DNA Repair Polymorphisms and Treatment Outcomes of Patients with Malignant Mesothelioma Treated with Gemcitabine-Platinum Combination Chemotherapy

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Introduction: Genetic polymorphisms that affect DNA repair capacity can modulate the efficacy and toxicity of cytotoxic agents. Therefore, the aim of our study was to evaluate the influence of genetic variability in DNA repair genes on treatment outcome in patients with malignant mesothelioma (MM) treated with gemcitabine-platinum combination chemotherapy.

Methods: In total, 109 patients with MM were genotyped for 10 polymorphisms in XRCC1, *NBN*, *RAD51*, and *XRCC3* genes. The influence of selected polymorphisms on tumor response and occurrence of treatment-related toxicity was determined by logistic regression analysis, whereas their influence on survival was estimated by Cox proportional hazards model.

Results: There were no associations between the investigated polymorphisms and tumor response, but we observed a significant association between *XRCC1* 399Gln allele and reduced overall survival (hazards ratio = 1.70; 95% confidence interval [CI] 1.06–2.73; p = 0.028). Interaction between *XRCC1* 399Gln allele and C-reactive protein levels revealed that carriers of at least one *XRCC1* 399Gln allele with C-reactive protein levels above median had significantly shorter overall survival time compared with other patients (12.9 months versus 25.3 months, log-rank $p < 0.001$). We also observed an association between *XRCC1* 399Gln and lower frequency of leukopenia (odds ratio [OR] = 0.25; 95% CI 0.09–0.67; p = 0.006), neutropenia (OR = 0.24; 95% CI 0.09–0.68; p = 0.007), and thrombocytopenia (OR = 0.27; 95% CI 0.09–0.84; p = 0.024). In addition, *NBN* 3474A>C, *XRCC3* -316A>G, and Thr241Met polymorphisms showed significant associations with treatment-related toxicity.

Conclusions: Our results support the hypothesis that DNA repair gene polymorphisms, particularly *XRCC1* Arg399Gln, may modify the response to gemcitabine-platinum combination chemotherapy and, for the first time, show this effect in patients with MM.

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Malignant mesothelioma (MM) is a rare tumor with an increasing incidence and a very poor prognosis. The 10-year average incidence in Slovenia is approximately 30 cases per year (www.slora.si, accessed on November 23, 2011) with a 1-year survival rate of 33%.[1] Recently, there have been important developments in the chemotherapy of MM, which have improved outcomes and prolonged survival of patients with MM. The pemetrexed-cisplatin combination chemotherapy has become a standard of care in MM treatment;[2] however, other similarly effective regimens, such as gemcitabine-cisplatin combination, are widely used.[3]

Gemcitabine exerts its cytotoxic effect mainly through inhibition of DNA synthesis by being incorporated into DNA and through inhibition of ribonucleotide reductase M1, resulting in a decrease of deoxyribonucleotide pools necessary for DNA synthesis. Incorporation of gemcitabine into DNA was reported to increase the stability of topoisomerase I cleavage complexes, leading to the accumulation of strand breaks.[4,5] Besides, platinum agents covalently bind to DNA, forming intrastrand DNA adducts or interstrand DNA crosslinks, which may also lead to generation of DNA strand breaks.[6] The synergistic cytotoxic effect of gemcitabine-cisplatin combination was observed in vitro[7] and it was suggested that this combination increases the accumulation of DNA strand breaks in MM cell lines.[8] These findings suggest that mechanisms involved in the repair of DNA strand breaks might play an important role in the response to gemcitabine-platinum treatment.

Single-strand breaks (SSBs) are repaired in a multistep process of the base-excision repair (BER) pathway. The central molecule of this pathway seems to be a scaffold protein x-ray repair crosscomplementing protein 1 (XRCC1), which coordinates repair of SSBs through interactions with other BER proteins.[9] Inadequate repair of SSBs because of a deficient BER mechanism can lead to more lethal double-strand breaks (DSBs).

The main mechanism involved in a high-fidelity repair of DSBs is the homologous recombination repair (HRR) pathway. The initial step is the recognition of DNA DSBs by meiotic recombination 11/RAD50/nibrin (MRE11/RAD50/NBN)

complex, followed by cleavage of 3′ ends of the DSB to form single-stranded tails, which invade the intact homologous DNA double helix. The RAD51 protein together with adaptor proteins, such as XRCC3, plays a central role in this process by facilitating initial steps of strand invasion. The 3′-single-stranded tails are extended by DNA polymerase and the resulting Holliday junctions are resolved to yield two intact DNA molecules.

