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MLH1 and *XRCC1* polymorphisms in Mexican patients with colorectal cancer

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ABSTRACT. DNA repair proteins maintain DNA integrity; polymorphisms in genes coding for these proteins can increase susceptibility to colorectal cancer (CRC) development. We analyzed a possible association of *MLH1* -93G>A and 655A>G and *XRCC1* Arg194Trp and Arg399Gln polymorphisms with CRC in Mexican patients. Genomic DNA samples were obtained from peripheral blood of 108 individuals with CRC (study group) at diagnosis and 120 blood donors (control group) from Western Mexico; both groups

were mestizos. The polymorphisms were detected by PCR-RFLP. Association was estimated by calculating the odds ratio (OR). We found that the *MLH1* and *XRCC1* polymorphisms were in Hardy-Weinberg equilibrium. The *MLH1* 655A>G polymorphism in the 655G allele was associated with a 2-fold increase risk for CRC (OR = 2.04 and 95% confidence interval (95%CI) = 1.12-3.69; P < 0.01), while the *MLH1* -93G>A polymorphism allele was associated with a protective effect (OR = 0.60, 95%CI = 0.40-0.89; P = 0.01 in the -93A allele and OR = 0.32, 95%CI = 0.13-0.79; P = 0.01 in the AA genotype). The *XRCC1* Arg194Trp and Arg399Gln polymorphisms did not show any significant associations. In conclusion, we found that *MLH1* -93G>A and 655A>G polymorphisms are associated with CRC in Mexican patients.

Key words: *MLH1* gene; *XRCC1* gene; Colorectal cancer; Mexican population

INTRODUCTION

Colorectal cancer (CRC) is characterized by genomic instability produced by chromosomal instability, aberrant DNA methylation, and defects in DNA repair. These mechanisms induce the accumulation of multiple tumor-specific mutations (Markowitz and Bertagnolli, 2009). The tumor suppressor gene *MLH1* [*mutL homolog 1*, *colon cancer*, *non-polyposis type 2* (*Escherichia coli*)] is located at 3p22.2, contains 19 exons, and codes for a protein of the replication repair complex that corrects mismatched bases; in fact, MLH1 deficiency has been associated with hereditary non-polyposis colorectal cancer or Lynch syndrome (Silva et al., 2009). The *MLH1* -93G>A (rs1800734) and 655A>G (rs1799977, I219V) polymorphisms are located in the promoter and exon 8, respectively (Allan et al., 2008; Silva et al., 2009). The *XRCC1* (*X-ray repair complementing defective repair in Chinese hamster cells 1*) gene is mapped at 19q13.2-13.3 and encodes a 70-kDa protein involved in the repair of DNA single-strand breaks produced by ionizing radiation and alkylating agents (Caldecott, 2003; Hung et al., 2005). Its polymorphisms Arg194Trp (rs1799782) and Arg399Gln (rs25487) are localized in exons 6 and 10, respectively. In this study, we evaluated the association of these four polymorphisms with CRC in Mexican patients.

SUBJECTS AND METHODS

Subjects

The patient group was composed of 108 individuals diagnosed with sporadic CRC. The average age was 62 years (range 20-96) and 52% were men. The control group was constituted by 120 healthy people randomly selected from blood donors. All subjects were mestizos from Western Mexico and provided written informed consent before collection of blood samples. Five milliliters of peripheral blood with EDTA as anticoagulant

was obtained from each individual.

Genomic DNA extraction and genotyping

Genomic DNA was extracted by means of the DTAB-CTAB method (Gustincich et al., 1991). All variants were detected via PCR-RFLP assays. The primers for the *MLH1* 655A>G polymorphism were those described by Mei et al. (2006), while the primers for the *MLH1* -93G>A polymorphism were designed in the Oligo Primer Analysis Software v6.71 and were 5'-CGCCAGATCACCTCAGCAGA-3' (forward) and 5'-CGCCAGAAGAGCCAAGGAAA-3' (reverse). PCR conditions consisted of an initial denaturation at 94°C for 10 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 70° (for -93G>A) or 55°C (for 655A>G) for 30 s, and extension at 72°C for 45 s, and final elongation at 72°C for 10 min. The restriction analysis for *MLH1* -93G>A and 655G>A was done overnight at 37°C with *Pvu*II and *Bcc*I, respectively. Both enzymes recognize the wild allele. The digestion products were separated on polyacrylamide gels. The *XRCC1* Arg194Trp and Arg399Gln polymorphisms were detected according to Zhang et al. (2005).

Statistical analysis

Allele and genotype frequencies were established by counting and the distribution of genotypes in both groups was compared by the chi-square test or the Fisher exact test. Hardy-Weinberg equilibrium (HWE) was evaluated by the chi-square test. Association was estimated by the odds ratio (OR) and 95% confidence interval (CI) in the SPSS v10.0 software (the wild allele was taken as reference). $P < 0.05$ was considered to be significant.

