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'-CATTGCCCAGCA 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 *Hpa*II (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 r^2 were calculated using Haploview 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 χ^2 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 (r^2=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).

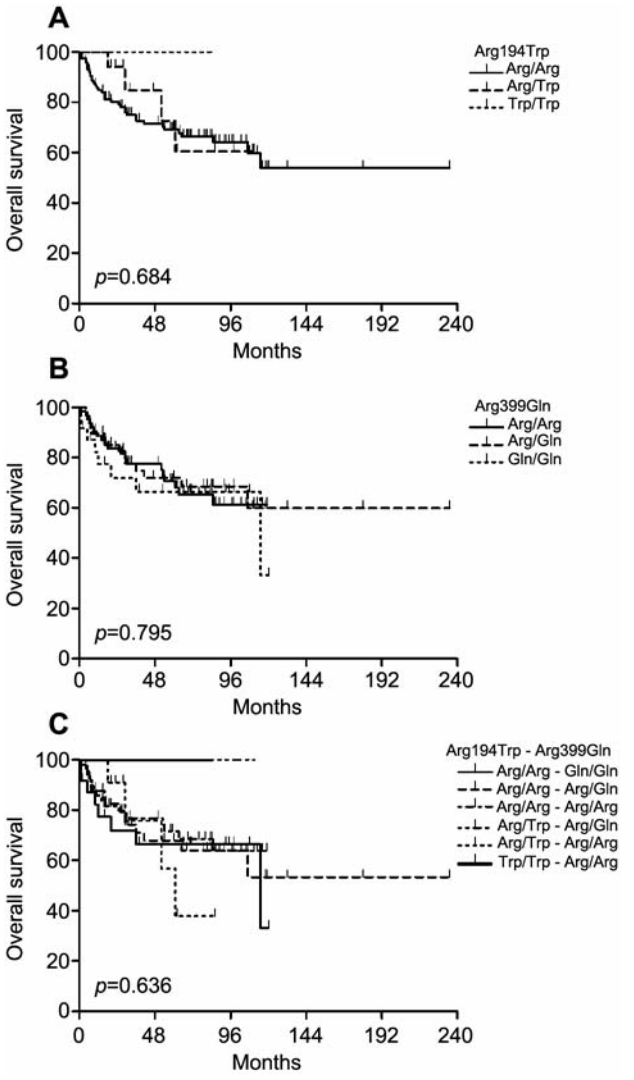

Figure 1. Kaplan-Meier curves showing overall survival of 142 RCC patients dependent on A, XRCC1 Arg194Trp; B, XRCC1 Arg399Gln; and C, XRCC1 Arg194Trp/Arg399Gln diplotypes, respectively.

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

Table I. *Clinicopathological characteristics at primary diagnosis and Arg194Trp genotype distribution in patients with renal cell cancer.*

	All	*XRCC1* Arg194Trp genotype			*p*-Value
		Arg/Arg	Arg/Trp	Trp/Trp	
N (%)	142	123 (86.6)	18 (12.7)	1 (0.7)	
Gender (male/female)	94/48	84/39	9/9	1/0	0.239
Age, years (mean±SD)	60.6±11.0	60.8±11.4	60.2±7.7	47	0.460
Median follow-up, months (range)	44.0 (1-235)	50.0 (1-235)	30.5 (9-111)	84	0.685*
Tumor stage					
pT_1	17 (12.0)	13 (76.5)	4 (23.5)	0	
pT_2	67 (47.2)	63 (94.0)	4 (6.0)	0	0.126
pT_{3+4}	58 (40.8)	47 (81.0)	10 (17.2)	1 (1.7)	
Lymph node status					
pN_0	115 (81.0)	99 (86.1)	15 (13.0)	1 (0.9)	0.853
pN_+	27 (19.0)	24 (88.9)	3 (11.1)	0	
Grade (n=113)					
1	20 (17.7)	17 (85.0)	3 (15.0)	0	0.873
2	75 (66.4)	63 (84.0)	12 (16.0)	0	
3	18 (15.9)	16 (88.9)	2 (11.1)	0	

Data are numbers, with percentages given in brackets. *Kruskal-Wallis test for nonparametric variables.

