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Impact of Interleukin-10 Gene Polymorphisms on Survival in Patients with Colorectal Cancer

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Chronic inflammation is thought to be the leading cause of colorectal cancer, and interleukin-10 (IL10) has been identified as a potent immunomodulatory cytokine that regulates inflammatory responses in the gastrointestinal tract. Although several single nucleotide polymorphisms (SNPs) in *IL10* have been associated with the risk of colorectal cancer, their prognostic significance has not been determined. Two hundred and eighty-two colorectal cancer patients were genotyped for two candidate cancer-associated SNPs in *IL10*. The associations of these SNPs with distant metastasis-free survival and overall survival were evaluated by Kaplan-Meier analysis and Cox regression model. The minor homozygote GG genotype of *IL10* rs3021094 was significantly associated with a 3.30-fold higher risk of death compared with the TT+TG genotypes ($P = 0.011$). The patients with *IL10* rs3021094 GG genotype also had a poorer overall survival in Kaplan-Meier analysis (log-rank $P = 0.007$) and in multivariate Cox regression model ($P = 0.044$) adjusting for age, gender, carcinoembryonic antigen levels, tumor differentiation, stage, lymphovascular invasion, and perineural invasion. In conclusion, our results suggest that *IL10* rs3021094 might be a valuable prognostic biomarker for colorectal cancer patients.

Key Words: Colorectal Neoplasms; Inflammation; Interleukin-10; Polymorphism; Survival

INTRODUCTION

Chronic inflammation is a well-established risk factor for colorectal cancer. However, the mechanisms involved in this process are still poorly understood. The current opinion is that there is an overreaction of the immune system toward antigens of the gut microbiota leading to chronic inflammation (1). Chronic accumulation of activated immune cells is accompanied by the release of proinflammatory cytokines. These cytokines can lead to the generation of oxygen and nitrogen reactive species, which are known to induce dysplasia by inducing DNA modifications in intestinal epithelial cells (2). These processes have been associated with altered expression of genes contributed to tumor growth such as *p53, APC,* and *K-ras* (3). However, activation of the immune system has also been shown to cause dysplastic cell elimination and preventing tumor progression under certain conditions (4).

The action of cytokines can be crudely described as pro-inflammatory (e.g., interleukin [IL]-6 and tumor necrosis factor-α) or anti-inflammatory (e.g., IL10 and transforming growth factor-β). The main biological function of IL10 is to limit inflammatory responses and is crucial for maintaining the immune homeostasis of the gastrointestinal tract (5). Animal studies showed that IL10-deficient mice spontaneously developed colorectal cancer when infected with enteric bacteria. Administration of exogenous IL10 could prevent colorectal cancer development in this model, indicating that IL10 is pivotal in the control of inflammation and inflammation-related colorectal cancer (6). *IL10* gene is polymorphic and common single nucleotide polymorphisms (SNPs) of *IL10* have been associated

with colorectal cancer risk (7-9). Here, we investigated the prognostic significance of two candidate haplotype tagging SNPs (tSNPs) in *IL10* on distant metastasis-free and overall survival in a cohort of 282 colorectal cancer patients.

MATERIALS AND METHODS

Patient recruitment and data collection

This cohort was generated from the tumor tissue bank at China Medical University Hospital, Taiwan. All patients seen at China Medical University Hospital, Taiwan with a diagnosis of cancers were approached to participate. Two hundred and eighty-two patients with histopathologically confirmed colorectal cancer were identified between 2001 and 2007; had consented to provide information and tissue; and had undergone blood collection for research purposes. The clinical data and outcomes were obtained from patients' clinical records and pathological reports. Among patients receiving curative surgery (stage I-III, n = 233), distant metastasis-free survival was defined as the time from surgery to the date of distant metastases or when censored at the latest date. Overall survival was defined as the time from diagnosis (n = 282) to the date of death from any cause or when censored at the latest date if patients were still alive. The survival data were updated most recently in 2008.

SNP selection and genotyping

Two candidate tSNPs in *IL10* were selected based on the evidence of association with risk of colorectal cancer (9). The tSNP rs3024496 within the 3´ untranslated region of *IL10* captures 6 SNPs, rs1800890, rs2222202, rs3024491, rs3024493, rs3024495, and rs3024505, located across promoter, intron 1, intron 3, intron 4, and 3´ downstream in linkage disequilibrium according to HapMap CHB (Han Chinese in Beijing, China) population data (10). The tSNP rs3021094 locates within the first intron of the *IL10*.

