DNA Repair Gene XRCC1 Polymorphisms and Outcome of Renal Cell Carcinoma in Caucasian Patients

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Abstract. Background: The human X-ray repair cross-complementing group 1 (XRCC1) enzyme plays an important role in response to DNA damage. Two common polymorphisms in XRCC1, Arg194Trp and Arg399Gln, have been repeatedly associated with risk for and outcome of numerous types of cancer treated with radio- and chemotherapy. Recently, a Japanese study suggested these polymorphisms both as risk factors and outcome predictors in renal cell carcinoma (RCC).

Patients and Methods: In the present study, 142 Caucasian patients suffering from RCC were genotyped and analyzed for tumor-related and overall survival and time to metastasis and progression. Results: Analyses revealed absence of the pre-described risk haplotype (194Trp/399Gln) as well as a lack of a statistically significant difference between the different endpoints and genotypes and diplotypes, respectively.

Conclusion: We conclude that in Caucasian patients, XRCC1 polymorphisms do not influence the outcome of RCC.

A complex system of DNA repair enzymes is needed to protect the genome from the consequences of exogenous and endogenous mutagenic influences (1). Deficiency of repair enzymes for DNA damage is known to increase the risk of developing neoplasms (2). Exposure to endogenous active oxygen, ionizing radiation or alkylating agents leads to single-strand breaks followed by base excision repair (BER) in which the enzyme X-ray repair cross-complementing group 1 (XRCC1) is involved (3). Mutations of the corresponding gene XRCC1 are causative for insufficient DNA repair in animal models (4). Therefore, human XRCC1 is a common object of polymorphism studies in cancer.

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XRCC1 is located on chromosome 19q13.2 and consists of 17 exons. Shen et al. sequenced the gene for polymorphisms and found, amongst others, two common variants in exon 6 (rs1799782), where T substitutes C, and in exon 10 (rs25487), where A substitutes G, which lead to the amino acid substitutions Arg194Trp and Arg399Gln, respectively (5). Since then, these two polymorphisms have been repeatedly associated with risk of numerous types of cancer (6). However, some studies have reported no association of XRCC1 genotype with cancer risk (7-9). Some investigators showed distinct functions of the variants at the molecular level, suggesting that these polymorphisms are not only risk markers but could also influence tumor biology (10, 11). Therefore, the prognostic impact of these polymorphisms was investigated and showed associations with outcome in treatment subgroups, both platinum-containing chemotherapy and radiotherapy, e.g. in lung cancer (12, 13). Occasionally, the polymorphisms were associated with outcome in entire study populations, e.g. in patients with lung and pancreatic cancer (14, 15).

Until recently, XRCC1 polymorphisms were not well analyzed in renal cell carcinoma (RCC), probably due to the known chemo- and radioinsensitivity of RCC (16). However, these therapies have become more important for RCC patients because of their possible effective combinations with modern targeted therapies (17). A Japanese group found an association of 399Gln/Gln genotype and 194Trp/399Gln haplotype with an increased risk for RCC (18). In a second study, they failed to replicate this association but suggested XRCC1 polymorphisms to be outcome predictors, especially of tumor progression (19).

The aim of the present study, therefore, was to evaluate the possible influence of the two polymorphisms upon disease-free and overall survival, as well as time to metastasis and progression in a Caucasian population.

Patients and Methods

Patients and controls. A total of 142 consecutive patients (94 male and 48 female) with clear cell RCC who were treated at the Department of Urology, University Hospital Essen, were included in the present study. Entry criteria were histopathological
diagnosis of clear cell kidney cancer and availability of genomic DNA. Classification of tumors, study design and data collection of this series were described previously (20). Thereby, the study population has been confirmed to be representative of Caucasian patients with RCC. The study was approved by the local Ethics Committee of the University Hospital Essen and performed in accordance with the Declaration of Helsinki.

**Genotyping.** DNA was extracted from whole blood or tumor-free fractions of fresh frozen tissue specimens using a commercially available kit (QIAamp; Qiagen, Hilden, Germany). Genotypes of the XRCC1 Arg194Trp polymorphism were determined by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis as previously described (18). PCR conditions for Arg399Gln genotype determination included primers 5’-GCCTTTGCCAACACCCCCAA-3’ and 5’-CATGGCCGAGCA CAGGATAAG-3’ applied in 37 cycles of amplification using Taq DNA Polymerase Master Mix RED (Ampliqon, Herlev, Denmark) at 64˚C annealing temperature. The 161-bp PCR products were digested with HpaII (New England Biolabs, Ipswich, MA, USA) and analyzed by 2% agarose gel electrophoresis. The completely restricted products (108 and 53 bp) represented the GG genotype. Re-genotyping of 40 randomly selected samples revealed complete concordance with previous results.

