Regulatory Cytokine Gene Polymorphisms and Risk of Colorectal Carcinoma

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ABSTRACT: It is well established that cancer arises in chronically inflamed tissue, and this is particularly notable in the gastrointestinal tract. Classic examples include *Helicobacter pylori*–associated gastric cancer, hepatocellular carcinoma, and inflammatory bowel disease–associated colorectal cancer. Growing evidence suggests that these associations might be not casual findings. Focusing on individual cytokines has generated evidence that anti-inflammatory cytokine interleukin (IL)-10 and transforming growth factor-beta1 (TGF-β1) may have a complex role in gastrointestinal carcinogenesis. As an example, IL-10-deficient mice develop severe atrophic gastritis and a chronic enterocolitis, developing colorectal cancer similar to human inflammatory bowel disease–associated neoplasia. TGF-β1 is a multifunctional signaling molecule with a wide array of roles. Animal experiments suggest that TGF-β1 plays a biphasic role in carcinogenesis by protecting against the early formation of benign epithelial growths, but promoting a significant stimulation of tumor growth invasion and metastasis during tumor progression. We assessed association of functional polymorphisms (–1082G/A;

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–592C/A) and TGF-β1 (–509C/T; +869C/T) influencing the IL-10 production to colorectal cancer risk in a case–control study of 62 patients and 124 matched controls. No significant differences were observed among cancer patients and controls for IL-10 –1082G/A; –592C/A genotype frequencies. Evaluation of odds ratios (OR) for the TGF-β1 +869C/T genotypes showed a significant increased risk for individuals bearing +869CC genotype compared to +869CT- and +869TT-positive individuals. These results suggest that the +869C allele, responsible for a Leu→Pro substitution in the signal peptide sequence of the TGF-β1 protein, may have a predisposing role in the development of colorectal cancer.

KEYWORDS: **colorectal cancer; gene polymorphisms; TGF-β1; IL-10**

INTRODUCTION

Immune response has a significant impact on the potential for malignancy. In particular, it is well established that cancer may arise in chronically inflamed tissue, and this is particularly notable in the gastrointestinal tract.[1–3] The importance of type I inflammatory response, in particular, is also demonstrated by experiments that show that B cell–deficient *Helicobacter*-infected mice are not protected from severe atrophy and metaplasia.[2] In colorectal adenomas and carcinomas, there is a predominance of CD4- and CD3-positive cells.[3] Thus CD4 T lymphocytes and their cytokine products are extremely important in the malignant transformation of chronically inflamed tissue.[4–6] Cytokines play a central role in the regulation of inflammatory response. Focusing on individual cytokines has generated evidence that anti-inflammatory cytokines, such as interleukin (IL)-10 and transforming growth factor-beta1 (TGF-β1), may have a complex role in gastrointestinal carcinogenesis.[5] As an example, IL-10-deficient mice develop severe atrophic gastritis and a chronic enterocolitis, developing colorectal cancer similar to human inflammatory bowel disease–associated neoplasia.[6,7] On the other hand, IL-10 is an immunosuppressive cytokine that may facilitate the development of cancer by supporting tumor escape from the immune response. TGF-β1 is a multifunctional signaling molecule with a wide array of roles.[8,9] Experimental models suggest that TGF-β1 plays a biphasic role in carcinogenesis by not only protecting against the early formation of benign epithelial growths, but also by promoting malignant transformation invasion and metastasis during tumor progression.[10,11]

We typed 62 colorectal cancer cases and 124 age- and sex-matched controls for four main functional polymorphisms of the IL-10 (–1082G/A; –592C/A) and TGF-β1 (–509C/T; +869C/T) genes, influencing level of cytokine production, to assess the association of these SNPs with the susceptibility to colorectal cancer.

MATERIALS AND METHODS

Blood samples from 62 patients were collected at the Department of Oncology and Surgical Disciplines, University of Palermo, where colon cancer diagnosis and grading were clinically assessed. The control group consisted of 124 unrelated age- and sex-matched healthy subjects, recruited in the same geographic area. Written informed consent was obtained from all the subjects according to Italian laws. Genomic DNA was isolated from peripheral blood leukocytes by a standard method using proteinase K digestion followed by standard salting-out technique.[12]

TGF-β1 –509C/T and +869C/T SNPs[13] were investigated by means of the amplification refractory mutation system (ARMS)-PCR technique, using two allele-specific PCR reactions for each SNP per DNA sample.[14] IL-10 –1082G/A and –592C/A SNPs were genotyped as previously described.[14]

Allele and genotype frequencies were analyzed for differences in distribution between patients and healthy controls by means of chi-square exact test with Yates correction. Odds ratio with the 95% confidence interval was obtained using Woolf's approximation method. The obtained *P*-values were multiplied for the number of possible genotypes of each SNP typed. *P*-corrected (*P*c) < 0.05 was considered the significance limit.

