Polymorphisms in \textit{RAD51}, \textit{XRCC2} and \textit{XRCC3} genes of the homologous recombination repair in colorectal cancer—a case control study

Renata Krupa · Tomasz Sliwinski · Maria Wisniewska-Jarosinska · Jan Chojnacki · Maja Wasylecka · Łukasz Dziki · Jan Morawiec · Janusz Blasiak

Received: 19 February 2010 / Accepted: 8 November 2010 / Published online: 20 November 2010 © The Author(s) 2010. This article is published with open access at Springerlink.com

Abstract XRCC2 and XRCC3 proteins are structurally and functionally related to RAD51 which play an important role in the homologous recombination, the process frequently involved in cancer transformation. In our previous work we show that the 135G>C polymorphism (rs1801320) of the \textit{RAD51} gene can modify the effect of the Thr241Met polymorphism (rs861539) of the \textit{XRCC3} gene. We tested the association between the 135G>C polymorphism of the \textit{RAD51} gene, the Thr241Met polymorphism of the \textit{XRCC3} gene and the Arg188His polymorphism (rs3218536) of the \textit{XRCC2} gene and colorectal cancer risk and clinicopathological parameters. Polymorphisms were evaluated by restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR) in 100 patients with invasive adenocarcinoma of the colon and in 100 sex, age and ethnicity matched cancer–free controls. We stratified the patients by genotypes, tumour Duke’s and TNM stage and calculated the linkage of each genotype with each stratum. Carriers of Arg188Arg/Me241tMet, His188His/Thr241Thr and His188His/G135G genotypes had an increased risk of colorectal cancer occurrence (OR 5.70, 95% CI 1.10–29.5; OR 12.4, 95% CI 1.63–94.9; OR 5.88, 95% CI 1.21–28.5, respectively). The C135C genotype decreased the risk of colorectal cancer singly (OR 0.06, 95% CI 0.02–0.22) as well as in combination with other two polymorphisms. TNM and Duke’s staging were not related to any of these polymorphisms. Our results suggest that the 135G>C polymorphism of the \textit{RAD51} gene can be an independent marker of colorectal cancer risk. The Thr241Met polymorphism of the \textit{XRCC3} gene and the Arg188His polymorphism of the \textit{XRCC2} gene can modify the risk of colorectal cancer.

Keywords Colorectal cancer · Genetic polymorphism · \textit{RAD51} · \textit{XRCC2} · \textit{XRCC3}

Introduction

Genetic polymorphisms in homologous recombination repair (HRR) genes, which can lead to protein haplinsufficiency have been associated with cancer risk [1]. The RAD51, XRCC2 and XRCC3 proteins are core components of DNA double strand breaks (DSBs) repair by HRR. XRCC2 and XRCC3 genes are structurally and functionally related to the \textit{RAD51} gene [2]. Cell deficient with any of these genes product are defective in homologous recombination and demonstrate genomic instability [3–6]. Hamster cell lines deficient in XRCC2 and XRCC3 genes had an elevated frequency of aneuploidy compared with wild-type cells and mutant cells transfected with an appropriate human gene [7]. XRCC2- and XRCC3-deficient hamster
cell lines show also a high frequency of multiple chromosomes and abnormal spindle formation [4]. CHO cell lines defective in XRCC2 and XRCC3 had lower spontaneous frequency of sister chromatid exchange than wild type cells [8]. The most frequent polymorphism in the XRCC3 gene is a C>T transition resulting in an amino acid substitution of Thr to Met at codon 241. Carriers of the Met allele had relatively high DNA adducts level in lymphocyte DNA, which could be the result of lower DNA repair capacity [9, 10]. Conflicting results have been published on the association with colon cancer [9, 11–15]. XRCC3-241Thr genotype was associated with adverse progression-free survival of colorectal cancer patients in one study [13]. This polymorphism was also associated with a better prognosis for colorectal cancer patients [14].