RESULTS

All four polymorphisms were in HWE. In CRC patients, the frequencies of the *MLH1* 655G allele and GG genotype were significantly higher even if the OR could not be analyzed due to absence of that genotype in the control group. As for the -93G>A polymorphism, both AA genotype and A allele were related to a significantly decreased risk. The *XRCC1* Arg399Gln and Arg194Trp polymorphisms did not show any significant difference (Table 1). Allele and genotype frequencies of the control group were described by Meza-Espinoza et al. (2009).

DISCUSSION

The analysis shows that *MLH1* polymorphisms are associated with CRC in Mexican patients. Actually, the 655G allele appears to confer a 2-fold increased risk to develop CRC in Mexican patients, a finding similar to that observed in 140 Spanish patients with sporadic CRC (Nejda et al., 2009). According to immunohistochemical analysis, it has been suggested that this polymorphism correlates with protein expression (Kim et al., 2004). *In vitro* mismatch repair assays led to classification of the 655A>G polymorphism as MMR+ (mismatch repair activity >60%) and a normal protein function was revealed by SIFT analysis (Takahashi et al., 2007).

Table 1. Genotype and allele frequencies of *MLH1* and *XRCC1* polymorphisms in the control group and colorectal cancer (CRC) patients from Western Mexico.

Genotype/allele	Control group		CRC patients			
	N	Frequency (%)	N	Frequency (%)	OR (95%CI)	P*
MLH1						
-93G>A	115		108			
GG	39	34	51	47	1.0 (Reference)	
GA	55	48	48	44	0.66 (0.37-1.17)	0.16
AA	21	18	9	9	0.32 (0.13-0.79)	0.01
G	133	58	150	69	1.0 (Reference)	
A	97	42	66	31	0.60 (0.40-0.89)	0.01
655A>G	100		102			
AA	81	81	71	70	1.0 (Reference)	
AG	19	19	26	25	1.56 (0.79-3.05)	0.19
GG	0	0	5	5	NA	0.01
A	181	90	168	82	1.0 (Reference)	
G	19	10	36	18	2.04 (1.12-3.69)	0.01
XRCC1						
Arg194Trp	120**		107			
Arg/Arg	86	72	86	80	1.0 (Reference)	
Arg/Trp	31	26	21	20	0.67 (0.36-1.27)	0.22
Trp/Trp	3	2	0	0	NA	0.08
Arg	203	85	193	90	1.0 (Reference)	
Trp	37	15	21	10	0.59 (0.33-1.05)	0.07
Arg399Gln	120**		103			
Arg/Arg	65	54	48	46.5	1.0 (Reference)	
Arg/Gln	47	39	48	46.5	1.38 (0.79-2.39)	0.24
Gln/Gln	8	7	7	7	1.18 (0.40-3.49)	0.75
Arg	177	74	144	70	1.0 (Reference)	
Gln	63	26	62	30	1.21 (0.80-1.83)	0.36

*Chi-square or Fisher exact tests. **Frequencies described by Meza-Espinoza et al. (2009). NA = not analyzed.

Our results also show that both the AA genotype and A allele of *MLH1* -93G>A polymorphism protect against CRC development. In a recent meta-analysis study, no association with CRC was observed (Pan et al., 2011). However, Allan et al. (2008) reported that *MLH1* -93A was associated with a 3-fold increased risk of CRC, negative for the MLH1 protein detectable by immunohistochemistry (OR = 3.30, 95%CI = 1.46-7.47), while Raptis et al. (2007) documented a 3-fold increased risk ($P < 0.001$) of CRC with MSI-H (microsatellite instability high) in a population from Ontario (OR = 3.23, 95%CI = 1.65-6.30) and an 8-fold increased risk ($P < 0.001$) in Newfoundland people (OR = 8.88, 95%CI = 2.33-33.9). To explain these discordant results, we propose that the *MLH1* -93G>A polymorphism may be cis-acting with unidentified variants in the minimal region for transcription - defined between -302 and -76 by Arita et al. (2003) - and co-modulates *MLH1* expression. Actually, these authors proved that single mutants in the *MLH1* -96 to -91 region show the lowest promoter activity (approximately 13 to 17%) and that double or triple mutants (-163 to -158, -145 to -139 and -96 to -91) exhibit a moderate activity (30%) (Arita et al., 2003). Yet, there is no conclusive evidence that the -93A allele reduces the activity of the promoter, and no nuclear factor for the *MLH1* transcription has been identified (Mei et al., 2010; Perera et al., 2011).

The non-association of the *XRCC1* Arg399Gln and Arg194Trp polymorphisms with CRC in Mexican patients agrees with the results of a recent meta-analysis (Wang et al., 2010). Such polymorphisms have also been assessed in other types of cancer. Although Wei et al. (2011) reported no association with prostate cancer, when they stratified by ethnicity, an in-

creased risk for the 399Gln allele in Asian men has been reported. Chen et al. (2012) related the Trp/Trp genotype of the *XRCC1* Arg194Trp polymorphism to an increased risk of gastric cancer, while Huang et al. (2009) associated dominant and recessive models for *XRCC1* Arg399Gln polymorphism with breast cancer.

