothesis that the XPC–PAT+ allele may contribute to the risk of developing head and neck cancer. In another study of the exonic polymorphism of XPC (Lys939Gln), individuals homozygous for the variant C-allele were at an almost 2-fold increase risk of bladder cancer compared to the wild-type allele [100].

To evaluate platinum-sensitivity or resistance in ovarian cancer, a panel of XP polymorphisms and chemotherapy response was assessed in the study by Adewole et al. (Table 8, Ref. [105]). Patients were classified into two groups based on treatment response as responders and non-responders. Although no statistical significant difference between responders and non-responders and the XP genotypes was seen, there was a protective trend in the risk of recurrence and death among the homozygous variant alleles when compared to the heterozygous variant and the wild-type alleles in XPC, XPD exon10, and XPD exon23 polymorphisms in patients with advanced epithelial ovarian cancer.

**XPD/ERCC4**

Several XPD polymorphisms have been recently identified [107]. These common polymorphisms include C22541A (156Arg) of exon 6 and C35326T (711Asp), which do not cause amino acid changes. However, the two most frequently studied XPD polymorphisms have been exon 10 Asp312Asn (G/A transition) and exon 23 Lys751Gln (C/A transversion) [108–110]. Spitz et al. [111] have previously reported a suboptimal DNA repair capacity in head and neck cancer patients with the variant A allele of exon10 SNP (Asp312Asn). Results of the analysis by Adewole et al. (Table 8, see Ref. [105]) of the XPD polymorphisms show that carriers of at least one variant allele of the XPD exon10 (Asp312Asn) SNP had a significantly reduced risk of death compared with carriers of the wild-type allele (HR 0.19, 95% CI 0.05–0.68); the association was similar for XPD exon23 (Lys751Gln) SNP (HR 0.17, 95% CI 0.01–0.96). Again, these findings support the hypothesis that DNA repair genotypes play an opposing role in cancer risk as well as clinical outcome.

**XPE/DDB1/2**

No variants of DDB1 have been described to our knowledge. Two variants of DDB2 in the same exon have been reported, but their allele frequencies are not known [112]. There are no known studies in ovarian cancer.
XPF/ERCC4

At least 10 SNPs have been identified in the promoter and coding region of the XPF gene [94,113]. However, most of the variants occur at very low allele frequencies except for Pro379Ser and Arg415Gln. Both of these SNPs have been investigated in lung cancer and breast cancer, respectively, but none has been shown to be associated with cancer risk or anti-cancer drug efficacy [114,115].

XPG

Although there are three validated XPG SNPs, only two have been studied with relative frequency [116,117]. In the more common SNP, a single nucleotide substitution (G → C) on exon 15 causes an amino acid change (His1104Asp) at codon 1104. The other two non-conserved SNPs in the coding sequence of the XPG gene (Arg1053Gly and Arg1080Gly) occur relatively uncommonly. The His1104Asp polymorphism of the XPG gene with an allele frequency of 25–30% has been studied in several cancers. One report showed that the variant allele was more common in cases of breast cancer than in controls and the results were significant after combining heterozygous and variant genotypes [118]. Further, a protective effect of the variant allele was shown in studies of bladder and lung cancer [100,119].

The role of XPG in predicting ovarian cancer outcome is also interesting (Table 8, see Ref. [105]). Carriers of at least one variant allele of His1104Asp SNP had a significantly increased risk of death compared with carriers of the wild-type allele in a cohort of 146 patients with advanced epithelial ovarian cancer. Furthermore, individuals with a homozygous variant XPG allele had a significantly shorter median survival (8.3 months, p = 0.006) compared with individuals with the homozygous XPG wild-type allele (24.6 months). The functional impact of this XPG SNP however, is unclear.

CSA/CKN1 and CSB/ERCC6

Recently, a novel SNP for the CSA/CKN1 gene has been identified [120]. The common T471C SNP was confirmed in a young female CS proband and may prove useful in pharmacogenetic studies of developmental and/or aging related pheno-types. No other CS SNP has been reported to date in ovarian cancer.

Future perspectives

In summary, platinum chemotherapy exerts its cytotoxic effect by forming DNA adducts and subsequently inhibiting DNA replication. Removing platinum–DNA adducts is a complex process that includes the XP complementation group of genes in the NER pathway. These studies reviewed suggest that concurrent up-regulation of genes involved in NER may be important in clinical resistance to platinum-based chemotherapy in ovarian cancer. It is unclear at this time whether coordinated regulation within NER may be effected by one gene or its SNP in this process, however, such questions are the subject of current investigations in our laboratory. Evaluation of genetic polymorphisms in cancer susceptibility may help us to understand the significance of these polymorphisms in the identification of individuals at higher risk of developing resistance to anti-cancer drug therapies. In the future, a more complete understanding of relevant genetic factors may result in improved strategies for determining both chemotherapy choice and efficacy in clinical trials that will translate to the bedside. A forthcoming manuscript parallels this work and focuses on implications in cervical cancer.

Conflict of interest statement
We declare that we have no conflict of interest.

References


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6 July 2007