**Statistical analyses.** Hardy-Weinberg equilibrium (HWE), haplotype frequencies, D’ and r² were calculated using Haplovew 4.0 (21). Kaplan-Meier plots and the log-rank test were used to retrospectively evaluate the relationship between genotypes, diplotypes, and the analyzed clinical outcomes (overall survival, tumor-related death, time to progression, and time to metastasis). Contingency tables and the χ² test were used to compare categorical variables. ANOVA was used for comparison of continuous variables. All statistical analyses were carried out using SPSS 15.0 (SPSS, Chicago, IL, USA) and GraphPad Prism 4.0 (GraphPad Software, San Diego, CA, USA).

**Results**

Clinicopathological characteristics of patients in relation to XRCC1 genotypes are summarized in Tables I and II. There was no association of polymorphism with clinicopathological parameters of RCC patients. Genotype distributions were compatible with HWE. Because the true genetic environment is reflected best when the interaction of polymorphisms is considered, haplotypes were constructed. Haplotype distribution revealed a strong linkage disequilibrium (D’=1.0) with a weak correlation (r²=0.03) of the two polymorphisms. Of particular interest is the complete absence of the pre-described risk haplotype (194Trp/399Gln; 18) in the study cohort (haplotype frequencies: 194Arg/399Arg 0.578; 194Arg/399Gln 0.352; 194Trp/399Arg 0.070).

Figure 1 shows the patients’ overall survival dependent on genotypes and diplotypes. Arg194Trp genotypes were not associated with survival (Figure 1A, p=0.684). The subgroup of Trp/Trp genotype carriers was event free but consisted of only one patient. Overall survival was not dependent on Arg399Gln genotype either (Figure 1B, p=0.795). Six haplotype pairs (diplotypes) could be constructed out of the 3 haplotypes. None of the possible diplotypes revealed a statistically significant difference for overall survival (Figure 1C, p=0.636). Additional analyses for tumor-related death, time to metastasis and progression revealed no differences between genotypes and diplotypes (data not shown).

Kaplan-Meier curves and log-rank tests were corrected for variables that showed a genotype-dependent association stronger than p=0.1, i.e. nodal status and grade for Arg399Gln, but revealed no differences between Arg399Gln genotypes in overall survival (p=0.443 and p=0.224). All analyses were likewise performed for the
other endpoints (tumor-related death, time to metastasis and progression) and did not vary statistically significantly (data not shown).

Discussion

RCC is associated with the worst clinical outcome among tumors of the urinary system (22). To date, the only effective treatment is surgical resection in localized disease. Although many different therapies were tested in clinical trials, only immunotherapy with interleukin-2 is an approved but unconvincing therapy for advanced stages. Therefore, targeted therapies (e.g. kinase inhibitors of angiogenesis) gain importance for the treatment of RCC patients because it has been suggested that their combination with previously unsuccessful standard therapies leads to better responses (17). XRCCI influences risk and outcome of different tumors most likely by altering their capacity for BER (4). Chemo-
and radiotherapy effects seem to be especially XRCC1 genotype-dependent (12, 13). Hence, it is of further interest whether XRCC1 DNA repair genotype influences the disease course of RCC. In this study, the outcome of German patients with RCC (overall survival, tumor-related death, time to metastasis, and time to progression) was not correlated with two prominent polymorphisms (genotypes as well as diplotypes) in XRCC1.

Recently, XRCC1 genotypes and haplotypes were suggested as risk and outcome predictors in Japanese RCC patients, with partly contradictory results in two studies (18, 19). The detected risk haplotype, 194Trp/399Gln, was absent from our study cohort, which might be due to ethnic differences. Therefore, it was not possible to detect the effects of this haplotype. One of the potential pathways of XRCC1 in influencing cancer risk and outcome is via aflatoxin-induced DNA damage, which is known to be XRCC1 genotype-dependent (11). The 399Gln allele was associated with increased levels of aflatoxin B1 DNA adducts, representing increased levels of DNA damage that might be due to a reduced BER function. Screenings in African, Asian and European countries revealed that aflatoxin metabolites were detectable in sera of African and Asian children and adults but not in those of European participants (23). Therefore, an effect of XRCC1 genotypes on RCC only in some ethnicities and/or cultural regions of the world cannot be excluded.

It is also possible that our results were influenced by chance or biased by genotyping failures. However, our study population was representative of Caucasian RCC patients: re-genotyping showed 100% concordance, genotype distributions were in concordance with HWE, and XRCC1 genotype distributions were comparable to those previously reported in studies with Caucasian participants (6).

In conclusion, our study indicates that effects of XRCC1 genotypes and haplotypes on prognosis of conservatively treated Caucasian RCC patients are unlikely. However, it cannot be ruled out that these polymorphisms may influence outcome of targeted therapies in Caucasian RCC patients due to the known radio- and chemo-sensitizing effects of such therapies. Therefore, further independent studies involving Caucasian as well as Asian patients will need to confirm these and previous results and are essential to elucidate the possible correlation between polymorphisms in XRCC1 and clinical outcome in RCC patients.

References