In conclusion, our results suggest that the *MLH1* -93G>A and 655A>G polymorphisms are associated with CRC in Mexican patients.

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REFERENCES

Allan JM, Shorto J, Adlard J, Bury J, et al. (2008). MLH1 -93G>A promoter polymorphism and risk of mismatch repair deficient colorectal cancer. *Int. J. Cancer* 123: 2456-2459.

Arita M, Zhong X, Min Z, Hemmi H, et al. (2003). Multiple sites required for expression in 5'-flanking region of the hMLH1 gene. *Gene* 306: 57-65.

Caldecott KW (2003). XRCC1 and DNA strand break repair. *DNA Repair* 2: 955-969.

Chen B, Zhou Y, Yang P and Wu XT (2012). Polymorphisms of XRCC1 and gastric cancer susceptibility: a meta-analysis. *Mol. Biol. Rep.* 39: 1305-1313.

Gustincich S, Manfioletti G, Del SG, Schneider C, et al. (1991). A fast method for high-quality genomic DNA extraction from whole human blood. *Biotechniques* 11: 298-300, 302.

Huang Y, Li L and Yu L (2009). XRCC1 Arg399Gln, Arg194Trp and Arg280His polymorphisms in breast cancer risk: a meta-analysis. *Mutagenesis* 24: 331-339.

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

Kim JC, Roh SA, Koo KH, Ka IH, et al. (2004). Genotyping possible polymorphic variants of human mismatch repair genes in healthy Korean individuals and sporadic colorectal cancer patients. *Fam. Cancer* 3: 129-137.

Markowitz SD and Bertagnolli MM (2009). Molecular origins of cancer: Molecular basis of colorectal cancer. *N. Engl. J. Med.* 361: 2449-2460.

Mei M, Liu D, Dong S, Ingvarsson S, et al. (2010). The MLH1 -93 promoter variant influences gene expression. *Cancer Epidemiol.* 34: 93-95.

Mei Q, Yan HL, Ding FX, Xue G, et al. (2006). Single-nucleotide polymorphisms of mismatch repair genes in healthy Chinese individuals and sporadic colorectal cancer patients. *Cancer Genet. Cytogenet.* 171: 17-23.

Meza-Espinoza JP, Peralta-Leal V, Gutierrez-Angulo M, Macias-Gomez N, et al. (2009). XRCC1 polymorphisms and haplotypes in Mexican patients with acute lymphoblastic leukemia. *Genet. Mol. Res.* 8: 1451-1458.

Nejda N, Iglesias D, Moreno AM, Medina A, V, et al. (2009). A MLH1 polymorphism that increases cancer risk is associated with better outcome in sporadic colorectal cancer. *Cancer Genet. Cytogenet.* 193: 71-77.

Pan XM, Yang WZ, Xu GH, Bai P, et al. (2011). The association between MLH1 -93 G>A polymorphism of DNA mismatch repair and cancer susceptibility: a meta-analysis. *Mutagenesis* 26: 667-673.

Perera S, Mrkonjic M, Rawson JB and Bapat B (2011). Functional effects of the MLH1-93G>A polymorphism on MLH1/EPM2AIP1 promoter activity. *Oncol. Rep.* 25: 809-815.

Raptis S, Mrkonjic M, Green RC, Pethe VV, et al. (2007). MLH1 -93G>A promoter polymorphism and the risk of microsatellite-unstable colorectal cancer. *J. Natl. Cancer Inst.* 99: 463-474.

Silva FC, Valentin MD, Ferreira FO, Carraro DM, et al. (2009). Mismatch repair genes in Lynch syndrome: a review. *Sao Paulo Med. J.* 127: 46-51.

Takahashi M, Shimodaira H, Andreutti-Zaugg C, Iggo R, et al. (2007). Functional analysis of human MLH1 variants using yeast and *in vitro* mismatch repair assays. *Cancer Res.* 67: 4595-4604.

Wang B, Wang D, Huang G, Zhang C, et al. (2010). XRCC1 polymorphisms and risk of colorectal cancer: a meta-analysis.

Int. J. Colorectal Dis. 25: 313-321.
Wei B, Zhou Y, Xu Z, Ruan J, et al. (2011). XRCC1 Arg399Gln and Arg194Trp polymorphisms in prostate cancer risk: a meta-analysis. *Prostate Cancer Prostatic Dis.* 14: 225-231.
Zhang Z, Wan J, Jin X, Jin T, et al. (2005). Genetic polymorphisms in XRCC1, APE1, ADPRT, XRCC2, and XRCC3 and risk of chronic benzene poisoning in a Chinese occupational population. *Cancer Epidemiol. Biomarkers Prev.* 14: 2614-2619.