A relatively rare polymorphism in the XRCC2 gene, a G>A transition resulting on Arg to His substitution at codon 188, was not found to be related with colorectal cancer (CRC) risk in two studies [15, 16].

Recently, we have shown that the 135G>C (c. –98 G>C; rs 1801320; Genbank accession number NT 010194) polymorphism can modify the effect of polymorphisms of the BRCA2 and XRCC3 genes on breast cancer occurrence [17–19].

In the present work we checked a potential influence of this polymorphism on the Thr241Met (c. 722 C>T; rs 861539, Genbank accession number NT 026437) polymorphism of the XRCC2 gene and the Arg188His (c. 563 G>A; rs3218536, Genbank accession number NT 007914) polymorphism of the XRCC3 gene on colorectal cancer occurrence and clinicopathological parameters in a Polish subpopulation.

Materials and methods

Patients

Blood samples were obtained from 100 patients (36 men and 64 women, median age 65, quartiles: 57, 75 years) with CRC treated during the study periods (2000–2001 and 2006–2007) at The Medical University of Lodz, Department of Gastroenterology and Internal Diseases and Department of Surgical Oncology, N. Copernicus Hospital, Lodz, Poland. Incidental patients consist of 90% of studied population. All patients had histologically confirmed invasive adenocarcinoma of the colon (Supplementary Table S1). A hundred sex- and age (±1 year)-matched individuals hospitalized due to their complains related to the lower gastrointestinal tract were enrolled as controls. They were examined by colonoscopy and subsequent histology of biopsies with no sign of colorectal cancer. Particularly, all controls had no macroscopic lesions of the colon mucosa revealed in the colonoscopy. Despite this, biopsies were taken every 10 cm along the whole colon and rectum from apparently normal mucosa in order to exclude individuals with early stages of mucosal dysplasia from the control group. All patients as well as controls were Caucasian. They accepted to cooperate and signed the informed consent. The protocol of the study was reviewed and approved by the Ethic Committee of the Medical University of Lodz and the experiment was conducted with the understanding and the consent of the human subject.

Genotype determination

Genomic DNA was prepared using the guanidine-isothiocyanate method as described previously [18]. The polymorphisms were genotyped with restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR). RAD51 genotyping was analysed by amplification of a 157-bp region surrounding the 135th nucleotide. This region contains a single MviI site that was abolished in the C135C variant. Wild type alleles were digested by MviI (Fermentas, Vilnius, Lithuania) producing 86 and 71 bp length products. The C135C variant of RAD51 was not digested by the enzyme, giving a single 157 bp PCR product. PCR was performed in a MT Research, INC thermal cycler with the following primers: 5′-TGGGAA CTGCAACTCATCTGG-3′ and 5′-GGCTCTGGAAGGCACTGCTC AGCTCACGCACC-3′ PCR). RAD51 polymorphism was analysed by amplification of a 157-bp region surrounding the 135th nucleotide. This region contains a single MviI site that was abolished in the C135C variant. Wild type alleles were digested by MviI (Fermentas, Vilnius, Lithuania) producing 86 and 71 bp length products. The C135C variant of RAD51 was not digested by the enzyme, giving a single 157 bp PCR product. PCR was performed in a MT Research, INC thermal cycler with the following primers: 5′-TGGGAA CTGCAACTCATCTGG-3′ and 5′-GGCTCTGGAAGGCACTGCTC AGCTCACGCACC-3′. The 136 bp PCR product was digested overnight with 3U of the restriction enzyme NcoI. The homozygous Thr/Thr genotype produced 39 and 97 bp fragments, heterozygous genotype three fragments: 136, 97 and 39 bp and the homozygous His/His genotype produced one 136 bp fragment. Restriction fragments were analysed on 3% agarose gels stained with ethidium bromide.

