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ORIGINAL RESEARCH

Genetic Variants of the *MIF* Gene and Susceptibility of Rectal Cancer

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Background: Rectal cancer (RC) has been documented to be a highly invasive malignant neoplasm worldwide. Macrophage migration inhibitory factor (MIF) is a multifunctional cytokine involved in cell-mediated immunity, immunoregulation, inflammation. In vitro and in vivo studies have identified that MIF was involved in the carcinogenesis and progression of RC.

Patients and Methods: This case–control study evaluated associations of genetic variants of the MIF gene and serum level of MIF with susceptibility of RC.

Results: We found MIF level was associated with an increased risk of RC (OR for per unit: 1.38, 95% CI:1.32–1.44; P < 0.001). Both MIF rs2012133 (OR = 1.30; 95% CIs = 1.08–1.58; P = 0.007) and rs755622 (OR = 1.45; 95% CIs = 1.15–1.82; P = 0.002) were significantly associated with increased risk of RC. Besides, we also found MIF rs5844572 was significantly associated with increased susceptibility of RC, with OR for per CATT repeat of 1.28 (95% CIs: 1.16–1.41; P < 0.001). Further, we found all three variants of the MIF gene, rs5844572, rs2012133 and rs755622, could increase serum level of MIF.

Conclusion: This study suggests that MIF plays an important role in the carcinogenesis of RC and could be used as a biomarker for early detection and prediction of RC.

Keywords: rectal cancer, genetic, MIF, susceptibility, case–control

Introduction

Rectal cancer (RC), a highly invasive malignant neoplasm derived from rectal tissue, ranks as one of the leading causes of death worldwide.[1] According to the Cancer Statistics, 2020, it was estimated that 43,340 new RC cases would occur in United States in 2020.[2] Although diet, environmental exposures, and lifestyle factors were considered as the risk factors for RC carcinogenesis.[3] However, genetic factors of RC still need to be explored, as there is a critical need to identify additional screening biomarkers for early diagnosis of RC.

Cumulating evidence has indicated that genetic variants of inflammatory cytokines could modulate the susceptibility of individuals to cancers.[4–11] Macrophage migration inhibitory factor (MIF), also known as glycosylation-inhibiting factor (GIF), encodes a lymphokine involved in cell-mediated immunity, immunoregulation, and inflammation.[12–14] It was implicated in the pathogenesis of many cancers, sepsis, and inflammatory and autoimmune diseases.[12,15] Two focused variants of MIF, rs755622 (−173G/C), and rs5844572 (−794 CATT 5–8 microsatellite repeat) have been identified to be associated with multiple cancers, acute lymphoblastic leukemia, systemic lupus erythematosus, tuberculosis and so on.[16–28] However,

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none have systematically evaluated the roles of genetic variants of MIF in the carcinogenesis of RC. Here, we hypothesized that six tagSNPs of MIF (rs5760090, rs2012133, rs755622, rs12628766, rs5760088, and rs3063367), together with the microsatellite repeat variant rs5844572, would be associated with serum level of MIF, further the susceptibility of RC. Hereby, we conducted this case–control study in a Chinese population to address this concern.

Patients and Methods

Study Subjects

The totally study population included 420 pathological confirmed RC patients (all the cases are adenocarcinoma, including 129 women and 291 men), as well as 490 frequency-matched healthy controls by age and gender who visited the hospital for routine healthy examination during the same period (142 women and 348 men). Participants with acute infection or recent antibiotic treatment were excluded. All the participants were asked to participate in the project voluntarily and to complete a questionnaire, in addition to providing 5 mL of their peripheral blood samples for DNA extraction and assays of serum level of MIF. The study was approved by the institutional review board of Liaoning Cancer Hospital (00123). The research was conducted in accordance with the World Medical Association Declaration of Helsinki, and all the participants provided written informed consent.