Genomic DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) and stored at -80°C until the time of study. Genotyping was performed as described previously (11-16) using Sequenom iPLEX matrix-assisted laser desorption/ionization-time of flight mass spectrometry technology at the National Center for Genome Medicine, Academia Sinica, Taiwan. The average genotype call rate for these SNPs was 99.6% and the frequencies of the SNPs were in Hardy-Weinberg equilibrium ($P > 0.01$).

Statistical analysis

Patient clinicopathologic characteristics were summarized as number and percentage of patients or median and interquartile range (IQR) of values. Age was dichotomized at the median value within the cohort. Carcinoembryonic antigen (CEA) level was dichotomized at 5 µg/L because of its correlation with an increasing stage of the colorectal cancer (17). The associations of *IL10* tSNPs and clinical characteristics with distant metastasis-free and overall survival were assessed using the Cox proportional hazards regression model and Kaplan-Meier analysis with log-rank test. We tested different genetic models, dominant, recessive, and additive models for each tSNP. The model with the most significant P value was considered the best-fitting model. Multivariate analyses to determine the interdependency of genotypes and other known prognostic factors, such as age at diagnosis, gender, CEA levels, tumor differentiation, stage, lymphovascular invasion, perineural invasion, and lymph node involvement, were carried out using Cox proportional hazards regression model. Statistical Package for the Social Sciences software version 19.0.0 (IBM, Armonk, NY) was used for other statistical analyses. A two-sided P value of < 0.05 was considered statistically significant.

Ethic statement

This study was approved by the institutional review board of the China Medical University Hospital (DMR98-IRB-210). Written informed consent was obtained from all patients.

RESULTS

The clinicopathologic characteristics of 282 colorectal cancer patients are summarized in Table 1. Twenty-five (10.7%) patients developed distant metastasis after curative surgery with a median follow-up of 24.8 months. Eighteen (6.4%) patients died after a median follow-up of 28.2 months. Younger age, higher stage, positive lymphovascular invasion, and lymph node involvement had a significantly higher risk of developing distant metastasis ($P \le 0.033$). Gender, CEA levels, stage, and lymph node involvement were significantly associated with overall survival ($P \le 0.046$).

We assessed the association of *IL10* tSNPs with distant metastasis-free and overall survival using a univariate Cox model (Table 2). We identified that the *IL10* rs3021094 GG genotype had a 3.30-fold higher risk of death compared with the TT+TG genotypes ($P = 0.011$). Kaplan-Meier survival curves and log-rank test also showed that the *IL10* rs3021094 GG genotype was significantly associated with poorer overall survival compared with the TT+TG genotypes ($P = 0.007$; Fig. 1).

To determine the interdependency of *IL10* rs3021094 with overall survival, a multivariate Cox proportional hazards model, including age, gender, CEA levels, tumor differentiation, stage, lymphovascular invasion, perineural invasion, lymph node involvement, and *IL10* rs3021094, was performed (Table 3). None of the factors reached significance when all clinical factors were included in the multivariate analysis ($P > 0.05$; Model 1). However, *IL10* rs3021094 remained as a significant predictor for overall survival without adjusting for lymph node involvement

