Pharmacogenomics and gemcitabine

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Approximately half of lung cancer patients present with metastases, and a large proportion will develop recurrent disease, with median survival to cisplatin-based chemotherapy of 11 months. No predictive factor of response to cisplatin-based chemotherapy is yet available in clinical practice. The nucleotide excision repair system plays a major role in repairing a variety of distorting lesions, notably platinum-induced DNA adducts. ERCC1 is a leading gene in repairing cisplatin DNA damage. We carried out three different studies examining individually the role of ERCC1, RRM1, and then both, mRNA expression in paraffin-embedded pretreatment bronchial biopsies from gemcitabine/cisplatin-treated advanced non-small-cell lung cancer (NSCLC) patients. Median survival was significantly prolonged in patients with low levels of ERCC1 or RRM1. BRCA1 is involved in homologous recombination repair, and we observed that low levels of BRCA1 mRNA significantly increased survival in gemcitabine/cisplatin-treated patients. Our observations lead us to recommend that tumors be regularly assessed for ERCC1 and BRCA1 mRNA expression in order to customize gemcitabine/cisplatin treatment.

Key words: BRCA1, cisplatin, ERCC1, gemcitabine, non-small-cell lung cancer, nucleotide excision repair

In 2004 in Europe, there were an estimated 2,886,800 incident cases of cancer diagnosed, including data recorded in the 25 member states of the European Union. Lung cancer was the most common form, with a total of 381,500 cases (13.2%) and an estimated mortality of 341,800 cases (20% of total) [1]. Around 85% of lung cancers are non-small-cell lung cancer (NSCLC), and the majority of patients present as advanced disease, with dismal prognosis. A landmark study in NSCLC showed that the combination of gemcitabine plus cisplatin attained a median survival of 9.1 months in comparison with 7.6 months with cisplatin alone [2]. A phase III trial of the European Organization for Research and Treatment of Cancer (EORTC) showed similar results with paclitaxel/cisplatin and gemcitabine/cisplatin, with median survival times of 8.1 months and 8.9 months, respectively. A third arm of paclitaxel/gemcitabine showed a shorter survival of 6.7 months [3]. A recent meta-analysis, which included a study encompassing 4556 patients, showed a median survival of 9 months for gemcitabine-based chemotherapy in contrast with 8.2 months for non-gemcitabine combinations [4]. One of the mechanisms of tumor resistance to cisplatin is increased nucleotide excision repair (NER) activity, in particular increased levels of ERCC1. The 5th incision made by the ERCC1-XPF complex is thought to be a rate-limiting step in the NER pathway, as shown by an increase in excision activity in extracts from non-cisplatin-resistant cells after addition of purified ERCC1-XPF protein, compared with no increase in excision activity after addition of ERCC1-XPF to extracts from cisplatin-resistant cells [5]. The potential use of ERCC1 mRNA expression as a predictive marker for the effectiveness of cisplatin-based chemotherapy is an important area of clinical translational research. Together with ERCC1, ribonucleotide reductase subunit M1 (RRM1) is also involved in gemcitabine metabolism as a participant in the NER pathway, providing nucleotides that fill gaps created by ERCC1 excision of the DNA strands containing cisplatin bulky adducts. Tumor overexpression of RRM1 mRNA in gemcitabine/cisplatin-treated NSCLC patients was associated with a short median survival of 3.6 months in contrast with 13.7 months for patients with low RRM1 mRNA expression \( P = 0.009 \) [6]. This data is ushering in a new era of customized chemotherapy based on NER transcripts.

Patients with lower DNA repair capacity are more chemosensitive than those who carry a proficient DNA repair system [7–9]. In 1995, it was shown that elevated DNA repair capacity is associated with drug resistance in lung cancer cell lines, and it was suggested that modulation of DNA repair mechanisms, such as the incorporation of specific DNA repair inhibitors in therapeutic regimens, could help to improve therapeutic strategies. The overall DNA repair capacity was estimated by the ability of cells to reactivate a plasmid damaged by cisplatin (host cell reactivation assay) [10]. Cytotoxicity from cisplatin and other platinum-containing drugs results from the formation of platinum DNA adducts, and clinical outcome is better in patients with higher levels of these adducts, indicating that these patients have lower DNA repair capacity. Nucleotide excision repair (NER) is the major mechanism for repairing platinum DNA adducts [7–9], involving the coordinated
activity of more than 20 enzymes that remove a segment of DNA containing a bulky adduct and then restore that segment by replicating the intact complementary strand. The predictive potential of cisplatin adducts in buccal cells was demonstrated in NSCLC patients receiving daily combined treatment with concomitant cisplatin and radiotherapy. Patients who had higher adduct levels by immunocytochemistry had a median survival of 30 months, in comparison with only 5 months for patients with low adduct levels [11]. It was hypothesized that patients with genetically determined effective DNA repair activity would be more likely to effectively repair DNA adducts in tumor tissue than would patients with genetically determined suboptimal DNA repair. To test this hypothesis, a functional assay of DNA repair capacity – the ability to repair benzo[α]pyrene diol epoxide (BPDE)-induced DNA adducts – in peripheral lymphocytes, rather than in tumors, was used. Median survival was 8.9 months for patients whose DNA repair capacity was in the top quartile, compared to 15.8 months for patients whose DNA repair capacity was in the bottom quartile (P = 0.04) [12].

