

The (-590 C/T) Polymorphism in the Interleukin-4 Gene is Associated with Increased Risk for Early Stages of Colorectal Adenocarcinoma

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Abstract. *Background:* In the light of the known association between several carcinomas and the -590C/T polymorphism, which affects transcription of the antitumor interleukin-4 (IL-4) gene, the purpose of this study was to investigate the possible contribution of this polymorphism to the development of colorectal cancer. *Materials and Methods:* The -590C/T polymorphism was examined in DNA samples of 93 patients with colorectal cancer (adenocarcinomas) and 108 healthy controls of comparable ethnicity, age and gender. *Results:* The detected allele and carrier frequencies for the high expression T allele in the patient group were significantly decreased in comparison with that of the control group (13.44% versus 22.22%, and 21.51% versus 36.11%, respectively, $p < 0.01$). The same pattern was observed between controls and patients in initial cancer stages. *Conclusion:* These findings indicate that IL-4 gene expression-related polymorphism is associated with the development of initial stages of colorectal cancer, while in advanced stages IL-4 levels appear to be less important.

Colorectal carcinomas accounted for 1 million new cases in 2002 (9.4% of the total cancer cases worldwide) and are the third most prevalent cancer type in women and fourth in men (1). Several genetic factors such as mutant oncogenes or tumor suppressor genes have already been

shown to play an important role in carcinogenesis, in addition to other factors such as poor hygiene, diet, drug abuse and smoking (2-4). Many functional gene polymorphisms such as common DNA polymorphisms in low penetrance genes have been implicated in the individual susceptibility to many different types of cancer (5-25).

A factor known to play an important role in carcinogenesis is interleukin-4 (IL-4), a cytokine produced by memory T cells that induces antibody production by B cells (26-29). IL-4 has an inhibitory role in inflammation and angiogenesis, as well as in tumor growth in some carcinomas such as oral, breast, gastric, renal, colon, and in multiple myelomas (27, 28, 30-38).

A functional C to T single nucleotide polymorphism located in the promoter region of the IL-4 gene (at position -590) is known to affect transcription (39). The "high expression" T allele confers increased gene transcription and elevated total serum levels of IL-4 (39). The T allele frequency ranges between 15 and 25% in Europeans (40). This allele has been associated with some types of cancer (41-43). In this study, the possible association of the IL-4 -590 C/T gene polymorphism with risk for colorectal cancer in Greek patients in comparison with healthy control subjects was investigated.

Materials and Methods

The study included 93 Greek patients who had been surgically treated for colorectal cancer (adenocarcinomas) within the last 5 years and 108 age and gender matched healthy controls of Greek origin. DNA was extracted from patient biopsies and from blood samples of the controls. Each biopsy was characterized pathologically, as well as in regard to cancer stage, according to Dukes' stage.

Molecular detection of the (-590 C/T) polymorphism in the IL-4 gene was performed by restriction fragment length

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Key Words: Colorectal cancer, adenocarcinomas, interleukin-4, polymorphism, inflammation, angiogenesis, tumor growth, metastasis.

Table I. Prevalence of IL-4 (-590 C/T) polymorphism in healthy controls as well as patients with oral cancer and their subgroups in regard to cancer stage (initial stages A & B and advanced C & D according to Dukes' stage).