Table 1. Demographic and clinical characteristics of colorectal cancer patients

Characteristics	Distant metastasis-free survival				Overall survival			
	No. (%)*	No. (%) of events*	HR (95% CI)	P	No. (%)*	No. (%) of events*	HR (95% CI)	P
All patients	233	25			282	18		
Age (yr)								
Median (IQR)	66 (56-73)				65 (54-73)			
< 65	106 (45.5)	17 (68.0)	1.00		133 (47.2)	10 (55.6)	1.00	
≥ 65	127 (54.5)	8 (32.0)	0.39 (0.17-0.90)	0.027	149 (52.8)	8 (44.4)	0.71 (0.28-1.81)	0.476
Gender								
Male	119 (51.1)	13 (52.0)	1.00		148 (52.5)	14 (77.8)	1.00	
Female	114 (48.9)	12 (48.0)	1.00 (0.46-2.20)	0.994	134 (47.5)	4 (22.2)	0.31 (0.10-0.95)	0.040
CEA (µg/L)								
Median (IQR)	3.5 (1.7-10.2)				4.4 (2.0-14.9)			
< 5	129 (59.7)	14 (60.9)	1.00		136 (52.3)	4 (23.5)	1.00	
≥ 5	87 (40.3)	9 (39.1)	0.93 (0.40-2.16)	0.870	124 (47.7)	13 (76.5)	3.72 (1.21-11.4)	0.022
Differentiation								
Well	43 (20.0)	4 (17.4)	1.00		51 (19.4)	2 (11.1)	1.00	
Moderate	153 (71.2)	13 (56.5)	0.87 (0.28-2.66)	0.800	185 (70.3)	14 (77.8)	2.01 (0.46-8.83)	0.357
Poor	19 (8.8)	6 (26.1)	3.30 (0.92-11.9)	0.067	27 (10.3)	2 (11.1)	1.93 (0.27-13.7)	0.511
Stage†								
I-II	150 (64.7)	10 (40.0)	1.00		-			
III	82 (35.3)	15 (60.0)	3.04 (1.36-6.78)	0.007	-			
I-III	-				232 (82.6)	11 (61.1)	1.00	
IV	-				49 (17.4)	7 (38.9)	3.16 (1.22-8.14)	0.018
Lymphovascular invasion								
Negative	179 (76.8)	13 (52.0)	1.00		201 (71.3)	10 (55.6)	1.00	
Positive	54 (23.2)	12 (48.0)	3.18 (1.45-6.98)	0.004	81 (28.7)	8 (44.4)	2.06 (0.81-5.23)	0.127
Perineural invasion								
Negative	202 (86.7)	20 (80.0)	1.00		234 (83.0)	15 (83.3)	1.00	
Positive	31 (13.3)	5 (20.0)	1.64 (0.61-4.37)	0.324	48 (17.0)	3 (16.7)	0.97 (0.28-3.36)	0.966
Lymph node involvement								
Negative	143 (64.1)	9 (42.9)	1.00		154 (56.8)	5 (31.3)	1.00	
Positive	80 (35.9)	12 (57.1)	2.57 (1.08-6.12)	0.033	117 (43.2)	11 (68.8)	2.94 (1.02-8.45)	0.046

*Column subtotals do not sum to No. of patients and No. of events due to missing data; †According to the American Joint Committee on Cancer - Cancer Staging Manual (version 6.0). P < 0.05 are in boldface. CEA, carcinoembryonic antigen; IQR, interquartile range; HR, Hazard ratio; CI, confidence interval.

Table 2. Association of *IL10* polymorphisms with distant metastasis-free survival and overall survival

SNP ID	Position	Location	Allele	Genotype distribution*	Best-fitting genetic models					
					Distant metastasis-free survival			Overall survival		
					Model	HR (95% CI)	P	Model	HR (95% CI)	P
rs3024496	Chr1:206941864	3′ UTR	A > G	259/22/0	Dominant	0.43 (0.06-3.18)	0.408	Dominant	0.04 (0.00-69.4)	0.405
rs3021094	Chr1:206944952	Intron 1	T > G	72/139/70	Recessive	1.78 (0.78-4.04)	0.168	Recessive	3.30 (1.31-8.31)	0.011

*The number represents major allele homozygotes, heterozygotes, and minor allele homozygotes, respectively. P < 0.05 are in boldface. SNP, single nucleotide polymorphism; HR, Hazard ratio; CI, confidence interval; UTR, untranslated region.

(P = 0.044; Model 2) because of a weak association between *IL10* rs3021094 and lymph node involvement (P = 0.107; data not shown). We did not observe any other noteworthy association between *IL10* tSNPs and other clinical characteristics listed in Table 1.

DISCUSSION

This translational study discovered that the *IL10* tSNP, rs3021094, might serve as a potential prognostic determinant for colorectal cancer. The result remained significant after adjusting for most of the major clinicopathologic characteristics. To our knowl-

edge, this study might provide the first indication that a tSNP of *IL10*, a key cytokine involved in immune homeostasis, is significantly associated with the clinical outcomes in colorectal cancer patients.

