Journal of Tumor

Online Submissions: http://www.ghrnet.org/index.php/jt
doi:10.17554/j.issn.1819-6187.2016.04.96

Journal of Tumor 2016 December; 4(5-6): 478-481
ISSN 1819-6187

ORIGINAL ARTICLE

Association of P53 Codon 72 with Colon Cancer: the Role of Genetic Factors

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Conflict-of-interest statement: The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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Received: July 18, 2016
Revised: October 23, 2016
Accepted: October 28, 2016
Published online: December 18, 2016

ABSTRACT

AIM: Studies on the association between p53 codon 72 and colon cancer have given contrasting results: in some populations a positive association has been found with *Pro variant, in other with *Arg variant and in other populations no association has been observed. We have studied the possible effect of genetic variability within four common polymorphisms on the association between p53 codon 72 and colon cancer.

METHODS AND MATERIAL: 106 subjects with colon cancer and 476 controls from the White population of Rome were studied. p53 codon 72, ACP_1, PTPN22, ADA_2 and ADA_6 polymorphisms were determined by DNA analysis. Statistical analyses were carried out by SPSS programs.

RESULTS: The proportions of the joint genotypes of *Pro allele carriers with *B/*B genotype of ACP_1, with carriers of *T allele of PTPN22, with the ADA_1*1/*1 genotype and with carriers of ADA_6*1 allele are higher in colon cancer than in controls. A statistically significant positive correlation is observed in colon cancer between the proportion of *Pro allele carriers and the number of the four genetic factors considered. Sex and cancer grade influence this correlation.

CONCLUSIONS: Common genetic polymorphisms influence the strength of correlation between p53 codon 72 and colon cancer suggesting a possible explanation of the contrasting result observed between different populations. Moreover, the results support the multi-factorial origin of cancer.

Key words: p53 codon 72; ACP1; PTPN22; ADA_2; ADA_6; Colon cancer

Spina C, Gloria-Bottini F, Coppeta L, Neri A, Magrini A, Bottini E. Association of P53 Codon 72 with Colon Cancer: the Role of Genetic Factors. *Journal of Tumor* 2016; 4(5-6): 478-481 Available from: URL: http: //www.ghrnet.org/index.php/jt/article/view/1789

INTRODUCTION

Studies on the association between p53 codon 72 and colon cancer have given contrasting results: in some populations a positive association has been found with *Pro variant[1,2], in other with *Arg variant[3,4] and in other populations no association has been observed[5].

p53 codon 72 is a polymorphic site within the p53 gene resulting in two protein variants with arginine and with proline respectively in the aminoacid sequence. The arginine variant (*Arg) is a strong apoptosis inducer while the proline variant (*Pro) is a strong transcriptional activator[6]. There are three genotypes: *Arg/*Arg, *Arg/*Pro and *Pro/*Pro.

Previous studies have shown an association of colon cancer with ADA[7] and with ACP_1 genetic polymorphism[8]. We reasoned that the proline variant with its strong transcriptional activity could interact positively with ADA and ACP_1 increasing the risk of colon cancer. In this paper we have studied the interaction of p53 codon 72 with ACP_1 and with ADA concerning their effects on susceptibility to

colon cancer. A possible interaction of p53 codon 72 with PTPN22 has been also considered. The role of these polymorphisms on the strength of the association between p53 codon 72 and colon cancer has been analyzed.

Acid phosphatase locus1 (ACP_1) gene encodes for the protein cLMWPTP (cytosolic Low Molecular Weight Protein Tyrosine Phosphatase) and shows a polymorphism due the presence of three codominant alleles *A,*B,*C at an autosomal locus. *C is the minor allele. There are six genotypes with total enzymatic activity decreasing in the order: *C/*C >*C/*B>*C/*A>*B/*B>*A*B>*A/*A. *A allele is associated to the lowest while *C allele to the highest enzymatic activity[9,10]. ACP_1 is involved in the regulation of glucose metabolism and flavo-enzymes activity. Moreover, the enzyme dephosphorylates a negative regulatory phosphorylation site of ZAP70 tyrosine kinase in T cells resulting in an increased activity of this kinase and enhanced signaling from T cell receptors suggesting an involvement of ACP_1 in immune disorders[11]. CLMWPTP is composed by two isoforms F and S that show different concentrations among genotypes: *B/*B genotype shows the highest concentration of F isoform.

