Analysis of *XRCC2* and *XRCC3* gene polymorphisms in pancreatic cancer

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Received May 6, 2015; Accepted October 26, 2015

DOI: 10.3892/br.2015.550

Abstract. The double-strand break DNA repair pathway, including *XRCC2* and *XRCC3* genes, is implicated in maintaining genomic stability and therefore could affect the pancreatic cancer risk. The aim of the present study was to evaluate the clinical significance of the *XRCC2* and *XRCC3* gene polymorphisms in patients with pancreatic cancer. The present study included 203 patients: 101 with pancreatic cancer and 102 healthy controls. The Arg188His *XRCC2* and the Thr241Met *XRCC3* gene polymorphisms have been studied in DNA isolated from blood samples. The associations of the analysed genotypes and clinical data at diagnosis have been evaluated. The frequencies of the genotypes of the Arg188His *XRCC2* and Thr241Met *XRCC3* polymorphisms did not differ significantly between patients and controls. The study did not identify a correlation between the *XRCC2* and *XRCC3* genes polymorphisms and tumor size or localisation. Analysed polymorphisms were also not associated with the gender and age of the patient, or the presence of regional or distant metastases. In conclusion, the present study did not suggest an association between the Arg188His *XRCC2* and the Thr241Met *XRCC3* polymorphisms and the clinical data of patients with pancreatic cancer.

Introduction

There are several biochemical pathways that can lead to carcinogenesis, one of which involves DNA damage induced by exogenous carcinogens or by endogenous metabolic processes. The double-strand break DNA repair pathway, including *XRCC2* and *XRCC3* genes, is implicated in maintaining genomic stability and therefore could affect the cancer risk. Common genetic polymorphisms in DNA repair genes may affect protein function and thus the capacity of repair DNA damage, which in turn could lead to genetic instability (1,2).

Single-nucleotide polymorphisms were identified in nearly all human DNA repair genes that have been investigated thus far, and some of them were shown to modulate the levels of DNA damage, individual DNA repair capacity and cancer risk. Among them, polymorphisms of X-ray repair cross complementing group 2 (*XRCC2*) and *XRCC3* have been studied extensively (3-5).

The *XRCC2* gene, located at 7q36.1, is an essential part of the homologous recombination repair pathway and a functional candidate for involvement in cancer progression. Common variants within *XRCC2*, including Arg188His polymorphism, have been identified as potential cancer susceptibility loci in recent studies, although association results are controversial. The Arg188His polymorphism has been proposed to be a genetic modifier for pancreatic cancer and was associated with an increased risk of breast, laryngeal and oral cancers (6-9). Recently, a large number of studies have attempted to identify the association between this polymorphism and other types of human cancer, such as ovarian, thyroid and colorectal cancer. However, results of these studies remain inconsistent rather than conclusive (4,10).

The *XRCC3* gene, located at chromosome 14q32.3, interacts and stabilizes Rad51 and is involved in homologous recombination repair for double-strand breaks of DNA. The *XRCC3* Thr241Met gene polymorphism could be associated with impaired function of repair, as this polymorphism consists of a Met to Thr substitution, which may influence the function of the enzyme by removing a phosphorylation site. The *XRCC3* polymorphism was associated with the risks of numerous types of cancer, such as lung, ovarian or gastric cancer; however, there is limited information regarding the analysed gene polymorphisms in pancreatic diseases (2,11,12).

The purpose of the present study was to evaluate the clinical significance of the Arg188His *XRCC2* and the Thr241Met *XRCC3* gene polymorphisms in patients with pancreatic cancer.

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Key words: pancreatic cancer, *XRCC2*, *XRCC3*, polymorphism, DNA repair genes

Materials and methods

Patients. The study included 203 patients: 101 with pancreatic cancer (47 men and 54 women; age, 43-86 years) and 102 gender- and age-matched healthy controls. The analysed patients were hospitalised in the Department of Digestive Tract Diseases (Medical University of Lodz Hospital, Lodz, Poland) or in the Department of Digestive Tract Surgery of Silesian Medical University (Katowice, Poland) between 2005 and 2010. The study protocol was approved by the ethical committee of Lodz Medical University. Written informed consent was obtained from each participant.

Pathological diagnosis. Only patients with confirmed pathology diagnosis of ductal pancreatic adenocarcinoma were included in the study. The pathological diagnosis was confirmed following surgical treatment or pancreatic tissue biopsy in patients qualified for palliative chemotherapy. A total of 41 patients (40.6%) with pancreatic adenocarcinoma underwent Whipple resection or distal pancreatectomy, 34 (33.7%) underwent palliative surgery and 26 (25.7%) underwent palliative chemotherapy and/or endoscopic treatment. Tumor grade was classified into G1 (well-differentiated), G2 (moderately differentiated) and G3 (poorly differentiated).