RESULTS AND DISCUSSION

The distribution of genotypes for each polymorphism typed was in agreement with the expected values fitting in the Hardy–Weinberg equation. No significant differences were observed among cancer patients and controls for IL-10 –1082G/A, and –592C/A genotype frequencies (TABLE 1). These data are in agreement with results reported by Macarthur *et al*.[15] suggesting that investigated IL-10 polymorphisms do not play an important role in susceptibility to colorectal carcinoma. The two SNPs at –1082G→A and –592C→A taken into account are in strong linkage. Thus, the possible haplotypes are: –1082G/–592C, –1082A/–592C, and –1082A/–592A.[16] These haplotypes are associated with differential IL-10 production, as demonstrated by reporter gene assays, although, in some cases, differing results have been described in cells undergoing distinct stimuli.[17] These polymorphisms have been reported in association with development of inflammatory diseases, such as inflammatory bowel diseases,[18] suggesting a role as supporter of inflammation involved in development of colorectal cancer. Thus, larger studies to confirm present data are required.

Similar results were obtained evaluating –509C/T SNP (TABLE 1), which induced a YY1 consensus sequence in a region of the promoter associated with negative transcription regulation,[19] when we analyzed the differences of genotype distribution between cases and healthy controls. On the contrary, the evaluation of +869C/T SNP inducing Leu10pro substitution in TGF-β1 signal

TABLE 1. Percentage of homozygous and heterozygous subjects for the –1082G/A and –592C/A IL-10 and –509C/T TGF-β1 SNPs in 62 patients affected by colorectal carcinoma and in 124 healthy controls

	Genotypes	Patients	Controls	OR (CI 95%)	*P*c*
IL-10 1082G/A	–1082GG	12 (19.4)	26 (21.0)	0.90 (0.42–1.94)	ns
	–1082GA	34 (54.8)	60 (48.4)	1.29 (0.70–2.39)	ns
	–1082AA	16 (25.8)	38 (30.6)	0.79 (0.40–1.56)	ns
IL-10 −592C/A	–592CC	31 (50.0)	69 (55.6)	0.80 (0.43–1.47)	ns
	–592CA	28 (45.2)	48 (38.7)	1.30 (0.70–2.42)	ns
	–592AA	3 (4.8)	7 (5.7)	0.85 (0.21–3.41)	ns
TGF-β1 −509C/T	–509CC	19 (30.6)	44 (35.5)	0.80 (0.42–1.54)	ns
	–509CT	29 (46.8)	58 (46.8)	1.00 (0.54–1.84)	ns
	–509TT	14 (22.6)	22 (17.7)	1.35 (0.64–2.87)	ns
TGF-β1 +869C/T	+869CC	35 (56.4)	41 (33.1)	2.62 (1.40–4.91)	0.011
	+869CT	23 (37.1)	61 (49.2)	0.61 (0.33–1.14)	ns
	+869TT	4 (6.5)	22 (17.7)	0.31 (0.11–0.97)	ns

*P-corrected (Pc) value. the obtained P-value was multiplied for the number of the possible genotypes for each SNP.

TGF-β1 +869C/T genotype distribution was found significantly increased in colorectal cancer patients group compared to the control group 3×2 $P\text{c} = 0.012$.

peptide allowed us to identify a significant increased risk for colon–rectal carcinoma associated with +869CC genotype (TABLE 1). It has been reported that the amount of TGF-β in serum is higher for Pro10 homozygotes than Leu10 homozygotes.[19] So the effect of the Leu10Pro polymorphism on the amount of TGF-β1 secreted *in vivo* might suggest that genetically determined low TGF-β production might be one of the factors involved in the susceptibility for colon rectal cancer. On the other hand, data here presented should be considered preliminary data as the exiguous number of patients studied does not allow haplotype reconstruction for both IL-10 and TGF-β1 SNPs. Haplotypes are actually considered more powerful to detect susceptibility alleles than individual polymorphisms.[20] However, our data suggest that in spite of the well-defined powerful immunoregulatory IL-10 role, TGF-β1 might play a costarring role in the prevention of carcinoma in the colonic–rectal microenvironment.

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