Short Communication

Assessing Tumor Mutations to Gain Insight into Base Excision Repair Sequence Polymorphisms and Smoking in Colon Cancer

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Abstract

DNA repair enzymes function in major pathways to reverse DNA damage, including base excision repair (BER). Missense polymorphisms in BER repair genes may contribute to differences in DNA repair capacity, specific mutations, and susceptibility to cancer in the presence of exposure to carcinogens such as cigarette smoking. In a study of 1,604 incident colon cancer cases and 1,969 matched population-based controls genotyped for BER variants *OGG1* (S326C) and *XRCC1* (R194W, R280H, and R399Q), we found no associations with colon cancer overall. However, a 2-fold increased risk of *BRAF* V600E tumor mutation was observed in current and former cigarette smokers homozygous for the *OGG1* polymorphism (odds ratio, 2.2; 95% confidence interval, 1.02-4.9, recessive model); similar associations were not observed for microsatellite instability, CpG island methylator phenotype, *KRAS2* mutations, or *TP53* mutations. The *XRCC1* R194W polymorphism was associated with a modest increased risk of *TP53* tumor mutations in those who regularly smoked cigarettes (odds ratio, 1.4; 95% confidence interval, 1.02-1.9). These findings point to the importance of studying tumor mutations when examining DNA repair polymorphisms and cigarette smoke exposure to identify potentially relevant associations with colorectal cancer. (Cancer Epidemiol Biomarkers Prev 2009;18(12):3384–8)

Introduction

An extensive system of DNA repair enzymes function in major pathways to reverse damage to DNA including base excision repair (BER), double-strand break repair, and nucleotide excision repair. Missense polymorphisms in DNA repair genes may contribute to differences in DNA repair capacity in these pathways and influence susceptibility to cancer, particularly in the presence of exposure to carcinogens such as cigarette smoke (1).

In BER, lesions consisting of modified DNA bases or single-strand breaks are repaired by one or more nucleotides through different protein networks (2). OGG1-initiated BER oxidative DNA damage acts to remove 8-oxoguanine from DNA and restore the original sequence (3). Reduced activity of oxoguanine DNA glycosylase (OGG) has been identified as a risk factor for lung, and head and neck cancers (4). A missense polymorphism in codon 326 of the *OGG1* gene (C>G change at position 1245) results in a cytosine substitution for serine (S326C, dbSNP no. rs1052133). As the colon may be subject to exposure to oxygen free radicals, reduced activity of OGG1 may be a risk factor in colon carcinogenesis. However, a limited number of studies of S326C and colon cancer have been conducted (5, 6). Another protein, XRCC1, is critical in the BER pathway, interacting with several BER enzymes to modify and stabilize their activity (2). Common *XRCC1* sequence variants, R194W (C>T; rs1799782), R280H (G>A; rs25489), and R399Q (GA; rs25487), have been previously implicated in risk of colorectal cancers (CRCs) and adenomas, although smoking did not seem to modify associations in previous reports with limited sample sizes that may have been too small to detect associations (7).

DNA repair polymorphisms and mediating effects of environmental exposures have been well studied in sporadic CRC; few reports have examined the association of common variants in these pathways with somatic tumor alterations. In previous reports, we examined DNA mismatch repair gene polymorphisms in *MLH1* and *MSH6* and risk of genetic and epigenetic changes in colon tumors with risk factors including tobacco smoke (8, 9). We have also presented findings to support that smoking

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can preferentially predispose to transversion mutations in *TP53* and *KRAS2*, and the *BRAF* V600E mutation, as well as microsatellite instability (MSI) and a CpG island methylator phenotype (CIMP) in CRC tumors (10-14). This investigation is, to our knowledge, the first large population-based case-control study in which both BER coding polymorphisms of incident colon cancers characterized for tumor marker status, including MSI, CIMP, *BRAF, KRAS2*, and *TP53* mutations, and cigarette smoking exposure were collected.

