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World J Gastroenterol 2006 March 7; 12(9):1426-1429
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Evaluation of *p53* codon 72 polymorphism in adenocarcinomas of the colon and rectum in La Plata, Argentina

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Received: 2005-09-13 Accepted: 2005-10-26

Abstract

AIM: To evaluate the potential association between *p53* codon 72 polymorphism and sporadic colorectal adenocarcinoma development, and human papillomavirus (HPV) infection.

METHODS: One-hundred and nine controls and 53 patients with colon cancer from the city of La Plata, Argentina were analyzed. *p53* codon 72 genotypes and HPV infection were identified using allele-specific polymerase chain reaction and nested polymerase chain reaction, respectively.

RESULTS: The differences in the distribution of *p53* codon 72 polymorphism between cases and controls were statistically significant. The arginine allele had a prevalence of 0.65 in controls and 0.77 in cases. The corresponding odds ratio for the homozygous arginine genotype was 2.08 (95% *CI*, 1.06-4.05; $P<0.05$). Lack of association was found between *p53* polymorphism and HPV infection in the set of adenocarcinomas.

CONCLUSION: The findings of the present study indicate that *p53* codon 72 arginine homozygous genotype may represent a genetic predisposing factor for colon cancer development. However, further studies are needed in order to elucidate the role of *p53* codon 72 polymorphism in colorectal cancer.

Key words: *p53* codon 72 polymorphism, human papillomavirus, colorectal cancer.

Pérez LO, Abba MC, Dulout FN, Golijow CDI. Evaluation of *p53* codon 72 polymorphism in adenocarcinomas of the colon and rectum in La Plata, Argentina. *World J Gastroenterol* 2006; 12(9): 1426-1429

http://www.wjgnet.com/1007-9327/12/1426.asp

INTRODUCTION

Sporadic carcinoma of the colon and rectum is a common cause of cancer deaths in the more developed countries, accounting for 44/100 000 new cases per year in males from the United States, and 33.1/100 000 in women. The incidence of colon cancer is lower in South America, ranging from 16.4/100 000 in males and 14.8/100 000 in females. By contrast, Argentina has increased rates of colon cancer compared with other South American countries, which represents the second and the third cause of cancer deaths in women and men, respectively[1].

Colorectal cancer development is a complex, gradual, multistep process, in which many factors are known to be implicated. The molecular and histological changes involved are also well described, though not well understood. Most of these cancers are adenocarcinomas (95%), showing a high frequency of *p53* mutations, mainly at advanced stages of colorectal cancer progression[2]. Regarding the etiology, epidemiological studies have associated several risk factors with colorectal cancer, including alcohol consumption, low-fiber or high-fat diet intake, hereditary conditions, familial history of colorectal cancer, personal history of colonic polyps and bowel inflammatory diseases. Subjects with the highest risk for colorectal cancer have either a hereditary condition or a bowel inflammatory disease. However, it is worth mentioning that this group comprises only 6% of the general population, while the remaining of colorectal cancer occurs in individuals with no known risk factors[3].

p53 is an important tumor suppressor gene involved in the regulation of cell growth, DNA maintenance and apoptosis. Also, experimental evidence suggests that the p53 protein is related to cell aggressiveness and tumor metastasis[4]. Recent studies have shown that a common polymorphism at codon 72 of the *p53* gene results in two alleles, the arginine (*Arg*) and proline (*Pro*) isoforms, which differ biologically and biochemically[5]. In this sense, it appears that this polymorphism may be associated with differential susceptibility to cancer. Several studies conducted in different countries reported significant associations

Table 1 Genotype frequencies of *p53* codon 72 polymorphism in colorectal controls and tumors according to Dukes staging