The Arg188His polymorphism of the XRCC2 was determined using the following primers: sense, 5′-GCCTGTGGTGTCATGACT-3′; antisense, 5′-ACAG GGCTCTGGAAGGCACGTCACGACCACGATTGCTG-3′. The 290 bp PCR product was digested overnight with 3U of the restriction enzyme HphI. The homozygous His/His genotype produced 148 and 142 bp fragments, heterozygous genotype three fragments: 290, 148 and 142 bp and the homozygous Arg/Arg genotype produced one 290 bp fragment. Restriction fragments were analysed on 8% polyacrylamide gels stained with ethidium bromide. We identified the product of particular reaction by comparing them with standards. We have
chosen representative pictures of gels. Examples of gels are given on Fig. 1.

Statistical analysis

Statistical analysis was performed using STATISTICA 8.0 package (Statsoft, Tulsa, USA). Distributions of genotypes and alleles between groups were tested using Fisher’s exact test. A linkage between SNP, cancer and clinicopathological parameters was assessed by the unconditional logistic regression (quasi-Newton method). For each SNP, odds ratio (OR) (single stage odds ratio for Duke’s and TNM staging) was estimated. Wild type alleles or additional homozygous variants were used as reference groups. The Peto method was used for estimating odds ratios in cases with no events in one or both groups. Moreover, ORs for colorectal cancer were estimated in association with combinations of each two genotypes, defined on the basis of three SNPs in the XRCC3, XRCC2 and RAD51 genes. OR for each combination was calculated with homozygous wild type variants combination as the reference. All tests were two tailed. In all tests P values of less than 0.05 were considered statistically significant.

Results and discussion

We categorized all DNA samples according to the polymorphic variants and cancer occurrence and further in the case of cancer patients—also according to clinicopathological parameters. Table 1 displays the distribution of genotypes of the 135G>C, Thr241Met and Arg188His polymorphisms. Among the controls, all genotype distributions did not differ significantly (P > 0.05) from those expected by the Hardy–Weinberg equilibrium. The frequencies of genotypes of the Arg188His and Thr241Met polymorphisms did not differ significantly between patients and controls. Using the logistic regression we did not find any association between these polymorphisms and colorectal cancer occurrence. Our results on the association between the Thr241Met polymorphism of the XRCC3 gene and colorectal cancer are in agreement with recent meta-analysis performed on 3,183/3,926 cases/controls [20]. In case of the 135G>C polymorphism of RAD51 gene we found statistically significant differences among cases and controls. Colorectal cancer patients had lower frequency of C/C genotype (P < 0.0001, statistical power 100%). This protecting effect was indicated also by odds ratio analysis (OR = 0.06, 95% CI 0.02–0.22). The current results on the association of the 135G>C polymorphism of the RAD51 gene and colorectal cancer are in agreement with our preliminary work [21]. In the current work we added polymorphisms in two different genes, which enabled us to study the role of gene–gene interaction in colorectal cancer. Moreover, the control group was more age-homogeneous than that in the preliminary research, which allowed us to decrease potential variation in the efficacy of DNA repair related to age. Differences in effectivity of DNA repair processes resulting from naturally occurred polymorphisms can affect the cancer risk [22–25]. Polymorphic genes of DNA repair are in great part included to low penetrance genes, which means that single gene product most often slightly affects the disease occurrence risk, but accumulation of changed alleles can have essential significance for its development. The combined effect of
investigated XRCC2, XRCC3 and RAD51 polymorphisms on colorectal cancer occurrence has not been investigated, yet. The design of the study enabled us to investigate several gene–gene interactions in the context of general relationship between a gene and its structural analogues. Moreover, we performed our study on an ethnically homogenous population, which may contribute to our knowledge on the variation of genotype-phenotype relationship dependence on the population.