DNA Extraction and Genotyping

Genomic DNA was isolated from the peripheral blood leukocytes of each subject using the QIAamp Blood Mini Kit (Qiagen NV, Venlo, the Netherlands) for genotyping. TagSNPs (rs5760090, rs2012133, rs755622, rs12628766, rs5760088, and rs3063367) were selected using Haploview 4.2 software basing the 1000 genome Phase 3 data (Chinese Han population), with 1 kb flanking region of the MIF gene. We also included the microsatellite repeat variant rs5844572. Then, 6 tagSNPs were genotyped using the TaqMan real-time PCR method on an ABI Prism 7900HT instrument (Applied Biosystems). Variant rs5844572 was genotyped using PCR amplification followed by capillary electrophoresis using a forward primer (5′ -TGCAGGAACCAATACCCAT AGG −3′) and a tetrachlorofluorescein (TET) - labeled fluorescent reverse primer (TET-5′ – AATGGTAAACTCGGGGGAC −3′). In order to confirm the genotyping results, DNA sequencing was used to replicate 10% of the randomly selected samples, and got a consistency of 100%.

Serum Level of MIF

The fasting serum of all participants for measurement of MIF was collected at the first admission. Then, serum level of MIF was determined with Human MIF ELISA kit (R&D Inc., Minneapolis, USA). The test range of the MIF is between 2 ng/mL and 100 g/mL. The coefficients of variation (CV) for the intra- and inter-assay reproducibility were 4.2–6.1% and 6.4–8.8%, respectively. For quality control, the experiment operator was blinded for the disease status.

Statistical Analysis

All statistical analyses were performed using SAS software (version 9.4; SAS Institute, Cary, NC, USA). All statistical tests were two-sided, and $P<0.05$ indicated a difference of statistical significance. For descriptive statistics, data was expressed as frequencies (percentages) for categorical variables and means (standard deviation, SD) for the continuous variables. Two-sided χ^2 tests were used to analyze the categorical demographic data, while Student's *t*-test or Mann–Whitney U-test were used to compare the values of MIF in controls and RC cases. The Hardy-Weinberg equilibrium was assessed by goodness-of-χ^2 test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to evaluate associations between MIF polymorphisms and RC susceptibility.

Results

Comparisons of Basic Characteristics of RC and Control Groups

Table 1 presents the comparisons of basic characteristics of RC cases and healthy control groups. The frequency distributions of age, gender, smoking status, and drinking status showed no significant difference with controls ($P>0.05$), while the RC group and control group were not homogenous regarding diabetes ($P = 0.022$).

Association of Serum Level of MIF with RC Risk

Compared with the controls, RC cases had a significantly higher serum level of MIF (shown in Table 1, mean ± SD: 17.5±6.7 vs 8.8±3.5, $P<0.001$). In the univariate model, MIF level as a continuous variable was associated with an increased risk of RC (OR for per unit: 1.38, 95% CI:1.32–1.44; $P < 0.001$).

Table 1 Distributions of Selected Variables in RC Cases and Healthy Controls

	Cases (n=420)	Controls (n=490)	P value
Age			
<50	236 (56.0%)	268 (54.7%)	0.680
≥50	185 (44.0%)	222 (45.3%)	
Gender			
male	291 (69.3%)	348 (71.0%)	0.568
female	129 (30.7%)	142 (29.0%)	
Smoking status			
Yes	119 (28.3%)	121 (24.7%)	0.214
No	301 (71.7%)	369 (75.3%)	
Drinking status			
Yes	140 (33.3%)	149 (30.4%)	0.345
No	280 (66.7%)	341 (69.6%)	
diabetes			
Yes	81 (19.3%)	67 (13.7%)	0.022
No	339 (80.7%)	423 (86.3%)	
MIF (ng/mL)	17.5±6.7	8.8±3.5	<0.001

Note: P value in bold means statistically significant.