Associations of the genotypes and clinical data. The associations of the analysed genotypes and clinical data at diagnosis were evaluated. The following demographic and clinical data were analysed: Age, tumor size, lymph node involvement, histological grade, distant metastases, history of smoking, weight loss >10%, as well as selected laboratory parameters: carbohydrate antigen (CA) 19-9, total bilirubin and albumin levels. An individual who had never smoked or had smoked <100 cigarettes in their lifetime was defined as a never-smoker. Ever-smokers included former (those who had quit smoking for >1 year before recruitment) and current smokers. Cumulative smoking was calculated as pack-years: The number of packs smoked per day multiplied by years of smoking.

Polymerase chain reaction (PCR) analysis. Peripheral venous blood samples were obtained from all the analysed patients at the time of hospital admission. PCR products for the analysed variants were analysed by restriction fragment length polymorphism analysis. The Arg188His *XRCC2* and the Thr241Met *XRCC3* gene polymorphisms have been studied in DNA isolated from blood samples. The primers, 5'-TGTAGT CACCCATCTCTCTGC-3' and 5'-AGTTGCTGCCATGCC TTACA-3'; were used to amplify the region containing the Arg188His *XRCC2* variant. PCR amplification was performed in a final volume of 25 μl containing 80 ng DNA, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.2 mM dNTP, each primer at 1.0 μM and 1.0 unit Taq polymerase (Takara, Otsu, Japan) in a GeneAmp PCR system 9700 (Applied Biosystems) Thermocycler. In total, 10 μl of the PCR product was digested with 3 units of *Hph*I using the manufacturer's recommended protocol. PCR products were visualised on 3% agarose gels with 10% ethidium bromide.

The Thr241Met *XRCC3* gene single-nucleotide polymorphism was genotyped by allelic discriminating TaqMan PCR, using the following primers, 5'-GCCTGGTGGTCATCG ACTC-3' and 5'-ACAGGGCTCTGGAAGGCACTGCTCAGC TCACGCACC-3'. PCR and end-point analysis was performed in a volume of 25 μl containing 200 ng of DNA, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.2 mM dNTP, 0.05% Tween-20, 0.05% Nonidet-P40, each primer at 1.0 μM and 1.0 unit Taq polymerase (Takara) in a GeneAmp PCR system 9700. A total of 10 μl of the PCR product was digested with 3 units of *Nco*I using the manufacturer's recommended protocol. PCR products were visualised on 3% agarose gels with 10% ethidium bromide.

Statistics. To determine the differences between groups, standard χ^2 test or Fisher's exact test were used. The clinical significance of analysed polymorphisms was determined using logistic regression analysis and presented in tables as odds ratios with their 95% confidence intervals. The deviations from Hardy-Weinberg equilibrium were analysed using the χ^2 test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Patients. All patients involved in the study were Caucasian. The mean ages were not significantly different for patients with pancreatic cancer (65.7±3.1 years) and controls (63.2±4.3 years; P>0.05). In patients with pancreatic adenocarcinoma, the tumor size ranged from 1.5 to 6.5 cm (mean, 3.4±2.3 cm). For histological differentiation, 28, 31 and 35 patients were classified into G1, G2 and G3 respectively, whereas 7 patients had missing data. Lymph nodes metastases were observed in 63 patients with pancreatic cancer (62.4%) and liver metastases in 19 (18.8%). A total of 29 patients (28.8%) presented weight loss >10% with the mean weight loss of 9.3±0.7 kg during 6 months. There were significantly more ever-smokers and current smokers among cases compared to the controls (51.5 vs. 35.9%; P<0.05). Serum levels of CA19-9, as well as bilirubin levels, were higher in patients with pancreatic cancer compared to the control group (201.3±17.4 vs. 17.9±4.2 U/ml for CA19-9 and 4.1±1.4 vs. 0.8±0.2 mg/dl for bilirubin, respectively; P<0.001). By contrast, there was no statistically significant difference between the mean albumin levels in PA patients compared to the healthy volunteers.

Genotype distributions. The genotype distributions of the Arg188His *XRCC2* and the Thr241Met *XRCC3* gene polymorphisms are summarised in Table I. All the allele distributions were consistent with Hardy-Weinberg equilibrium. The distribution of gene variants was similar between all cases and controls, and a maximal difference of 5.1% was identified for the *XRCC3* polymorphism with the heterozygous Thr/Met genotype being less frequent in cases (37.6%) compared to controls (42.7%). This difference was, however, not significant (P>0.05).