Materials and Methods

Study Population. Participants in the study were from the Kaiser Permanente Medical Care Program of Northern California, the Twin Cities Metropolitan area in Minnesota, or an eight-county area in Utah (Davis, Salt Lake, Utah, Weber, Wasatch, Tooele, Morgan, and Summit counties). All eligible incident cases with a first primary colon tumor[6] within these defined populations were identified between October 1, 1991 and September 30, 1994 and recruited for the study. Cases with a previous colorectal tumor, familial adenomatous polyposis, ulcerative colitis, or Crohn's disease documented on the pathology report were not eligible for the study. In addition to these criteria, participants were between 30 and 79 y of age at time of diagnosis, English speaking, and mentally competent to complete the interview. Using the same eligibility criteria, controls were frequency matched to cases by sex and by 5-year age cohort. At Kaiser Permanente Medical Care Program of Northern California, controls were randomly selected from membership lists. At Minnesota and Utah, controls younger than 65 y were randomly selected from random-digit dialing or driver's license lists and controls 65 y and older were randomly selected from social security lists.

Institutional review board approval was obtained from all study centers. A total of 1,604 colon cancer cases (898 men and 706 women) and 1,969 controls (1,040 men and 929 women) with genotype data were included in the study. The race/ethnicity of the study population, self-reported at the time of interview, was primarily White, non-Hispanic (91% of cases and 93% of controls). The remainder were Hispanic (4% of cases and 4% of controls) or African-American (5% of cases and 4% of controls). The median age of cases at diagnosis and controls at selection was 64 y. Of all cases asked to participate, 75.6% cooperated; of controls selected, 63.7% participated as previously described (15).

Data Collection. Data were collected for cases and controls by trained and certified interviewers for a calendar-year reference period 2 y before year of diagnosis for cases or selection for controls; rigorous quality control methods were used (16). Anthropometrics and a detailed diet and life-style history were collected. Long-term vigorous physical activity data were collected using a detailed physical activity questionnaire (15). Participants were asked to report number of years they smoked, the age they stopped smoking (if former smoker), and usual number of cigarettes smoked per day while smoking regularly. Of cases, 14% were current and 45% were former smo-

[6] ICD-O 2nd edition codes 18.0 and 18.2 through 18.9, determined by the Surveillance Epidemiology and End Results Cancer Registries in Utah and Northern California.

