Platinum Resistance Related to a Functional NER Pathway

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Tobacco carcinogens induce DNA adducts that are repaired by the nucleotide excision repair (NER) pathway.[1] Inhaled combustion-derived particles, such as cigarette smoke, cause a local pulmonary inflammatory response that is characterized by the influx of neutrophils into the airways. On entering the lung, neutrophils are activated and release reactive oxygen species and an array of proteins, such as myeloperoxidase. A significant reduction of NER in human alveolar epithelial cells was observed when they were cocultured with activated neutrophils.[2]

NER, a highly versatile pathway for DNA damage removal, is often dysfunctional in non-small cell lung cancer (NSCLC) and could, therefore, be the Achilles heel for customizing chemotherapy. NER removes numerous types of DNA helix-distorting lesions, including cisplatin- and ultraviolet-induced photo products.[1] Inherited defects in the NER process cause serious repair disorders: xeroderma pigmentosum (XP), with extreme risk of ultraviolet-induced skin cancer, and Cockayne syndrome. NER functions by a "cut-and-paste" mechanism in which cisplatin damage recognition, local opening of the DNA helix around the lesion, damage excision, and gap filling occur in successive steps[1,3–5] (Figure 1). NER is composed of two subpathways: global genome NER (GG-NER) and transcription-coupled NER (TC-NER), which share the same core mechanism but differ in the way lesions are recognized.[6] NER-defective XP is classified into seven complementation groups, XPA to XPG. XPC and XPE are specifically defective in GG-NER, which repairs the damage on the nontranscribed strand, whereas the other XP groups involve deficiencies in both TC-NER and GG-NER.[7]

The first step in GG-NER is damage recognition by the heterodimer XPC/hHR23B, which binds with higher affinity to helix-distorting DNA lesions than to nondamaged, double-stranded DNA[1] (Figure 1A). Various NER factors, including transcription factor IIH (TFIIH, a general transcription factor for RNA polymerase II), XPA, replication protein A (RPA), and XPG work together to repair DNA damage (Figure 1B). TFIIH can be divided into two subcomplexes: the core TFIIH (composed of XPB, p62, p52, p44, p34, and p8) and a cdk-activating kinase subcomplex (containing cdk7, cyclin H, and MAT1).[7] Both subcomplexes are bridged by XPD. In NER, TFIIH unwinds the duplex DNA around the lesion to allow the recruitment of the NER factors XPA, RPA, XPG, and excision repair cross-complementing 1 (ERCC1)/XPF (Figure 1B). TFIIH, with the two helicases XPB and XPD, opens an approximately 30-base-long DNA segment around the platinum damage. This open intermediate is stabilized by RPA and XPA (Figure 1C). The DNA strand that contains the damaged base(s) is excised by the two NER endonucleases XPG and ERCC1/XPF (Figure 1D). XPG cleaves the damaged DNA strand 3′ from the lesion, and ERCC1/XPF cleaves the damaged strand 5′ from the lesion (Figure 1D). The resulting gap is filled by DNA polymerase δ or ε in the presence of replication factors[8] (Figure 1E). Importantly, the ERCC1/XPF structure-specific nuclease has an additional role in the repair of cisplatin adducts besides its function in NER: the recombination repair of interstrand cross-links.[9] Moreover, colocalization of ERCC1 foci and RAD51 foci in response to cisplatin treatment has recently been found and may represent recruitment of ERCC1/XPF to sites of recombination repair.[10] Nevertheless, in addition to NER, cisplatin-induced cytotoxicity requires the interaction of components from several different DNA damage-processing systems.[11]

CLINICAL STUDIES TESTING PLATINUM OUTCOME ACCORDING TO ERCC1, RRM1, AND BRCA1

In recent years, ERCC1 and other components of DNA damage-processing systems have been examined in the clinical setting (Figure 2). High tumor tissue levels of ERCC1 mRNA in ovarian and gastric cancer patients have been associated with cisplatin resistance.[12,13] When intratumoral ERCC1 mRNA derived from paraffin-embedded tumor specimens was measured by real-time reverse transcriptase polymerase chain reaction in metastatic colon cancer patients treated with oxaliplatin and 5-fluorouracil, high levels of ERCC1 significantly correlated with poor response and shorter survival.[14]

Several studies in stage IV gemcitabine/cisplatin-treated NSCLC show that patients with low ERCC1 or ribonucleotide reductase subunit M1 (RRM1) mRNA levels have a median survival of 15 months. Nevertheless, the predictive value of low ERCC1 mRNA levels found in our original study[15] was not borne out by our second study with