IL10 has a pivotal role in the immunity of the gastrointestinal tract, and IL10-deficient mice have been shown to develop colitis and colorectal cancer (6). A recent genome-wide association study implicated a 3′ downstream *IL10* SNP, rs3024505 (captured by rs3024496 in this study), to the susceptibility of ulcerative colitis, a subtype of inflammatory bowel disease (18). However, we did not observed significant association between *IL10* rs3024496 and clinical outcomes in colorectal cancer, sug-
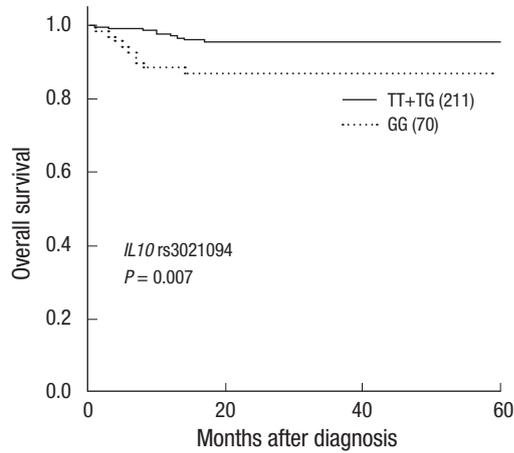
Fig. 1. Kaplan-Meier curves of overall survival by the genotypes at *IL10* rs3021094. Numbers in parentheses indicate the number of patients. Subtotal does not sum to 282 patients due to missing data.

gesting that IL10 might play different roles during colorectal cancer development and progression. Another candidate tSNP, rs3021094, is located in the first intron of the *IL10* gene, and is predicted to alter a putative transcription factor binding site for SP3 transcription factor, according to the SNPInfo analysis (19). It has been demonstrated that IL10 expression is regulated by the constitutively and ubiquitously expressed SP1 and SP3 as key transcription factors (20). Therefore, it is possible that *IL10* rs3021094 might influence IL10 expression by altering the consensus transcription factor binding site for SP3. Further fine-mapping and functional analyses are required to provide molecular biology evidence of how *IL10* rs3021094 translates into physiologic processes and affects colorectal cancer progression.

In conclusion, we observed a statistically significant association between a SNP, rs3021094, in the anti-inflammatory cytokine *IL10* and survival in colorectal cancer patients. We are cautious in our interpretation of these data, because the study is limited by its small size, short follow-up period, and homogeneous Chinese Han population. However, we view this finding as exploratory and given the emerging evidence on chronic inflammation in colorectal carcinogenesis. Further independent external studies are warranted and might lead to new intervention or prevention strategies for colorectal cancer.

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DISCLOSURE

Table 3. Multivariate analysis of factors associated with overall survival

Variables	Model 1		Model 2	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age (yr)				
< 65	1.00		1.00	
≥ 65	0.87 (0.30-2.54)	0.795	0.66 (0.24-1.82)	0.426
Gender				
Male	1.00		1.00	
Female	0.34 (0.10-1.12)	0.076	0.29 (0.09-0.95)	**0.041**
CEA (μg/L)				
< 5	1.00		1.00	
≥ 5	2.04 (0.57-7.29)	0.274	2.97 (0.88-10.1)	0.081
Differentiation				
Well	1.00		1.00	
Moderate	3.52 (0.45-27.7)	0.233	1.94 (0.43-8.81)	0.388
Poor	3.51 (0.31-40.3)	0.314	2.14 (0.28-16.1)	0.460
Stage				
I-III	1.00		1.00	
IV	1.64 (0.50-5.41)	0.418	1.89 (0.61-5.85)	0.269
Lymphovascular invasion				
Negative	1.00		1.00	
Positive	2.16 (0.60-7.82)	0.242	1.94 (0.59-6.37)	0.277
Perineural invasion				
Negative	1.00		1.00	
Positive	0.40 (0.09-1.72)	0.217	0.35 (0.08-1.50)	0.159
Lymph node involvement				
Negative	1.00			
Positive	1.95 (0.57-6.67)	0.288		
IL10 rs3021094				
TT+TG	1.00		1.00	
GG	2.03 (0.71-5.83)	0.187	2.72 (1.03-7.21)	**0.044**

P < 0.05 are in boldface. CEA, carcinoembryonic antigen; HR, Hazard ratio; CI, confidence interval; Multivariate models included age, gender, CEA levels, tumor differentiation, stage, lymphovascular invasion, perineural invasion, *IL10* rs3021094, or lymph node involvement.

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