Table 1. Association of BER sequence polymorphisms and colon tumors

Polymorphism		Genotype	Control	Case	All cases		Case	MSI+		Case	CIMP high		Case	BRAF V600E		Case	KRAS2 mutation		Case	TP53 mutation	
			n	n*	OR (95% CI)		*n*	OR (95% CI)		*n*	OR (95% CI)		*n*	OR (95% CI)		*n*	OR (95% CI)		*n*	OR (95% CI)	
OGG1	S326C	CC	1,172	918	1.0 (Reference)		120	1.0 (Reference)		184	1.0 (Reference)		60	1.0 (Reference)		201	1.0 (Reference)		310	1.0 (Reference)	
		CG	686	570	1.1 (0.9-1.2)		60	0.9 (0.7-1.2)		80	0.8 (0.6-1.0)		28	0.8 (0.5-1.3)		135	1.2 (0.9-1.5)		190	1.1 (0.9-1.3)	
		GG	93	94	1.3 (1.0-1.8)		10	1.0 (0.5-1.9)		13	0.9 (0.5-1.5)		8	1.7 (0.9-3.5)		20	1.2 (0.8-1.9)		27	1.0 (0.7-1.6)	
		P_{trend}		0.09			0.68			0.16			0.74			0.17			0.59		
		GG vs CC/CG[†]			1.3 (1.0-1.8)		10	1.1 (0.6-2.0)		13	1.0 (0.6-1.6)		8	1.8 (0.9-3.7)		20	1.1 (0.7-1.8)		27	1.0 (0.7-1.5)	
XRCC1	R194W	CC	1,724	1,369	1.0 (Reference)		166	1.0 (Reference)		245	1.0 (Reference)		82	1.0 (Reference)		300	1.0 (Reference)		450	1.0 (Reference)	
		CT/TT	226	213	1.2 (1.0-1.4)		24	1.0 (0.7-1.5)		32	0.9 (0.6-1.3)		14	1.2 (0.7-2.1)		56	1.3 (1.0-1.8)		77	1.2 (1.0-1.6)	
XRCC1	R280H	GG	1,766	1,441	1.0 (Reference)		172	1.0 (Reference)		254	1.0 (Reference)		85	1.0 (Reference)		321	1.0 (Reference)		487	1.0 (Reference)	
		GA/AA	184	141	0.9 (0.7-1.2)		18	1.0 (0.7-1.7)		23	0.9 (0.6-1.4)		11	1.3 (0.7-2.4)		35	1.1 (0.8-1.6)		40	0.8 (0.6-1.1)	
XRCC1	R399Q	GG	826	679	1.0 (Reference)		79	1.0 (Reference)		122	1.0 (Reference)		50	1.0 (Reference)		155	1.0 (Reference)		225	1.0 (Reference)	
		GA	872	725	1.0 (0.9-1.2)		93	1.1 (0.8-1.5)		122	1.0 (0.8-1.2)		33	0.6 (0.4-0.9)		169	1.1 (0.9-1.3)		242	1.0 (0.9-1.3)	
		AA	252	178	0.9 (0.7-1.1)		18	0.8 (0.5-1.3)		33	0.9 (0.6-1.3)		13	0.8 (0.5-1.5)		32	0.8 (0.5-1.1)		60	1.0 (0.7-1.3)	
		P_{trend}		0.43			0.44			0.41			0.13			0.26			0.73		

NOTE: Adjusted for age, sex, race, center, energy, body mass index, activity, long-term alcohol use, smoking, recent NSAIDs, and family history.
*Includes 1,210 cases with tumor marker data.
[†]Recessive model.

Table 2. Association of BER sequence polymorphisms and colon tumors in current or former smokers

			Control	Case	All cases	Case	MSI+
			n	n*	OR (95% CI)	*n*	OR (95% CI)
OGG1	S326C	CC	620	556	1.0 (Reference)	78	1.0 (Reference)
		CG	372	321	1.0 (0.8, 1.2)	35	0.8 (0.6-1.2)
		GG	45	54	1.4 (0.9, 2.2)	7	1.2 (0.5-2.5)
		P_{trend}		0.44		0.63	
		GG vs CC/CG†			1.5 (1.0, 2.2)		1.2 (0.6-2.6)
XRCC1	R194W	CC	925	804	1.0 (Reference)	104	1.0 (Reference)
		CT/TT	111	127	1.3 (1.0-1.7)	16	1.1 (0.6-1.7)
XRCC1	R280H	GG	946	847	1.0 (Reference)	111	1.0 (Reference)
		GA/AA	90	84	1.1 (0.8-1.5)	9	0.9 (0.5-1.8)
XRCC1	R399Q	GG	424	393	1.0 (Reference)	47	1.0 (Reference)
		GA	477	424	1.0 (0.8-1.2)	61	1.2 (0.8-1.7)
		AA	135	114	0.9 (0.7-1.3)	12	0.8 (0.4-1.5)
		P_{trend}		0.59		0.68	

NOTE: Adjusted for age, sex, race, center, energy, body mass index, activity, long-term alcohol use, recent NSAIDs, and family history.
*Includes 689 cases with tumor marker data.
†Recessive model.

kers; of controls, 13% currently smoked and 39% formerly smoked. Participants who reported nonsteroidal anti-inflammatory medication use (NSAID; ibuprofen or aspirin) regularly for 1 mo or more, within 2 y of the reference year, were considered recent users.