Table II. *Clinicopathological characteristics at primary diagnosis and Arg399Gln genotype distribution in patients with renal cell cancer.*

	All	*XRCC1* Arg399Gln genotype			*p*-Value
		Arg/Arg	Arg/Gln	Gln/Gln	
N (%)	142	65 (45.8)	54 (38.0)	23 (16.2)	
Gender (male/female)	94/48	45/20	33/21	16/7	0.604
Age, years (mean±SD)	60.6±11.0	61.8±10.3	59.4±12.4	59.9±9.6	0.483
Median follow-up, months (range)	44.0 (1-235)	53.0 (1-119)	34.5 (1-235)	38 (1-120)	0.877*
Tumor stage					
pT_1	17 (12.0)	6 (35.3)	7 (41.2)	4 (23.5)	0.841
pT_2	67 (47.2)	32 (47.8)	24 (35.8)	11 (16.4)	
pT_{3+4}	58 (40.8)	27 (46.5)	23 (39.7)	8 (13.8)	
Lymph node status					
pN_0	115 (81.0)	58 (50.4)	39 (33.9)	18 (15.7)	0.058
pN_+	27 (19.0)	7 (25.9)	15 (55.6)	5 (18.5)	
Grade (n=113)					
1	20 (17.7)	14 (70.0)	4 (20.0)	2 (10.0)	0.074
2	75 (66.4)	28 (37.3)	32 (42.7)	15 (20.0)	
3	18 (15.9)	9 (50.0)	8 (44.4)	1 (5.6)	

Data are numbers with percentages given in brackets. *Kruskal-Wallis test for nonparametric variables.

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). *XRCC1* 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 B_1 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

1 Hoeijmakers JH: Genome maintenance mechanisms for preventing cancer. Nature *411*: 366-374, 2001.

2 Kraemer KH, Levy DD, Parris CN, Gozukara EM, Moriwaki S, Adelberg S and Seidman MM: Xeroderma pigmentosum and related disorders: examining the linkage between defective DNA repair and cancer. J Invest Dermatol *103*: 96S-101S, 1994.

3 Thompson LH, Brookman KW, Jones NJ, Allen SA and Carrano AV: Molecular cloning of the human *XRCC1* gene, which corrects defective DNA strand break repair and sister chromatid exchange. Mol Cell Biol *10*: 6160-6171, 1990.

4 Shen MR, Zdzienicka MZ, Mohrenweiser H, Thompson LH and Thelen MP: Mutations in hamster single-strand break repair gene *XRCC1* causing defective DNA repair. Nucleic Acids Res *26*: 1032-1037, 1998.

5 Shen MR, Jones IM and Mohrenweiser H: Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. Cancer Res *58*: 604-608, 1998.

6 Hung RJ, Hall J, Brennan P and Boffetta P: Genetic polymorphisms in the base excision repair pathway and cancer risk: a HuGE review. Am J Epidemiol *162*: 925-942, 2005.

7 Deligezer U, Akisik EE and Dalay N: Lack of association of *XRCC1* codon 399Gln polymorphism with chronic myelogenous leukemia. Anticancer Res *27*: 2453-2456, 2007.

8 Narter KF, Ergen A, Agachan B, Gormus U, Timirci O and Isbir T: Bladder cancer and polymorphisms of DNA repair genes (*XRCC1, XRCC3, XPD, XPG, APE1, hOGG1*). Anticancer Res *29*: 1389-1393, 2009.

9 Improta G, Sgambato A, Bianchino G, Zupa A, Grieco V, La TG, Traficante A and Cittadini A: Polymorphisms of the DNA repair genes *XRCC1* and *XRCC3* and risk of lung and colorectal cancer: a case control study in a Southern Italian population. Anticancer Res *28*: 2941-2946, 2008.