The potential association between *XRCC2* and *XRCC3* genotype distribution and the clinical data of pancreatic adenocarcinoma patients was investigated. However, the current study did not show a correlation between analysed genes polymorphisms and tumor size, grade or localisation. *XRCC2* and *XRCC3* genes polymorphisms were also not associated with the gender and age of patients, or the presence of regional or distant metastases (Tables II and III).

Table I. Distribution of Arg188His *XRCC2* and Thr241Met *XRCC3* genotypes in the analysed group of patients.

Genotype	Patients, n (%) PA (n=101)	Control (n=103)	OR (95% CI)
Arg188His *XRCC2*			
Arg/Arg	38 (37.6)	28 (36.9)	Reference
Arg/His	43 (42.6)	41 (39.8)	1.76 (0.86-3.59)
His /His	20 (19.8)	24 (23.3)	1.44 (0.63-3.35)
Thr241Met *XRCC3*			
Thr/Thr	32 (31.7)	30 (29.1)	Reference
Thr/Met	38 (37.6)	44 (42.7)	0.79 (0.54-1.14)
Met/Met	31 (30.7)	29 (28.2)	1.37 (0.92-2.02)

PA, pancreatic adenocarcinoma; OR, odds ratio; CI, confidence interval.

Table II. Association between the Arg188His *XRCC2* polymorphism and clinical data of patients with pancreatic cancer.

Variables	His(+) allele (His/His and Arg/His) n=63, n (%)[a]	His(-) allele (Arg/Arg) n=38, n (%)[a]
Age, years		
<65	29 (46.0)	18 (47.4)
≥65	34 (54.0)	20 (52.6)
Gender		
Male	30 (47.6)	17 (44.7)
Female	33 (52.4)	21 (55.3)
Tumor size, cm		
≤3	27 (42.8)	19 (50.0)
>3	36 (57.2)	19 (50.0)
Tumor differentiation		
G1+G2	39 (61.9)	27 (71.1)
G3	24 (38.1)	11 (28.9)
Lymph nodes metastases		
Absent	28 (44.4)	12 (31.6)
Present	37 (55.6)	26 (68.4)
Weight loss, %		
<10	45 (71.4)	27 (71.1)
≥10	18 (28.6)	11 (28.9)
Smoking		
Yes	31 (49.2)	20 (52.6)
No	32 (50.8)	18 (47.4)
CA19-9, U/ml		
<37	17 (26.9)	10 (26.3)
≥37	46 (73.1)	28 (73.7)
Bilirubin, mg/dl		
<1.2	26 (41.3)	18 (47.4)
>1.2	37 (58.7)	20 (52.6)

[a]P>0.05. CA19-9, carbohydrate antigen 19-9.

Table III. Association between the Thr241Met *XRCC3* polymorphism and clinical data of patients with pancreatic cancer.

Variables	Thr(+) allele (Thr/Thr and Thr/Met) n=70, n (%)[a]	Thr(-) allele (Met/Met) n=31, n (%)[a]
Age, years		
<65	33 (47.1)	16 (48.4)
≥65	37 (52.9)	15 (51.6)
Gender		
Male	32 (45.7)	15 (48.4)
Female	38 (54.3)	16 (51.6)
Tumor size, cm		
≤3	31 (44.3)	15 (48.4)
>3	39 (55.7)	16 (51.6)
Tumor differentiation		
G1+G2	40 (57.1)	18 (58.1)
G3	30 (42.9)	13 (41.9)
Lymph nodes metastases		
Absent	28 (40.0)	10 (32.3)
Present	42 (60.0)	21 (67.7)
Weight loss, %		
<10	50 (71.4)	22 (70.9)
≥10	20 (28.6)	9 (29.1)
Smoking		
Yes	35 (50.0)	17 (54.8)
No	35 (50.0)	14 (45.2)
CA19-9, U/ml		
<37	19 (27.1)	8 (25.8)
≥37	51 (72.9)	23 (74.2)
Bilirubin, mg/dl		
<1.2	38 (54.3)	17 (54.8)
>1.2	32 (45.7)	14 (45.2)

[a]P>0.05. CA19-9, carbohydrate antigen 19-9.