Genotyping. Genomic DNA was extracted from peripheral WBC collected from blood drawn at the time of the study interview using the Puregene kit (Gentra Systems). From available DNA, genotyping was successfully conducted for 1,604 cases and 1,969 controls. Missing genotyping data were due to lack of amplification or ambiguous results (<0.5%). The *OGG1* S326C and polymorphisms in *XRCC1* (R194W, R280H, and R399Q) were detected by allelic discrimination using TaqMan assays on a 7900HT sequence detection system (Applied Biosystems). Positive controls for all the genotypes as well as four negative controls were included in each plate. For quality control purposes, genotyping for 94 randomly selected samples was repeated. There were no discrepancies. All genotypes were in Hardy-Weinberg equilibrium. Minor allele frequencies in cases and controls, by race/ethnicity, are shown in Supplementary Table S1 (online). As part of a larger study, *XRCC3* T241M, *ERCC2* (D312N and K751Q), *ERCC5* D1104H, and *MGMT* (L84F and I143V) were also genotyped using these methods as previously described (17).

Tumor Analysis. Tumor DNA was obtained from paraffin-embedded tissue. Tumors were characterized by their genetic profile that included *TP53* sequence data for mutation hotspots of exons 5 through 8; sequence data for *KRAS2* codons 12 and 13; five CpG Island markers that are considered to be markers of CIMP (18, 19), methylated in tumors MINT1, MINT2, and MINT31, *CDKN2A* (*p16*), and *MLH1*; the V600E *BRAF* mutation; and MSI status determined by *BAT26* and *TGFβRII*. For MSI, the majority of tumors were classified as MSI positive (MSI+) or microsatellite stable based on *BAT26* status; tumors that did not have a *BAT26* result were classified using *TGFβRII*; a very small subset of tumors that had neither *BAT26* nor *TGFβRII* results were classified using a panel of 10 tetranucleotide repeats. These methods have been previously described in detail (10, 20). Of 1,604 genotyped cases, tumor markers were assessed in 1,210 colon cancers.

Statistical Analysis. All statistical analyses were done using SAS version 9.2 (SAS Institute). Tumors were defined by specific alterations detected; MSI+, *BRAF* V600E, any *KRAS2* mutation, any *TP53* mutation, or CIMP high defined as methylation of two or more of five markers. Additionally, *KRAS2* or *TP53* status was further defined by the presence of any transition or any transversion mutation. Population-based controls were used to assess associations for the population overall while examining multiple outcomes defined by tumor status. A multiple logistic regression model was used to compare all interviewed cases, regardless of whether or not tumor tissue was obtained, to controls. To compare specific types of mutations to controls while adjusting for the other tumor mutations simultaneously, a generalized estimating equation with a multinomial outcome was used as case subjects could contribute from one to five outcome observations depending upon how many tumor alterations or mutations (MSI+, CIMP high, *BRAF, KRAS2, TP53*) an individual had (21). The generalized estimating equation accounts for correlation introduced by including subjects multiple times and was implemented using the GENMOD procedure as described by Kuss and McLerran (22). All models were adjusted for sex, age at diagnosis or selection, study center, race/ethnicity, total energy (kcal), body mass index in kg/m^2, long-term vigorous physical activity, long-term alcohol use, cigarette smoking (never, current, or former smoker) in combined analyses not stratified by smoking, recent NSAID use, and family history of CRC in first-degree relatives.

We assessed odds ratios (OR) and 95% confidence intervals (95% CI) in logistic regression models (colon cancer overall) and in generalized estimating equation models for tumor mutation outcomes. Analyses were stratified by cigarette smoking status dichotomized as never smoked and current/former smoker. *P* for trend was assessed over using ordered categories of variables and comparing the likelihood ratio of a model with the variable to the likelihood ratio of a model without the variable using a χ^2 test with one degree of freedom. *P* for interaction was determined by comparing a full model including an ordinal multiplicative interaction term to a reduced model without an interaction term, using a likelihood ratio test.