Genetic Variants of the MIF Gene and Susceptibility of RC

As shown in Table 2, The genotype frequencies of rs5760090, rs2012133, rs755622, rs12628766, rs5760088, and rs3063367 in control group fit the Hardy–Weinberg equilibrium (P > 0.05). Both MIF rs2012133 (OR = 1.30; 95% CIs = 1.08–1.58; P = 0.007) and rs755622 (OR = 1.45; 95% CIs = 1.15–1.82; P = 0.002) were significantly associated with increased susceptibility of RC. Besides, we also found MIF rs5844572 was significantly associated with increased susceptibility of RC, with OR for per CATT repeat being 1.28 (95% CIs: 1.16–1.41; P < 0.001). Even adjusted for the Bonferroni correction, the results were still significant (P < 0.05), which means the robustness of our findings.

Associations Between Genetic Variants of the MIF Gene and Serum Level of MIF

We also evaluated the associations between genetic variants of the MIF gene (rs5844572, rs2012133 and rs755622) and serum level of MIF in both RC cases and controls. As shown in Figure 1, serum level of MIF increases with the increase of CATT repeat (P<0.001). Minor allele C of rs2012133 and rs755622 are also associated with increased serum level of MIF (P<0.001).

Table 2 Genetic Variants of the MIF Gene and Susceptibility of RC

Genotype	Cases	Controls	Adjusted OR (95% CI)*	P value
rs5844572				
CATT 5/5	27	53	1.00 (reference)	
CATT 5/6	97	166	1.18 (0.64–2.17)	0.592
CATT 5/7	42	33	2.57 (1.36–4.85)	0.003
CATT 6/6	138	150	1.86 (1.11–3.11)	0.018
CATT 6/7	96	83	2.34 (1.37–4.00)	0.002
CATT 7/7	20	5	8.09 (3.09–21.17)	<0.001
Per CATT			1.28 (1.16–1.41)	<0.001
rs5760090				
GG	207	245	1.00 (reference)	
AG	195	215	1.09 (0.79–1.52)	0.586
AA	18	30	0.72 (0.42–1.25)	0.249
A vs G			0.99 (0.94–1.05)	0.780
rs2012133				
GG	108	171	1.00 (reference)	
CG	240	249	1.56 (1.16–2.10)	0.004
CC	72	70	1.66 (1.11–2.49)	0.014
C vs G			1.30 (1.08–1.58)	0.007
rs755622				
GG	248	333	1.00 (reference)	
CG	144	137	1.44 (1.08–1.92)	0.013
CC	28	20	1.92 (1.07–3.42)	0.028
C vs G			1.45 (1.15–1.82)	0.002
rs12628766				
GG	302	364	1.00 (reference)	
CG	111	120	1.14 (0.81–1.59)	0.456
CC	7	6	1.43 (0.47–4.33)	0.523
C vs G			1.15 (0.85–1.54)	0.366
rs5760088				
GG	222	274	1.00 (reference)	
AG	170	181	1.18 (0.88–1.59)	0.268
AA	28	35	1.01 (0.76–1.33)	0.961
A vs G			1.09 (0.84–1.41)	0.512
rs3063367				
GG	129	157	1.00 (reference)	
AG	233	254	1.14 (0.82–1.58)	0.440
AA	58	79	0.91 (0.66–1.26)	0.574
A vs G			1.02 (0.86–1.20)	0.824

Note: *Adjusted for age, gender, smoking, drinking status, and diabetes. P value in bold means statistically significant.