Cisplatin resistance is associated with increased expression of the excision repair cross-complementing 1 (ERCC1) gene [13]. Cancer tissues from ovarian cancer patients whose tumors were clinically resistant to therapy showed greater levels of ERCC1 mRNA [14]. cDNA derived from primary gastric tumors before chemotherapy was used to determine ERCC1 mRNA levels expressed as the ratio of the PCR product of the ERCC1 gene and the β-actin gene. Response and survival with cisplatin plus fluorouracil were significantly associated with levels of ERCC1 ≤5.8 [15]. More modern studies have used cDNA derived from paraffin-embedded tumor specimens to determine ERCC1 mRNA expression relative to the internal reference gene β-actin, using fluorescence-based, real-time reverse transcriptase PCR. Colorectal cancer patients treated with oxaliplatin plus fluorouracil with low ERCC1 expression had a significantly longer survival compared to patients with high intratumoral ERCC1 mRNA levels [16]. We carried out a study to examine the role of ERCC1 mRNA levels in advanced NSCLC patients treated with gemcitabine plus cisplatin. Patients with low ERCC1 mRNA levels attained a response rate of 52%, while in those with high levels, the response rate was 36%. This difference was not significant; however, when we used a cutoff of 5.8 for ERCC1 expression, median survival was 15 months for patients with low levels and only 5 months for those with high levels (P < 0.001) [17].

Based on these findings, we carried out an ERCC1 mRNA customized chemotherapy trial. More than 400 patients have been included and randomized to the control or the experimental arm. The control arm received docetaxel plus cisplatin, and patients in the experimental arm received either the same combination of docetaxel plus cisplatin if their ERCC1 mRNA levels were low or docetaxel plus gemcitabine if their levels were high (Figure 1). The preliminary results on 264 patients, presented at ASCO 2005 [18], showed that the response rate for patients with low ERCC1 levels was 56.6% while for patients in the control arm it was 40.4% (P = 0.02). When patients in the control arm were split according to the ERCC1 levels, those with low levels had a response rate of 47.3%, while those with high levels had a response rate of 26.1%.

The logistic regression model for tumor progression indicated a significant improvement for patients randomized to docetaxel plus cisplatin based on low ERCC1 levels. Although the results are still preliminary, time to progression and survival adjusted for age are significantly in favor of the group with low ERCC1 levels.

BRCA1 has also been recognized as a marker of chemoresistance. BRCA1 was overexpressed in the cisplatin-resistant breast cancer cell line MCF-7 [19]. BRCA1 is component of multiple DNA repair pathways and functions as a molecular determinant of response to a range of cytotoxic chemotherapeutic agents. It has been demonstrated that BRCA1 abrogates the apoptotic phenotype induced by a range of DNA-damaging agents, including cisplatin, etoposide and bleomycin, and induces dramatic responses to a range of antimicrotubule agents, including paclitaxel and vinorelbine. These landmark findings indicate that BRCA1 functions as a differential regulator of chemotherapy-induced apoptosis [20–22]. Sporadic cancers, such as breast, ovarian and NSCLC, can have the BRCA1 function abrogated by methylation or other mechanisms. In addition, methylation of FANCF has been observed in these tumors. These characteristics, known as ‘BRCAness’, increase sensitivity to cisplatin and related DNA cross-linking agents and may increase resistance to antimicrotubule drugs [23]. Recently, it has been shown that BRCA1 or BRCA1 dysfunction profoundly sensitizes cells to the inhibition of poly(ADP-ribose) polymerase (PARP) [24]. We have observed that BRCA1 mRNA expression closely correlates with ERCC1 mRNA expression and that BRCA1 mRNA expression predicts outcome in locally advanced NSCLC patients treated with neoadjuvant gemcitabine plus cisplatin followed by surgery. Median survival has not been reached in patients with the lowest BRCA1 mRNA levels, while survival was very poor in patients with the highest levels [25]. These findings are along the same lines as preclinical data, indicating that patients with high BRCA1 levels could respond more favorably, not to non-cisplatin regimens but to antimicrotubule drugs.

Although gemcitabine is a neutral drug for the BRCA1 mRNA effect [20], we have observed that elevation of ERCC1 and BRCA1 is closely related to high levels of ribonucleotide reductase, which is one of the principal mechanisms of resistance to gemcitabine [26].

Recently, EGFR mutations have been found to occur at a significantly higher frequency in hereditary breast cancer than in sporadic breast cancer [27]. As the authors state, this finding

**Figure 1.**

ERCC1-Custimized Chemotherapy in Advanced NSCLC

<table>
<thead>
<tr>
<th>GENOTYPE B1</th>
<th>docetaxel / cisplatin (low ERCC1 mRNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control arm</td>
<td>docetaxel / cisplatin</td>
</tr>
<tr>
<td>GENOTYPE B2</td>
<td>docetaxel / gemcitabine (high ERCC1 mRNA)</td>
</tr>
<tr>
<td>Experimental arm</td>
<td>ERCC1 levels</td>
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Table showing the potential combination of treatments based on ERCC1 mRNA levels.
may not be surprising given the functional effect of BRCA1 and BRCA2 mutations. Defects in BRCA1 and BRCA2 have been shown to disrupt the DNA repair mechanism, which leads to genomic instability. Along with others, we have observed that EGFR mutations are highly predictive of response and are a prognostic marker for survival in lung adenocarcinoma patients [28–32]. We have observed dramatic responses, including in brain metastases, with only treatment with tyrosine kinase inhibitors, without the need for radiotherapy or other therapeutic interventions. So far, the data has been collected retrospectively on gefitinib-treated patients after second- or third-line chemotherapy failure. In our experience, the preliminary evidence of EGFR mutations in lung adenocarcinoma patients in Spain [32] indicates a frequency of 20%. We have undertaken a prospective study of EGFR mutation assessment in advanced lung adenocarcinomas. The EGFR assay is performed rapidly with an average of five-day wait for results. Patients without EGFR mutations can be examined to determine their BRCA1 mRNA levels for customized chemotherapy (Figure 2).

disclosures
Dr Rosell has reported no financial relationships with companies whose products are mentioned in this article.

references