Lyp is a protein tyrosine phosphatase encoded by the PTPN22 gene that is involved in the regulation of T cell receptor signaling. The gene shows a single nucleotide polymorphism C/T at +1858 resulting in the W620 variant that is associated with autoimmune disorders. The variant is a gain of function of the enzyme that more strongly inhibits T cell receptor mediated signals and it has been suggested that increased susceptibility to autoimmune disorders is due to failure to delete autoreactive T cells during intratymic selection [12]. The PTPN22 polymorphism has two alleles: *C1858 (encoding R620 variant) and *T1858 (encoding W620 variant), correspondingly there are three genotypes *C/*C, *C*/*T and *T/*T.*T is the minor allele.

ADA_2 is a locus on intron 2 of ADA gene (nt 19466-19470) and its polymorphism is detected by the PstI restriction enzyme. ADA_2 shows two alleles: ADA_2*1 with higher frequency and ADA_2*2 with lower frequency. Correspondingly there are three genotypes: ADA_2*1/*1, ADA_2*1/*2 and ADA_2*2/*2.

ADA_6 is another polymorphic locus within ADA gene (exon 6 nt 31230-31235). Its polymorphism is detected by the MluNI restriction enzyme and shows two alleles ADA_6*1 with lower frequency and ADA_6*2 with higher frequency. Correspondingly there are three genotypes: ADA_6*1/*1, ADA_6*1/*2 and ADA_6*2/*2. The genetic variability in these loci of ADA gene could influence enzymatic activity and/or functions of ADA as ectoenzyme.

MATERIALS AND METHODS

We have studied 106 subjects admitted to the hospital for colon cancer and 476 control subjects of comparable age and sex proportion without cancer. All subjects were from the White population of Rome and gave informed consent to participate to the study that was approved by the Council of Department. These subjects have been considered also in previous studies [7,8]. The population of Rome is a mixture of people from all regions of Italy.

Genetic polymorphisms were determined by DNA analysis as previously described[13,14]. Three way contingency tables were analyzed by a log linear model according to Sokal and Rohlf[15]. Chi-square of independence and Odds Ratio analysis were carried out by SPSS package[16]. Eta (η) is a measure of the strength of association: η^2 indicates the proportion of variance in the dependent variable that is explained by the independent variable.

In both cases and controls the observed proportions of ACP_1, ADA_6 and PTPN22 genotypes do not show appreciable difference with the expected proportion assuming Hardy-Weinberg equilibrium. Statistically significant differences ($p < 0.05$) are observed for ADA_2 genotypes: in controls the observed proportion of *1/*1 genotype is 58.2% *vs* 60.8% for H.W. expected proportion while in colon cancer the expected proportion of this genotype is 66.0% *vs* 60.0% for expected proportion assuming H.W. equilibrium.

RESULTS

Table 1 shows the joint genotype distribution of p53 codon 72 with ACP_1, PTPN22, ADA_2 and ADA_6. Subjects carrying the *Pro allele of p53 codon 72 and *B/*B genotype of ACP_1 show a frequency higher in colon cancer than in controls. Subjects carrying the *Pro allele and the *T allele of PTPN22 show a frequency higher in colon cancer than in control. For these two joint genotypes however the difference between cancer and controls does not reach the level of statistical significance. Subjects carrying the *Pro allele and the ADA_1*1/*1 genotype and subjects carrying the *Pro allele and the ADA_6*1 allele show a frequency higher in colon cancer than in controls and these differences are statistically significant. The difference with respect to controls is very marked for ADA_6 polymorphism.

In table 2 we have analyzed in more detail the interaction among ADA_6, p53 codon 72 and colon cancer. There is a highly significant interaction among the three variables suggesting that the association between p53 codon 72 and colon cancer is strongly influenced by the ADA_6 genotype.

Table 2 The interaction among ADA_6, p53 codon 72 and colon cancer.