The frequencies of combined genotypes of XRCC2, XRCC3 and RAD51 genes are displayed in Supplementary Table S2. We found statistically significant differences between distribution of combined genotypes for colorectal cancer patients and control groups. Odds ratio analysis for a combination of the Arg188His polymorphism of XRCC2 with the 135G>C polymorphism of RAD51 indicates protecting role of the C/C homozygous genotype against colorectal cancer in a Polish population (OR = 0.03; 95% CI 0.00–0.26, P < 0.0001; statistical power 99.9%). Additional results were obtained for the combination of the Thr241Met polymorphism of the XRCC3 gene and 135G>C polymorphism of RAD51 gene (OR = 0.07; 95% CI 0.00–0.56, P = 0.0021; statistical power 95.8% for Thr241Thr and C135C genotype and OR = 0.13; 95% CI 0.03–0.61; statistical power 93.6% for Thr241Met and C135C genotype).

Combination of variant homozygous His188His genotype of XRCC2 gene with wild type variants of both XRCC3 and RAD51 polymorphisms increased the risk of colorectal cancer occurrence (OR = 12.4; 95% CI 1.63–94.9, P = 0.0259; statistical power 38.7% and OR = 5.88; 95% CI 1.21–28.5, P = 0.0391; statistical power 70.1%, respectively). This effect was also found for variant genotype of the XRCC3 polymorphism in combination with wild type homozygous genotype of the XRCC2 polymorphism (OR = 5.70; 95% CI 1.10–29.5, P = 0.0391; statistical power 52.4%).

Next, we divided colorectal cancer patients into groups depending on Duke’s and TNM staging status. We did not found any relation between any group and polymorphism in the single stage OR analysis (Table 2). To our knowledge it is the first study linking clinical parameters of colorectal cancer with XRCC2, XRCC3 and RAD51 polymorphisms.

We performed our study on relatively small populations of both patients and controls and we do not consider our results as definitive. Instead, they may be an important indicator for a larger cohort study, leading to establishing some more solid evidence. At present, we are unable to give a straight interpretation of some of our results. For example the XRCC2 Met241Met genotype was not associated with the occurrence of colon cancer, but it was associated with both increased risk in combination with XRCC3 Arg188Arg genotype and a decreased risk in combination with the RAD51 C135C genotype. This was probably due to a complex interaction between these polymorphisms, underlined by the mechanism requiring further studies.

Double strand DNA breaks are the most dangerous DNA damage. They occurred directly in cells as the result of endogenous and exogenous processes or as a result of a conversion of single strand breaks [26]. Unrepaired can result in amplification or loss of genetic material which can result in neoplastic transformation by activation of oncogenes, inactivation of suppressor genes or loss of heterosigosity. It can be result of decrease of HRR fidelity or switch of repair over less correct process of non homologous end joining (NHEJ). It is also possible that genomic instability resulted from defective repair of double strand DNA breaks is an effect of increase of the gene expression...
not its reduction. The localization of the 135G>C polymorphism of the RAD51 gene in 5’UTR region indicates, that this polymorphism can be related to mRNA stability and translation. The RAD51 gene transcript occurred in two main isoforms. Isoform I is 104 nucleotide longer, than isoform II, which is result of alternative splicing. Lost fragment of isoform II contains 77% of GC base pairs [27]. This sequence favors dimensional structures that negatively regulate translation [28, 29]. It seems to be possible that isoform II has greater translation potential. The isoform II level is lower in cell lines with the C/C genotype of 135G>C polymorphism, therefore this genotype can be related to lower level of RAD51 protein.

Our results led us to hypothesis that colorectal cancer occurrence may be in part result of underexpression of RAD51 gene. The cells with C/C genotype have low level of RAD51 protein. In this event other proteins such as XRCC2 and XRCC3 act in HRR process. Variant genotypes of these proteins have decreased repair capacity thus patients with this genotypes do not repair double strand DNA breaks efficiently by HRR.