	Genotype frequencies			Odds ratio	95% *CI*
	Arg/*Arg*	*Arg*/*Pro*	*Pro*/*Pro*		
Controls	44 (40.3%)	53 (48.6%)	12 (11.1%)	1 (reference)	
Carcinomas	31 (58.5%)	20 (37.7%)	2 (3.8%)	2.08[a]	1.06-4.05
Dukes A-B	13 (52.0%)	11 (44.0%)	1 (4%)	1.6	0.66-3.83
Dukes C-D	18 (64.3%)	9 (32.1%)	1 (3.6%)	2.66[a]	1.12-6.29

[a]$P<0.05$.

between *p53* polymorphism and human cancer. However, the data available for most cancers remain inconclusive, including for colorectal cancer[6-10]. In addition, the *Arg* isoform of the p53 protein was shown to be more susceptible to degradation by the human papillomavirus (HPV) E6 protein than the proline one, and homozygosity for the arginine allele was found at higher frequency in the germlines of individuals affected by HPV-linked cancer[11]. Although the presence of HPV-DNA in colorectal tissues and adenocarcinomas was reported in populations from different regions[12-16], the association of *p53* codon 72 polymorphism with colorectal cancer taking into account HPV infection was investigated only once[17]. In order to elucidate the potential significance of *p53* polymorphism in sporadic colorectal cancer, in association with high-risk HPV infection, a sample of 53 cases and 109 controls from the city of La Plata, Argentina was characterized, using PCR-based methods.

MATERIALS AND METHODS

Fifty-three patients with sporadic colorectal carcinomas and 109 healthy individuals were screened for *p53* codon 72 polymorphism variants. The age range for cases was 51-80 years, and the age range for the controls was 18-67 years. Colorectal adenocarcinomas were obtained from patients during surgical procedures. Liquid cytologies and gastrointestinal biopsies were taken from controls, consisting of subjects with no evidence of neoplastic disorders. Specimens were classified according to the Dukes staging system, 7 as Dukes A, 18 as Dukes B, 19 as Dukes C and 9 as Dukes D.

Paraffin embedded tissues were washed twice with xylene and then with 100% ethanol, air-dried and suspended in 300 μL of digestion buffer (Tris-HCL pH 8, Triton X-100 and Tween 20) with proteinase K (100 μg/mL) for 2 h at 56℃ (Promega, Madison, Wisconsin, USA). Liquid cytologies were suspended and washed twice with 1 mL PBS, resuspended in 400 μL of digestion buffer and digested with proteinase K for 2 h at 56℃. DNA was extracted from the lysates using the *salting out* (salt precipitation) procedure [18]. The samples were stored at –20℃ until used.

p53 codon 72 polymorphism analysis

The *p53* codon 72 polymorphism was studied using allele-specific PCR, as previously described[11], with minor modifications[19]. Detection of the amplicons was made by electrophoresis onto a 6% polyacrylamide minigel and ethidium bromide staining. Genotypes were finally determined under UV illumination.

Human papillomavirus detection and genotyping

HPV infection was investigated in colon tissues from 53 patients. HPV DNA was detected by nested PCR, according to the methods previously described[20]. The region L1 of the viral genome was amplified, using MY09/11 as outer primers, and GP05/06 as inner primers. HPV genotyping was performed using the low ionic strength-single strand conformational polymorphism (LIS-SSCP) procedure, as described elsewhere[21]. To determine DNA quality for PCR amplification, a fragment of the human thymidine kinase gene was amplified by PCR in all the samples[22].

Statistical analysis

Association between *p53* codon 72 genotypes, colorectal adenocarcinoma and HPV infection was assessed by the chi-square (χ^2) test. The basic significance level was fixed at *P value* < 0.05. The statistical analysis was performed using the statistical package SPSS™.