	COLON CANCER		CONTROLS	
	*Arg/*Arg	*Pro carriers	*Arg/*Arg	*Pro carriers
ADA_6*2/*2	28	24	146	160
Carriers of ADA_6*1	15	29	81	58

Three way contingency table analysis by a log linear model
x = p53 codon 72; y = ADA_6; z = colon cancer vs controls

	G	df	p
xyz interaction	7.105	1	0.009
xy independence	8.079	2	0.020
xz independence	8.583	2	0.015
yz independence	14.620	2	0.001
Independence of z from x and y	15.844	3	0.002

Table 1 Proportion of the joint genotype of p53 codon 72 with ACP_1, PTPN22, ADA_2 and ADA_6 in colon cancer and in controls.

	COLON CANCER		CONTROLS		Significance of difference
	%	Total n°	%	Total n°	
ACP_1 : Joint genotype: Carriers of *Pro allele of p53 codon 72 /*B/*B genotype of ACP1	28.10%	103	21.20%	476	P = 0.135
PTPN22: Joint genotype: Carriers of *Pro allele of p53 codon 72 /Carriers of *T allele of PTPN22	7.50%	106	4.00%	473	P = 0.120
ADA_2: Joint genotype: Carriers of *Pro allele of p53 codon 72 /Carriers of *ADA_2*1/*1 genotype	38.90%	85	26.40%	439	P = 0.015
ADA_6: Joint genotype: Carriers of *Pro allele of p53 codon 72 /Carriers of *ADA_6*1 allele	30.20%	96	13.00%	445	P = 0.00006

Table 3 The effect of sex and grade on the relationship between the proportion of *Pro carriers and the number of factors considered (PTPN22, ACP_1, ADA_1 and ADA_6).

	Linear correlation	η
FOUR FACTORS		
All patients	P = 0.010	0.326
Males	P = 0.254	0.290
Females	P = 0.010	0.611
Grade ≤ 2	P = 0.255	0.241
Grade >2	P = 0.024	0.471
TWO FACTORS (ADA_2 and ADA_6)		
All patients	P = 0.020	0.240
Males	P = 0.479	0.117
Females	P = 0.011	0.494
Grade ≤ 2	P = 0.185	0.186
Grade > 2	P = 0.042	0.317

Figure 1 shows the proportion of *Pro allele carriers in colon cancer in relation to the number of genetic factors considered (PTPN22, ACP_1, ADA_2 and ADA_6). There is a positive correlation suggesting that the strength of association between colon cancer and p53 codon 72 depends on the number of factors considered.

In Figure 2 we have considered only the sites of ADA gene. The results are similar to those shows in Figure 1 but the strength of association is lower ($\eta = 0.23$ considering only ADA_2 and ADA_6 while $\eta = 0.32$ considering also ACP_1 and PTPN22). This suggests that ACP_1 and PTPN22 give a significant contribution to the relationship.

Table 3 shows the effect of sex and cancer grade on the relationship between the proportion of *Pro carriers and the number of factors considered. The relationship is much more marked in females than in males and in grade > 2 than in grade ≤ 2.

DISCUSSION

Common genetic polymorphisms influence the strength of correlation between p53 codon 72 and colon cancer suggesting a possible explanation for the contrasting result observed between different populations. The present results support the multi factorial origin of colon cancer. Since all polymorphisms studied are involved in immunological functions our data suggest an involvement of immune system in the pathogenesis of colon cancer. In stress conditions there is an increase of adenosine concentration in both intra and extra cellular compartments and this inhibits the immune response through ADA2R and ADA3R adenosine receptors[17,18]. Since adenosine deaminase contributes to the control of adenosine concentration, this could explain the stronger effect of the two ADA gene polymorphism as compared to ACP_1 and PTPN22.

Both ACP_1 and ADA, however, show also important effects on glucose metabolism; therefore, the possibility that these metabolic effects could contribute to the association cannot be excluded.

The strong differences between sexes supports the hypothesis of an immunological mechanism since it is known[19] that most immune disorders are more frequent in females than in males.

The difference observed between low grade and high cancer grade patients supports the hypothesis that the factors considered have a significant role in colon cancer and make unlikely that the associations observed are a mere chance sampling artifacts.