In present work we showed that the 135G>C polymorphism of the RAD51 can modify the colorectal cancer risk alone as well as with association with other polymorphisms: the Thr241Met in XRCC3 gene and the Arg188His in XRCC2 gene. We showed also that all investigated polymorphisms 135G>C of RAD51, Arg188His and Thr241Met of XRCC3 should be simultaneously taken into account as a part of polygenic cause of colorectal cancer occurrence.

Acknowledgments This work was supported by the grant no. 505/376 from University of Lodz and the “Spoleczny Komitet Walki z Rakiem in Lodz” Foundation.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

1. Smilenov LB (2006) Tumor development: haploinsufficiency and local network assembly. Cancer Lett 240:17–28
2. Masson JY, Tarounas MC, Stasiak AZ, Stasiak A, Shah R, McIlwraith MJ, Benson FE, West SC (2001) Identification and purification of two distinct complexes containing the five RAD51 paralogs. Genes Dev 15:3296–3307
3. Thacker J (2005) The RAD51 gene family, genetic instability and cancer. Cancer Lett 219:125–135
4. Griffin CS (2002) Aneuploidy, centrosome activity and chromosome instability in cells deficient in homologous recombination repair. Mutat Res 504:149–155
5. Deans B, Griffin CS, O’Regan P, Jasim M, Thacker J (2003) Homologous recombination deficiency leads to profound genetic instability in cells derived from Xrc2-knockout mice. Cancer Res 63:8181–8187
6. Takata M, Sasaki MS, Tachiiri S, Fukushima T, Sonoda E, Schild D, Thompson LH, Takeda S (2001) Chromosome instability and defective recombinational repair in knockout mutants of the five Rad51 paralogs. Mol Cell Biol 21:2858–2866
7. Griffin CS, Simpson PJ, Wilson CR, Thacker J (2000) Mammalian recombination-repair genes XRCC2 and XRCC3 promote correct chromosome segregation. Nat Cell Biol 2:757–761
8. Nagasawa H, Wilson PF, Chen DJ, Thompson LH, Bedford JS, Little JB (2008) Low doses of alpha particles do not induce sister chromatid exchanges in bystander Chinese hamster cells defective in homologous recombination. DNA Repair 7:515–522
9. Matullo G, Guarera S, Carturan S, Peluso M, Malaveille C, Davico L, Piazza A, Vineis P (2001) DNA repair gene polymorphisms, bulky DNA adducts in white blood cells and bladder cancer in a case–control study. Int J Cancer 92:562–567
10. Matullo G, Palli D, Peluso M et al. (2001) XRCC1 gene polymorphisms, smoking and (32)P-DNA adducts in a sample of healthy subjects. Carcinogenesis 22:1437–1445
11. Krupa R, Blasiak J (2004) Analysis of the G/C polymorphism in the 5'-untranslated region of the RAD51 gene in breast cancer. Acta Biochim Pol 50:249–253
12. Sliwinski T, Krupa R, Majsterek I, Rykala J, Kolacinska A, Morawiec Z, Drzewoski J, Zadrozny M, Blasiak J (2005) Polymorphisms of the BRCA2 and RAD51 genes in breast cancer. Breast Cancer Res Treat 94:105–109
13. Ruzzo A, Graziano F, Loupakis F, Santini D, Catalano V, Bisonni R, Ficarelli R, Fontana A, Andreoni F, Falcone A, Canestrari E, Tonini G, Mari D, Lippe P, Pizzagalli F, Schiavon G, Alberghi P, Giustini L, Maltese P, Testa E, Menichetti ET, Magnani M (2008) Pharmacogenetic profiling in patients with advanced colorectal cancer treated with first-line FOLFIRI chemotherapy. Pharmacogenomics J 8(4):278–288