RESULTS

Histological classification of adenocarcinomas showed that 41.5% of the cases presented high differentiation, meanwhile 30.2% and 24.5% presented moderate and low differentiation, respectively. The remaining cases were unidentified (3.8%). All DNA samples were successfully amplified by PCR for TK gene, demonstrating that the DNA recovered from the paraffin embedded tissues had quality enough to be analyzed for gene polymorphisms and HPV infection. In control samples, the genotype distribution for *p53* polymorphism showed 40.3%, 48.6% and 11.1% for the *Arg*/*Arg*, *Arg*/*Pro* and *Pro*/*Pro* genotypes, respectively. Allelic frequencies corresponded to 0.65 for the arginine allele and 0.35 for the proline allele. The obtained genotype frequencies fitted the Hardy-Weinberg equilibrium ($P>0.05$). On the other hand, 58.5% of the cases were *Arg*/*Arg*, 37.7% were *Arg*/*Pro* and 3.8% were *Pro*/*Pro*. The corresponding frequencies were 0.77 for the arginine allele and 0.23 for the proline allele. A significant difference between cases and controls was found for the *Arg*/*Arg* genotype compared with (grouped) *Arg*/*Pro* and *Pro*/*Pro* genotypes. Table 1 shows the obtained risk estimation for

colorectal cancer with its corresponding confidence intervals.

HPV-DNA detection and genotyping was carried out using PCR-SSCP on a smaller set of adenocarcinomas (n = 53). HPV 16 was detected in 41.5% (22/53) of the tissues analyzed, HPV 18 in 24.5% (13/53) and HPV 33 in 7.5%. The distribution of the *p53* polymorphism in HPV negative samples was 53.8% (7/13) and 46.1% (6/13) for the *Arg/Arg* and *Arg/Pro* genotypes, respectively. None of the HPV negative samples tested positive for *Pro/Pro*. On the other hand, 60% (24/40) of the HPV positive samples were *Arg/Arg*, 35% (14/40) were *Arg/Pro* and 5% (2/40) were *Pro/Pro*. No significant differences were found between these two groups regarding *Arg* allele and HPV infection (OR, 1.28; 95% CI, 0.36-4.53; P>0.05).

DISCUSSION

In this study, a positive correlation of *p53* polymorphism and colorectal cancer was observed through analysis of a sample of 162 individuals from La Plata, Argentina. This observation is in agreement with the original study of Storey *et al.* (1998) on cervical cancer[11]. However, the distribution of *p53* genotypes according to type-specific HPV infections showed no significant association in this set of adenocarcinomas.

It is well known that the distribution of *p53* codon 72 polymorphism varies in different geographic regions and ethnicity. According to the literature, general populations from Latin America, United States and Europe exhibit high frequencies of the *Arg* allele compared to the *Pro* one, while lower prevalences of *Arg* are found in African and Asian populations [23-26]. In this study, the frequency for the arginine allele was estimated at 0.65. This result is concordant with that obtained for the control group in a previous case-control study on cervical cancer performed in the city of La Plata[19].

The present study provides additional evidence regarding the role of both HPV infection and *p53* codon 72 variants in colon tissues. In agreement with previous studies, HPV-DNA was detected in a high proportion of adenocarcinomas of the colon[12-16]. These findings may suggest a potential role for high-risk HPV in colorectal cancer. Although the present study did not examine HPV DNA in normal colon tissues, its prevalence was reported in a case-control study on colorectal cancer conducted in La Plata[16]. Using PCR-based techniques, the authors found that 33% (10/30) of the normal samples were positive for HPV DNA. Interestingly, eighty percent (8/10) of the positives were single infections of HPV 16.

With respect to the *p53* polymorphism, the frequency of the *Arg* allele in colorectal cancer lesions showed a two-fold increase compared with that in normal samples. The estimated risk (OR) of the *Arg/Arg* genotype for colorectal cancer was 2.08, and subjects with Dukes C and D reported the highest frequency of *Arg/Arg*. This finding suggests that the *Arg* allele may be associated with increased malignancy in colorectal cancer progression. When stratified by HPV infection, the frequency of *Arg/Arg* genotype in HPV positive adenocarcinomas was not statistically different from that in HPV negative samples. However, it should be considered that this finding may represent an artifact, possibly produced as a result of the small sample size.