The limitation of the present study is represented by the relatively small number of patients examined.

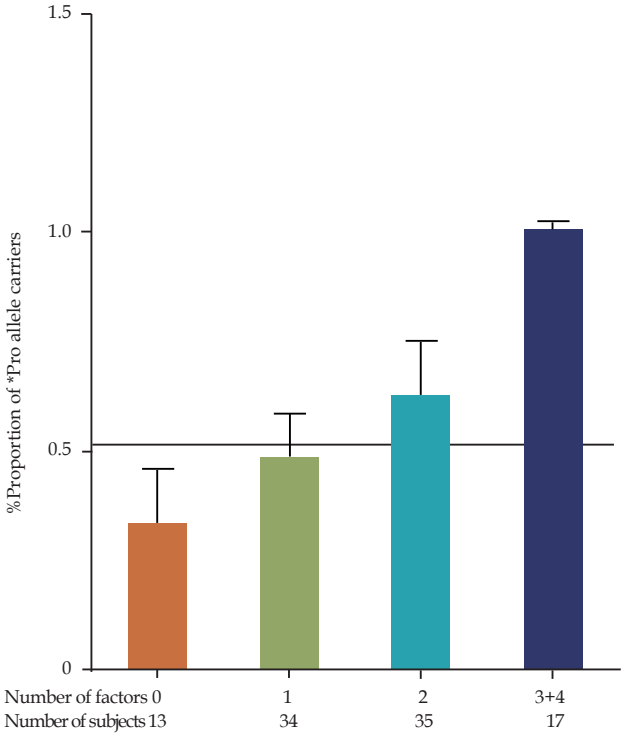

Figure 1 The relationship between the proportion of *Pro carriers in colon cancer and the number of factors associated positively with the disease (*B/*B genotype of ACP_1, carrier of *T allele of PTPN22, ADA_2*1/*1 genotype and carrier of ADA_6*1 allele). The horizontal line corresponds to the proportion of *Pro carriers in the general population. η= 0.321.
Number of factors 0 means that 13 individuals have none of the four factors.
Number of factors 1 means that 34 individuals have only 1 of the four factors.
Number of factors 2 means that 35 individuals have 2 of the four factors.
Number of factors 3+4 means that 17 individuals have 3 or 4 factors.

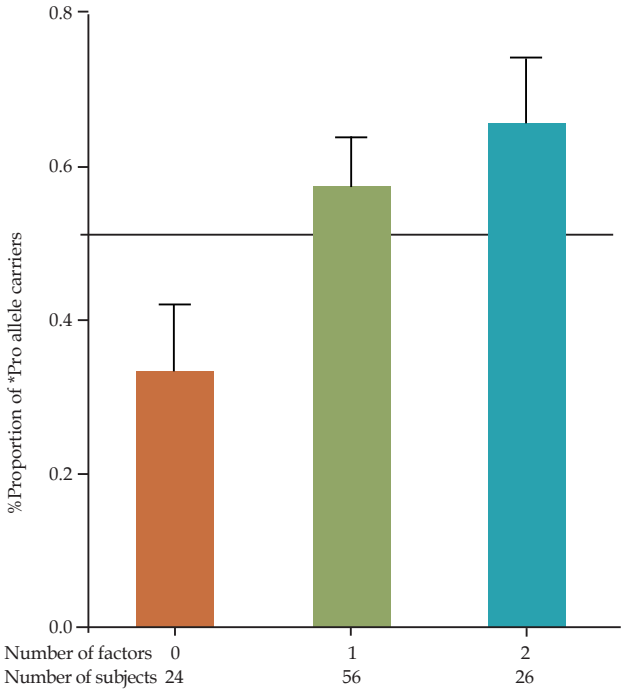

Figure 2 The relationship between the proportion of *Pro carriers in colon cancer and the number of factors considered (ADA_2*1/*1 genotype and carrier of ADA_6*1 allele).The horizontal line corresponds to the proportion of *Pro carriers in the general population. η = 0.232.

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Peer reviewer: Maaged Akiel; Guangwen Cao.