Table 2. Association of BER sequence polymorphisms and colon tumors in current or former smokers (Cont'd)

Case	CIMP high	Case	BRAF mutation	Case	KRAS2 mutation	Case	TP53 mutation
n	OR (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)
114	1.0 (Reference)	40	1.0 (Reference)	116	1.0 (Reference)	188	1.0 (Reference)
50	0.8 (0.6-1.1)	19	0.8 (0.5-1.4)	69	1.1 (0.8-1.4)	116	1.1 (0.9-1.4)
8	0.9 (0.5-1.8)	6	2.1 (0.9-4.7)	10	1.1 (0.6-2.2)	14	1.0 (0.5-1.7)
0.36		0.43		0.68		0.78	
	1.0 (0.5-1.9)		2.2 (1.02-4.9)		1.1 (0.6-2.2)		0.9 (0.5-1.7)
158	1.0 (Reference)	56	1.0 (Reference)	168	1.0 (Reference)	269	1.0 (Reference)
14	0.6 (0.4-1.0)	9	1.1 (0.6-2.2)	27	1.2 (0.8-1.7)	49	1.4 (1.02-1.9)
158	1.0 (Reference)	59	1.0 (Reference)	177	1.0 (Reference)	293	1.0 (Reference)
14	1.0 (0.6-1.7)	6	1.2 (0.5-2.5)	18	1.2 (0.7-1.9)	25	0.9 (0.6-1.4)
74	1.0 (Reference)	33	1.0	87	1.0 (Reference)	133	1.0 (Reference)
72	0.9 (0.7-1.2)	21	0.6 (0.3-0.9)	89	1.0 (0.7-1.3)	145	1.0 (0.8-1.3)
26	1.1 (0.8-1.7)	11	1.0 (0.5-2.0)	19	0.7 (0.5-1.2)	40	1.1 (0.7-1.5)
0.90		0.43		0.25		0.93	

Results

Associations of BER pathway missense polymorphisms in *OGG1* and *XRCC1* are shown in Table 1 for colon cancer overall and by tumor alteration or mutation. The largest effect size, a nonstatistically significant 1.7-fold increased risk of *BRAF* V600E mutation, was observed in homozygous carriers of the *OGG1* S326C variant. Coding polymorphisms in other DNA repair pathways that were examined, double-strand break repair (*XRCC3* T241M), nucleotide excision repair (*ERCC2* D312N and K751Q, *ERCC5* D1104H), and direct repair gene *MGMT* (L84F and I143V), were generally not associated with colon cancer or colon tumor subtypes compared with controls, and thus, data are not shown; however, a supplementary table of results is available online (Supplementary Table S2). To further examine associations of BER sequence variants, we examined associations among those who ever smoked cigarettes (Table 2, case-control comparison). In those who currently or formerly smoked cigarettes on a regular basis (53% of controls and 57% of cases with tumor marker data), homozygous carriers of the *OGG1* variant were twice as likely to harbor a *BRAF* tumor mutation compared with individuals with a genotype containing 0 or 1 variant allele. As heterozygous individuals did not exhibit any increased risk of *BRAF*, a recessive model may be appropriate, as shown; however, the number of cases homozygous for S326C is small. *BRAF* mutation in colon cancer often occurs in conjunction with MSI or CIMP; however, *OGG1* S326C did not confer an increased risk in these tumor outcomes (Table 2). An interaction term only approached statistical significance in a case-case comparison of *BRAF*-mutated and nonmutated tumors ($P = 0.08$) and was not significant in a case-control comparison ($P = 0.41$; data not shown). As ever smokers comprised over two thirds of cases with a *BRAF* tumor mutation (68%), the numbers may be small to detect an interaction due to relatively few nonsmokers in the analysis who exhibited this alteration.

Missense polymorphisms in *XRCC1* were generally not associated with risk of colon cancer; however, carriage of one or two variant R194W alleles was associated with a modest 1.4-fold increased risk of a *TP53* mutation in smokers (Table 2), with the association signal observed more prominently in transversion mutations (OR, 1.7; 95% CI, 0.9-3.2; data not shown). As the R194W and R280H minor alleles are relatively less common (frequencies of 0.06 and 0.05, respectively), a dominant model is shown. *XRCC1* haplotypes or combined genotype combinations across R194W, R280H, and R399Q were not associated with colon cancer or tumor subtype.