10 Duell EJ, Wiencke JK, Cheng TJ, Varkonyi A, Zuo ZF, Ashok TD, Mark EJ, Wain JC, Christiani DC and Kelsey KT: Polymorphisms in the DNA repair genes *XRCC1* and *ERCC2* and biomarkers of DNA damage in human blood mononuclear cells. Carcinogenesis *21*: 965-971, 2000.

11 Lunn RM, Langlois RG, Hsieh LL, Thompson CL and Bell DA: *XRCC1* polymorphisms: effects on aflatoxin B1-DNA adducts and glycophorin A variant frequency. Cancer Res *59*: 2557-2561, 1999.

12 Yoon SM, Hong YC, Park HJ, Lee JE, Kim SY, Kim JH, Lee SW, Park SY, Lee JS and Choi EK: The polymorphism and haplotypes of *XRCC1* and survival of non-small cell lung cancer after radiotherapy. Int J Radiat Oncol Biol Phys *63*: 885-891, 2005.

13 de las Penas R, Sanchez-Ronco M, Alberola V, Taron M, Camps C, Garcia-Carbonero R, Massuti B, Queralt C, Botia M, Garcia-Gomez R, Isla D, Cobo M, Santarpia M, Cecere F, Mendez P, Sanchez JJ and Rosell R: Polymorphisms in DNA repair genes modulate survival in cisplatin/gemcitabine-treated non-small cell lung cancer patients. Ann Oncol *17*: 668-675, 2006.

14 Sreeja L, Syamala VS, Syamala V, Hariharan S, Raveendran PB, Vijayalekshmi RV, Madhavan J and Ankathil R: Prognostic importance of DNA repair gene polymorphisms of *XRCC1* Arg399Gln and *XPD* Lys751Gln in lung cancer patients from India. J Cancer Res Clin Oncol *134*: 645-652, 2008.

15 Li D, Li Y, Jiao L, Chang DZ, Beinart G, Wolff RA, Evans DB, Hassan MM and Abbruzzese JL: Effects of base excision repair gene polymorphisms on pancreatic cancer survival. Int J Cancer *120*: 1748-1754, 2007.

16 Ritchie AW and Chisholm GD: The natural history of renal carcinoma. Semin Oncol *10*: 390-400, 1983.

17 Senan S and Smit EF: Design of clinical trials of radiation combined with antiangiogenic therapy. Oncologist *12*: 465-477, 2007.

18 Hirata H, Hinoda Y, Matsuyama H, Tanaka Y, Okayama N, Suehiro Y, Zhao H, Urakami S, Kawamoto K, Kawakami T, Igawa M, Naito K and Dahiya R: Polymorphisms of DNA repair genes are associated with renal cell carcinoma. Biochem Biophys Res Commun *342*: 1058-1062, 2006.
19 Sakano S, Hinoda Y, Okayama N, Kawai Y, Korenaga Y, Eguchi S, Nagao K, Ohmi C and Naito K: The association of DNA repair gene polymorphisms with the development and progression of renal cell carcinoma. Ann Oncol *18*: 1817-1827, 2007.
20 Frey UH, Lummen G, Jager T, Jockel KH, Schmid KW, Rubben H, Muller N, Siffert W and Eisenhardt A: The *GNAS1* T393C polymorphism predicts survival in patients with clear cell renal cell carcinoma. Clin Cancer Res *12*: 759-763, 2006.
21 Barrett JC, Fry B, Maller J and Daly MJ: Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics *21*: 263-265, 2005.
22 Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, Feuer EJ and Thun MJ: Cancer statistics, 2005. CA Cancer J Clin *55*: 10-30, 2005.
23 Wild CP, Jiang YZ, Allen SJ, Jansen LA, Hall AJ and Montesano R: Aflatoxin-albumin adducts in human sera from different regions of the world. Carcinogenesis *11*: 2271-2274, 1990.

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