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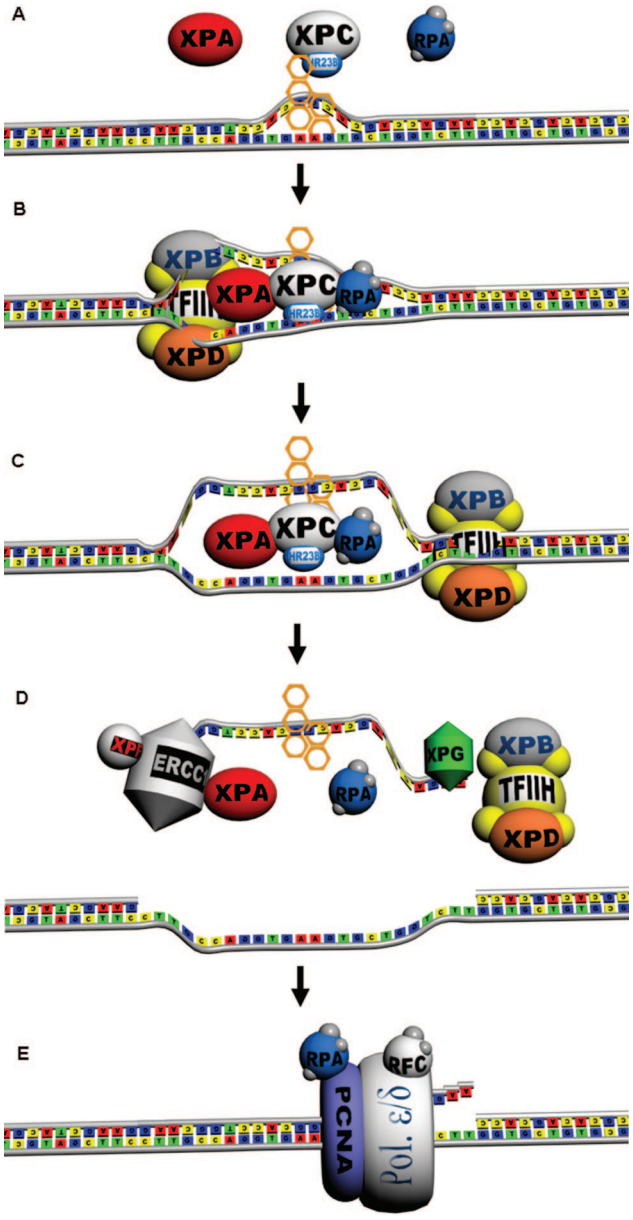

FIGURE 1. Functional nucleotide excision repair (NER) pathway and the repair of platinum damage. (*A*) Recognition of platinum adducts (shown as golden rings) by the heterodimer XPC/hHR23B. (*B*) TFIIH contains two helicases, which open an approximately 30-base-long DNA segment around the damage. (*C*) This open intermediate is stabilized by RPA and XPA. (*D*) The DNA strand that contains the damaged base(s) is excised by XPG and excision repair cross-complementing 1 (ERCC1)/XPF. XPG cleaves the damaged DNA strand 3′ from the lesion, and ERCC1/XPF cleaves the damaged strand 5′ from the lesion. (*E*) The resulting gap is filled by DNA polymerase δ or ε in the presence of replication factors.

Italian patients, in which low ERCC1 levels showed a nonsignificant trend toward better survival (unpublished data). Conversely, in these same Italian patients, low RRM1 mRNA levels were significantly associated with improved survival (15.5 versus 6.8 months; $p = 0.002$).[16] No differences were found according to RRM1 status in patients treated with paclitaxel/carboplatin or vinorelbine/cisplatin as part of the original phase III randomized trial.[17] Whereas no differences between the three different cisplatin doublets were found in the original trial, RRM1 identified patients with better survival in the gemcitabine/cisplatin-treated arm, which can be attributed to the effect of RRM1 on gemcitabine metabolism and on the NER pathway. Low RRM1 levels were associated with a significantly better survival[18] in NSCLC patients treated with gemcitabine/cisplatin as part of a large phase III randomized trial.[19] Nevertheless, the predictive value of RRM1 was not evident in the group of patients who received gemcitabine/cisplatin/vinorelbine triplets, raising the hypothesis that antimicrotubule drugs act on the NER pathway in a different way, as explained below. In another study[20] of patients treated with neoadjuvant gemcitabine/cisplatin and then surgery, the lowest RRM1 mRNA levels (bottom quartile) predicted significantly better survival in comparison with those with higher levels of RRM1, whereas no differences were observed according to ERCC1 or XPD mRNA levels.