Case and control studies conducted in Japan and Turkey failed to find an association between the prevalence of *p53* polymorphism and colorectal cancer[6,17]. In the study conducted by Murata *et al.* (1996), the allelic frequencies were concordant between the controls and two sets of colorectal and lung cancer cases, reaching a frequency of approximately 0.6 for the *Arg* allele. The authors only found a significant association between *p53* polymorphism and lung cancer in non-smoking patients[6]. Similar to our study, Sayhan *et al.* (2001) incorporated the analysis of HPV infection in a case-control study on colorectal cancer and *p53* codon 72 polymorphism. However, *p53* genotypes did not correlate with colon cancer, or with the prevalence of high-risk HPV infections[17]. On the other hand, a case-control study conducted in Spain showed a modest association between the *Pro* allele and colorectal cancer (OR, 1.34; P = 0.066). However, such an association was of borderline significance, and it was lost when the analysis was adjusted to another common polymorphism of *p53* examined in that study, *p53*PIN3[10].

The present study has several strengths, including the use of a representative group of controls from the city of La Plata and the careful examination of all stages of colon cancer. Regarding methodology, misclassification and allelic loss was avoided by the use of two separate allele-specific PCR reactions, so that low copies of one allele would not be affected by the presence of several copies of the other. On the other hand, the present study was not controlled for other potential predisposing factors, such as smoking or life-style habits. This is an important issue to be addressed in further studies in order to assess the role of *p53* polymorphism in this tissue.

The mechanisms which lead to the increase of the *Arg* allele in human cancers are not well-established. Another mechanism proposed to explain the epidemiological findings was postulated by Marin *et al.* (2000)[27]. In their study, the authors demonstrated that certain conformational p53 mutants bind and inactivate the p73 protein, a p53 homologue and transcription factor of some p53 target genes[28]. In experimental assays, the binding of the p53 *Arg* isoform to p73 equaled to or exceeded that promoted by the corresponding *Pro* isoform. Thus, it appears that the *p53* codon 72 polymorphism influences the interaction between p53 mutants and p73, and therefore its ability to activate some p53 target genes. In this sense, preferential mutation and retention of the *Arg* allele was found in a set of various cell cancers from *p53* codon 72 germline heterozygotes[27]. However, a recent study did not find that p53-p73beta interactions were influenced by the *p53* polymorphism[29]. Moreover, no significant differences were found in *p53* mutation prevalence between *Arg/Arg* (40/97) and *Pro/Pro* (7/16) genotypes in a set of colorectal tumors[7]. These contradictory findings implicate that the involvement of *p53* polymorphism in human cancer demands further study. More recently, Schneider-Stock *et al.* (2004) found preferential mutation of the *Arg* allele in a group of colorectal adenocarcinomas. They also reported selective loss of the *Pro* allele in tumors with loss of het-

erozygosity (LOH), resulting in a positive association between *Arg* prevalence and Dukes progression. The authors discarded the possibility of HPV as a potential mechanism for the higher frequency of *Arg* alleles in colorectal tumors and hypothesized that carcinogenic exposure may selectively affect the *p53 Pro* allele in the development of colon cancer[8].

Overall, the findings of the present study indicate that the *p53* codon 72 polymorphism may act as a predisposing factor to colorectal cancer but it is not associated with high-risk HPV infections. Clearly, the data available are still inconsistent and it would be unwise to draw conclusions. Further studies of larger sample sizes, including the analysis of the premalignant lesions and the status of the *p53* gene, are awaited in order to elucidate the magnitude of genetic susceptibility in sporadic colorectal carcinogenesis.

ACKNOWLEDGEMENTS

We thank Dr. Echeverría and Dr. Galosi from the Virology Department, Faculty of Veterinary Sciences, National University of La Plata for their technical help.

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S- Editor Wang J **L-Editor** Zhu LH **E- Editor** Zhang Y