Discussion

Several studies have investigated the possible role of *XRCC2* and *XRCC3* genes polymorphisms in neoplastic diseases. The 188His allele and 188His/His homozygous variant of the *XRCC2* Arg188His polymorphism were associated with an increased risk of breast cancer in the Polish population (12). Other studies observed that *XRCC2* polymorphisms may have an important role in colorectal cancer tumorigenesis, conferring susceptibility to rectal tumors (13). This polymorphism may be also associated with an increased risk of gastric and pharyngeal cancers (14,15). However, in a recently published meta-analysis there was a statistically significant association between *XRCC2* Arg188His polymorphisms and neoplastic diseases identified in ovarian cancer, but not in the other types of cancer studied (16).

Similarly, results from studies of the Thr241Met *XRCC3* gene polymorphism on the risk of various types of cancer

have been inconsistent. It was reported that the risk of colorectal cancer in individuals with the *XRCC3* Thr/Met and Met/Met genotype was ~2.5 times elevated compared to Thr/Thr-wild genotype (12). According to a recently published meta-analysis, the *XRCC3* Thr241Met polymorphism may also be a risk factor for gastric cancer among the Asian population, particularly in non-cardiac location (11). By contrast, the *XRCC3* Thr241Met genotype was not a risk factor for the development of chronic myeloid leukemia and ovarian cancer. Additionally, no association was observed between the prognostic factors and the *XRCC3* polymorphisms in those patients (4,17).

In the current study, the *XRCC2* Arg188His as well as Thr241Met *XRCC3* genotype distribution were similar in patients with pancreatic adenocarcinoma and the control group. According to the data, the analysed polymorphisms were also not associated with the tumor size, histological grade, regional or distant metastases, laboratory findings or gender and age. These results are in agreement with other studies concerning patients with breast cancer, differentiated thyroid carcinoma or chronic myeloid leukemia (4,6,16,18). Similar results were reported in a meta-analysis that included 7 studies with 1,070 patients with leukemia and 1,850 controls (19).

To the best of our knowledge, only a few studies have investigated the genetic variants of *XRCC2* and *XRCC3* genes and the risk of developing pancreatic cancer, however, those results were conflicting. Jiao *et al* (9) reported a risk-modifying effect of *XRCC2* Arg188His polymorphism and pancreatic cancer among smokers. Compared with never-smokers carrying *XRCC2* Arg188Arg, ever-smokers had a statistically significantly increased risk of pancreatic cancer. Additionally, they observed the association between the *XRCC2* polymorphism and the number of pack-years of smoking in modifying the risk of pancreatic cancer in ever-smokers. The subtle variation in the *XRCC2* polymorphism may possibly influence the susceptibility to pancreatic cancer in those with significant exposure to cigarette carcinogens. By contrast, no association between *XRCC3* polymorphism and the risk of pancreatic cancer was reported (9).

It is known that smoking is one of the only established environmental risk factors for pancreatic cancer; however, the precise mechanism of action in the pancreas is unclear. It is reasonable to assume that the effect of tobacco smoking on pancreatic tissues is a result of a complex combination of direct and indirect action of tobacco-associated carcinogens and metabolites that are known to damage DNA. Furthermore, there is mounting epidemiological evidence that DNA repair polymorphisms in combination with heavy tobacco smoking increase the pancreatic cancer risk. In the study of Duell *et al* (20), combinations of genetic variants of *XRCC3* Thr241Met and smoking were one of the best two-factor predictors for increasing the risk of pancreatic cancer.

Prior to this, Li *et al* (21) demonstrated that the heterozygous and homozygous variant alleles of *XRCC2* R188H or the homozygous mutant allele of *XRCC3* 17893 were associated with significantly decreased overall survival of patients with pancreatic cancer. The genotype effect was present in patients with localised disease but absent in those with metastases. They concluded that individuals with metastatic disease may already have too many genetic alterations driving tumor progression, so that any subtle effect of genotypes to alter DNA repair capacity is overwhelmed.

The present study is one of the few studies concerning the role of repair gene polymorphisms in pancreatic cancer. However, the results did not confirm previous results. The possible explanation of this difference may be the heterogeneity of the patient population tested with different ethnic background. Furthermore, a single study may be limited due to a relatively small sample size. A larger patient study population may aid in the more accurate evaluation of clinical significance of examined *XRCC2* and *XRCC3* genes polymorphism.

In conclusion, the current evidence did not suggest that the analysed *XRCC2* and *XRCC3* polymorphisms were directly associated with pancreatic cancer risk. The study did not show any correlation between those polymorphisms and the clinical data of pancreatic cancer patients. These results should be explained with certain caution and re-evaluated in the future with more studies that contain larger sample sizes.

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