Discussion

Similar to other reports, we found little evidence to support a strong involvement of common nonsynonymous variants in the BER, double-strand break repair, nucleotide excision repair, and direct DNA repair pathways in overall colon cancer etiology. However, within tumor subphenotypes, there was a suggestive association of *OGG1* in the BER pathway and *BRAF* colon tumor mutation in those who ever smoked cigarettes, with current or former smokers comprising the majority of BRAF-mutated cases. Although some studies have reported no difference in 8-OHdG levels or repair activity between S326C genotypes in functional assays, the ability to suppress spontaneous mutagenesis has been shown to be significantly lower for OGG1 protein encoded by the variant C allele than for the S allele in lung cancer cell lines (4, 5). Smoking in combination with lower OGG activity has been associated with higher cancer risk for lung and esophageal cancer. Among smokers, homozygous carriers of the 326C allele who putatively have a decreased ability to cope with oxidative DNA damage are more susceptible to lung cancer than smokers who carry no or one copy of the variant (5).

Interestingly, *OGG1* S326C was not associated with MSI+ or CIMP-high colon cancers overall or in current and former smokers in our study; *BRAF* V600E mutations are less common in colon cancers (9%) than either MSI+ or CIMP-high tumors (14% and 27%, respectively; ref. 23). A majority of BRAF-mutated tumors exhibit CIMP (and additionally, MSI) and are thought to be a less frequent event in the CIMP-high pathway (24). As we used generalized estimating equations to model multiple tumor outcomes in which tumors can exhibit more than one somatic alteration event to account for correlation introduced by including cases multiple times, the estimate of increased risk of *BRAF* colon cancer in homozygous S326C carriers who smoked seems to be independent of whether or not their tumor also exhibited a CIMP-high phenotype. Smoking has been previously associated with hyperplastic polyps (25), and *BRAF* mutations seem to be involved in the formation of hyperplastic polyps, which may be the precursor for sporadic MSI+ and CIMP-high tumors (26).

We previously reported smoking in association with both CIMP-high and *BRAF* mutations in colon cancers (13); More than two thirds of cases (68%) with a *BRAF* mutation were current or former smokers. Our finding that smoking may interact with *OGG1* S326C to increase risk is suggestive and must be interpreted with caution. Although our case-control study of colon tumors was large, numbers to detect a statistically significant interaction with both *OGG1* genotype and smoking in *BRAF* mutated tumors were small. Our results indicate the possibility that decreased enzyme activity from a nonsynonymous variant in the BER pathway may be involved in predisposing colon tumors to *BRAF* V600E in current and former regular users of tobacco.

Common polymorphisms in BER or other DNA repair pathways have been examined in colorectal tumor subtypes in a very limited number or reports. Park et al. (6) reported that the S326C polymorphism was not associated with tumor location or MSI status in CRC patients, and Sliwiski et al. (27) did not find a correlation between this variant and CRC occurrence or progression. In a study of 125 cases and 247 matched controls, Kim et al. (5) previously reported the homozygous variant S326C genotype was associated with colon cancer in smokers. Our findings lend further support to the hypothesis that risk of colon tumors may be influenced by *OGG1* genotype in the presence of environmental exposures such as smoking (4); however, the increased risk was observed in tumors with positive *BRAF* V600E mutation status. We recognize that in our hypothesis-based investigation, the associations we observed may be due to chance as a number of comparisons were made; thus, our findings should be interpreted with caution and replication in other studies is warranted.

This investigation points out that putative functional risk alleles and cigarette smoke exposure in sporadic CRC needs to be studied in tumors assessed for mutation subtypes to detect potentially relevant smoking-related associations that may otherwise be overlooked in candidate gene case-control studies of genetic and life-style factors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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