A growing body of evidence has suggested that DNA repair mechanism can modulate the anticancer activity of cytotoxic agents and, therefore, genetic polymorphisms that affect DNA repair capacity might influence the efficacy and toxicity of gemcitabine-platinum combination chemotherapy in patients with MM. There are some reports regarding the influence of BER polymorphisms on treatment response to gemcitabine-platinum combination chemotherapy,[10,11] but evidence of the association between HRR polymorphisms and treatment outcome is insufficient. Moreover, the influence of BER or HRR polymorphisms on treatment outcome in MM patients treated with gemcitabine-platinum combination chemotherapy has not been established so far.

The aim of our study was to evaluate the influence of single-nucleotide polymorphisms (SNPs) in *XRCC1*, *NBN*, *RAD51*, and *XRCC3* genes and their corresponding haplotypes on tumor response, survival, and treatment-related toxicity in patients with MM treated with gemcitabine-platinum combination chemotherapy.

PATIENTS AND METHODS

Study Design

Separate case-control studies were designed for the analysis of tumor response and toxicity. For analysis of tumor response, cases were defined as patients with stable disease (SD) or progressed disease (PD), whereas controls were defined as patients with complete response (CR) or partial responses (PR) to treatment. For toxicity analyses, cases were defined as patients who developed specific treatment-related toxicities, whereas controls were defined as patients who did not develop that toxicity. For survival analysis, a Cox model was used.

Patients

The study group consisted of 109 patients with histologically confirmed MM. All the patients were diagnosed between 1997 and 2010 at the University Clinic of Pulmonary and Allergic Diseases in Golnik, Slovenia and at the University Clinical Centre Maribor, Slovenia. The inclusion criteria for the selection of patients and details of clinical data collection were described previously.[12]

All the patients who were alive at the time of data collection gave their written informed consent to participate in the study. The study was approved by the Slovenian Ethics Committee for Research in Medicine (approval ref. no. 04/02/09) and was carried out according to the Declaration of Helsinki.

Treatment

All patients with MM were treated at the Institute of Oncology, Ljubljana, Slovenia; therefore, treatment, outcome assessment, and follow-up were centralized for all subjects. Patients were treated with gemcitabine in combination with a platinum agent according to one of the two following regimens: gemcitabine in prolonged infusion in combination with cisplatin or carboplatin;[13] or gemcitabine in standard infusion in combination with cisplatin.[14] We also included patients who received gemcitabine-platinum combination chemotherapy as a part of multimodality treatment with surgery and/or palliative radiotherapy.

Response, Survival, and Toxicity Assessment

Tumor response was evaluated as described previously.[14] Progression-free survival (PFS) time was defined as time from day 1 of first-line gemcitabine-platinum chemotherapy to the day of documented disease progression according to the Response Evaluation Criteria In Solid Tumors or to death from any cause, whichever occurred first. Overall survival (OS) time was defined as time from day 1 of first-line gemcitabine-platinum chemotherapy to death from any cause. Patients without documented progression or death at the last follow-up evaluation (September 2011) were censored at that time. Hematologic toxicities, nephrotoxicity, alopecia, and nausea/vomiting were evaluated according to the National Cancer Institute-Common Toxicity Criteria, version 4.0 (http://ctep.info.nih.gov/reporting/ctc.html, accessed on November 23, 2011). Hematologic toxicities were defined by decreased serum hemoglobin levels (anemia), decline of: white blood cells (leukopenia), neutrophil (neutropenia), and platelet count (thrombocytopenia). Nephrotoxicity was defined by elevated levels of serum creatinine concentration. The highest grade of individual toxicity during first-line chemotherapy was chosen as the endpoint for toxicity analyses. Toxicities of grade 2 or higher were considered as clinically relevant. Thrombocytopenia and nephrotoxicity was categorized only as present or absent because of the very low frequency of grade 2 or higher toxicities in the study group.

SNP Selection

SNP search in *XRCC1*, *NBN*, *RAD51*, and *XRCC3* genes was assessed using the scientific literature database PubMed (http://www.ncbi.nlm.nih.gov/pubmed), National Center for Biotechnology Information SNP database,[15] and HapMap database.[16] Previously investigated functional SNPs and/or putatively functional SNPs that tag haplotype blocks with minor allele frequencies greater than 5% were selected. The exploration of possible SNPs' functionality and haplotype-tagging was carried out by Web-based SNP prediction tools.[17,18]

DNA Extraction and Genotyping

Tumor tissue specimens or peripheral blood samples were collected at the time of diagnosis. Tumor tissue specimens were routinely formalin fixed and paraffin embedded. Genomic DNA from formalin-fixed and paraffin-embedded tissue was extracted as previously described.[19] A Qiagen FlexiGene kit (Qiagen, Hilden, Germany) was used for the extraction of genomic DNA from frozen whole-blood samples.

Genotypes of *XRCC1* were determined by TaqMan SNP genotyping method (Applied Biosystems, Foster City, CA),