Genotype	Controls		Patients		Patients with cancer stages A & B			Patients with cancer stages C & D		
	(%)	(%)	<i>p</i>	OR (CI)	(%)	<i>p</i>	OR (CI)	(%)	<i>p</i>	OR (CI)
TT	9 (8.33%)	5 (5.38%)	<i>p</i> =0.4017	0.96 (0.20-4.65)	2 (4.35%)	<i>p</i> =0.3251	1.27 (0.20-7.96)	3 (6.38%)	<i>p</i> =0.7499	1.51 (0.23-10.08)
CC	69 (63.89%)	73 (78.49%)		1 (referent)	40 (86.96%)		1 (referent)	33 (70.21%)		1 (referent)
C/T	30 (27.78%)	15 (16.13%)	<i>p</i>=0.0402	0.62 (0.27-1.43)	4 (8.7%)	<i>p</i>=0.0056	0.46 (0.15-1.48)	11 (23.40%)	<i>p</i> =0.5547	0.88 (0.33-2.33)
Total	108 (100%)	93 (100%)			46 (100%)			47 (100%)		
Prevalence of T allele										
T allele frequency	22.22%	13.44%	<i>p</i>=0.0272	0.71 (0.36-1.40)	8.70%	<i>p</i>=0.0056	0.66 (0.26-1.71)	18.09%	<i>p</i> =0.4510	0.95 (0.42-2.14)
Carrier frequency of T allele	36.11%	21.51%	<i>p</i>=0.0295	0.64 (0.29-1.40)	13.04%	<i>p</i>=0.0037	0.51 (0.18-1.49)	29.79%	<i>p</i> =0.4684	0.91 (0.36-2.29)

Fischer's *p*-value corresponds to genotype comparisons and allele frequency comparisons; significant *p*-value is given in bold; odds ratios (OR) are age-adjusted; CI: 95% confidence interval.

polymorphism typing and gel electrophoretic analysis, as previously described (44). Statistical analysis was performed by Fisher's Exact test and age-adjusted odds ratios with a 95% confidence interval (CI). A *p*-value of <0.05 was considered statistically significant.

Results

Table I shows the detected IL-4 (-590 C/T) genotypes in healthy controls and cases with colorectal cancer. The genotype distributions were in Hardy-Weinberg equilibrium in the control group.

Significantly lower carrier and allele frequencies of the "high expression" T allele were observed in the whole group of patients (21.51%; *p*=0.0295, and 13.44%; *p*=0.0272, respectively) and the subgroup of patients with initial stages of cancer (Dukes's stage A and B)

(13.04%, *p*=0.0037, and 8.70%; *p*=0.0056, respectively) in comparison to controls (Table I).

The homozygotes TT for the "high expression" T allele in the patient group were not significantly different compared to the controls (Table I). However, the frequency of the CT genotype in the patient group was lower in comparison to that of the controls (16.13% and 27.78%, respectively *p*=0.0402). Furthermore, a statistically significant decrease in the CT genotypes of the IL-4 -590 C/T polymorphism was also observed in the subgroup of patients with early cancer stages (*p*=0.0056) (Table I).

Discussion

Interleukin-4 is a multifunctional cytokine with an ambiguous role in cancer. Although it exerts an inhibitory effect on breast, gastric, renal and colon

carcinoma cell lines, it stimulates head and neck squamous cell carcinoma, prostate cancer and different types of lymphoma (27, 28, 30-38). The present study revealed that the -590 C/T polymorphism, affecting IL-4 gene expression, is associated with the development of colorectal cancer.

The observed "high expression" T allele and carrier frequencies were significantly lower in the patient group, and in the subgroup of patients with early stages of cancer, in comparison to controls. These findings indicate that the T allele may play a protective role against colorectal tumor growth, since increased IL-4 levels have a strong inhibitory effect on cancer cells by induction of apoptosis (27). In advanced cancer stages the anti-tumor role of IL-4 seems to be less important as this association study implies, in accordance to a previous one of the same polymorphism in oral cancer (43).

Interestingly, only the CT heterozygote genotypes were found to be significantly fewer in patients than controls, while the same trend was also observed for TT homozygote genotypes but it did not reach a statistically significant level. This fact might be due to the sharp decrease of T alleles in the group of studied patients, whose number was rather modest.

Since several cytokines and other factors have been previously associated with colorectal cancer (17, 18, 20, 22, 24), further investigations should be performed for elucidation of the underlying pathogenic mechanisms and determination of protective measures for specific individuals.

Acknowledgements

The authors would like to thank Zoe Serefoglou for helpful discussion about the methodology of this study.

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Received July 12, 2007

Revised August 29, 2007

Accepted October 1, 2007