The predictive role of ERCC1 in cisplatin response was tested in a customized chemotherapy trial based on tumor ERCC1 mRNA levels. Patients in the control arm received docetaxel plus cisplatin. Patients in the customized arm received treatment based on ERCC1 mRNA levels: those with low levels received docetaxel plus cisplatin, and those with high levels received non–cisplatin-based treatment (docetaxel plus gemcitabine). Objective response was observed in 53 patients (39.3%) in the control arm and 107 patients (50.7%) in the customized arm ($p = 0.019$).[21] This study shows that assessment of ERCC1 mRNA expression in patient tumor tissue is feasible in the clinical setting and predicts response to docetaxel plus cisplatin. Nevertheless, the response benefit did not translate into improved survival, which can be partly explained by the fact that antimicrotubule drugs may not be the best partner for cisplatin in the presence of low ERCC1 levels. The combination of ERCC1 and RRM1 gene expression levels in frozen tumor specimens has also been used to select chemotherapy[22]: patients with low levels of both genes received carboplatin plus gemcitabine; those with high levels of both genes received docetaxel plus vinorelbine; those with high ERCC1 but low RRM1 received gemcitabine plus docetaxel; and those with low ERCC1 and high RRM1 received docetaxel plus carboplatin. This study confirmed the feasibility of this approach and paved the way for additional studies.

GG-NER might not correctly detect cisplatin DNA adducts, because it has been shown to possess a low affinity for these adducts.[23,24] On the other hand, defects in TC-NER (Figure 1) render cells markedly hypersensitive to cisplatin.[8] Unlike ERCC1, breast cancer susceptibility gene 1 (BRCA1) is involved in TC-NER,[25,26] and BRCA1-deficient cells are hypersensitive to cisplatin.[27] BRCA1 expression confers differential chemosensitivity in cell lines,[28] and low levels of

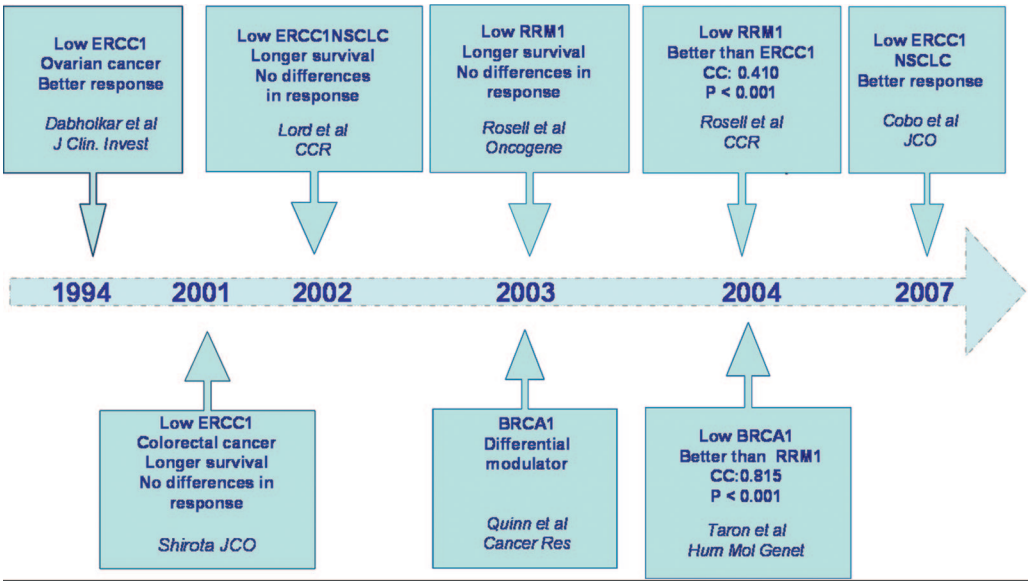

FIGURE 2. Clinical studies of excision repair cross-complementing 1 (ERCC1) and other components of DNA damage-processing systems.

BRCA1 correlated with increased survival in stage III NSCLC patients treated with neoadjuvant cisplatin/gemcitabine and then surgery.[29]

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