14. Moreno V, Gemignani F, Landi S, Gioia-Patricola L, Chabrier A, Blanco I, González S, Guino E, Capellá G, Canzian F (2006) Polymorphisms in genes of nucleotide and base excision repair: risk and prognosis of colorectal cancer. Clin Cancer Res 12:2101–2108
15. Tranah GJ, Giovannucci E, Ma J, Fuchs C, Hankinson SE, Hunter DJ (2004) XRCC2 and XRCC3 polymorphisms are not associated with risk of colorectal adenoma. Cancer Epidemiol Biomarkers Prev 13(6):1090–1091
16. Curtin K, Lin WY, George R, Katory M, Shorto J, Cannon-Albright LA, Smith G, Bishop DT, Cox A, Camp NJ, Colorectal Cancer Study Group (2009) Genetic variants in XRCC3: new insights into colorectal cancer tumorigenesis. Cancer Epidemiol Biomarkers Prev 18(9):2476–2484
17. Blasiak J, Przybylowksa K, Czechowska A, Zadrozy M, Pertynski T, Rykala J, Kolacinska A, Morawiec Z, Drzewoski J (2003) Analysis of the G/C polymorphism in the 5'-untranslated region of the RAD51 gene in breast cancer. Acta Biochim Pol 50:249–253
18. Sliwinski T, Krupa R, Majsterek I, Rykala J, Kolacinska A, Morawiec Z, Drzewoski J, Zadrozny M, Blasiak J (2005) Polymorphisms of the BRCA2 and RAD51 genes in breast cancer. Breast Cancer Res Treat 94:105–109
19. Krupa R, Synowiec E, Pawlowska E, Morawiec Z, Sobczuk A, Zadrozy M, Wozniak K, Blasiak J (2009) Polymorphism of the homologous recombination repair genes RAD51 and XRCC3 in breast cancer. Exp Mol Pathol 87(1):32–35
20. Jiang Z, Li C, Xu Y, Cai S (2010) A meta-analysis on XRCC1 and XRCC3 polymorphisms and colorectal cancer risk. Int J Colorectal Dis 25(2):169–180
21. Wiśniewska-Jarosińska M, Sliwiński T, Krupa R, Stec-Michalska K, Chojnacki J, Blasiak J (2009) The role of RAD 51 gene polymorphism in patients with colorectal cancer in the Polish subpopulation. Pol Merkur Lekarski 26(155):455–457
22. Akisik E, Yazici H, Dalay N (2010) ARLTS1, MDM2 and RAD51 gene variations are associated with familial breast cancer. Mol Biol Rep. doi:10.1007/s1103301001133
23. Li C, Jiang Z, Liu X (2010) XPD Lys(751)Gln and Asp(312)Asn polymorphisms and bladder cancer risk: a meta-analysis. Mol Biol Rep 37:301–309
24. Stanczyk M, Sliwinski T, Cuchra M, Zubowska M, Bielecka-Kowalska A, Kowalski M, Szemraj J, Mlynski W, Majsterek I (2010) The association of polymorphisms in DNA base excision repair genes XRCC1, OGG1 and MUTYH with the risk of childhood acute lymphoblastic leukemia. Mol Biol Rep. doi:10.1007/s110330100127x
25. Wu J, Wang D, Song L, Li S, Ding J, Chen S, Li J, Ma G, Zhang X (2010) A new familial gastric cancer-related gene polymorphism: T1151A in the mismatch repair gene hMLH1. Mol Biol Rep. doi:10.1007/s110330109891
26. Kowalska-Loth B, Bubko I, Komorowska B, Szumiel I, Staron K (1998) Contribution of topoisomerase I to conversion of single-strand into double-strand DNA breaks. Mol Biol Rep 25:21–26
27. Antoniou AC, Similnikova OL, Simard J et al. (2007) RAD51 135G>C modifies breast cancer risk among BRCA2 mutation carriers: results from a combined analysis of 19 studies. Am J Hum Genet 81:1186–1200
28. Hughes TA (2006) Regulation of gene expression by alternative untranslated regions. Trends Genet 22:119–122
29. Zaker M (2003) Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res 31:3406–3415