Discussion

The current study explored the association between genetic variants of the MIF gene and susceptibility of RC using a case–control study in a Chinese population. First, we found serum level of MIF as a continuous

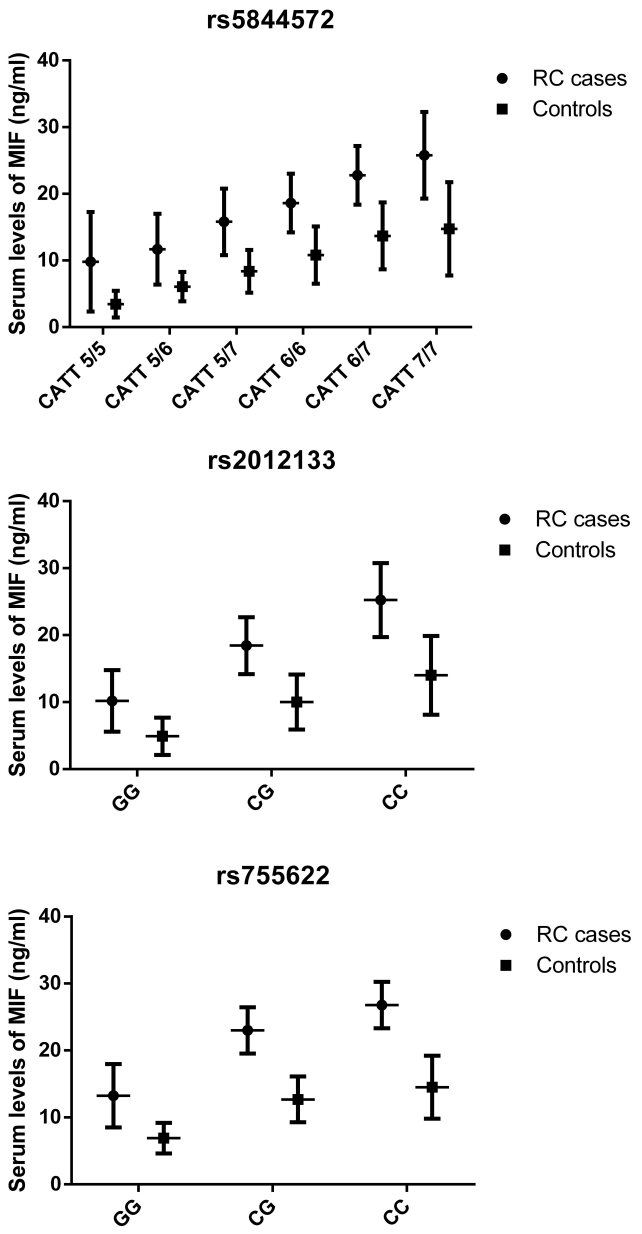

Figure 1 Associations between genetic variants of the *MIF* gene and serum level of MIF.

variable was significantly associated with an increased risk of RC. Second, three variants of the MIF gene, rs5844572, rs2012133 and rs755622, could increase the serum level of MIF, further influencing the susceptibility of RC. To the best of our knowledge, this should be the first study which aims to evaluate the potential genetic function of the MIF gene in the carcinogenesis process of RC at the population level.

Cytokines play a complex role in the initiation and progression of inflammation and tumorigenesis.[14,29] Meanwhile, genetic variants of many cytokine genes, including TNF-α, TGF-β, tumor necrosis factor-a (TNF-α), Interleukin 1β (IL1β), have been evaluated for their associations with cancer susceptibility.[30–32] This cumulative evidence confirmed the crucial role of genetic variants of the cytokine genes in the carcinogenesis process of RC, and provided clues for further exploration.[33,34]

The MIF gene (Homo sapiens) has been mapped on to 22q11.23, and contains three exons, two introns, and several putative transcription factor binding sites.[35] Elevated serum level of MIF has been associated with higher risk of RC, autism, alopecia areata, and active pulmonary tuberculosis.[36–42] In our study, we found MIF level was associated with an increased risk of RC (OR for per unit: 1.38, 95% CI:1.32–1.44; $P < 0.001$). Meanwhile, MIF rs755622 and rs5844572 have been evaluated to be associated with multiple cancers and other diseases.[23–35] In our study, the CATT5 allele of rs5844572 exhibits the lowest MIF level, while CATT6–7 alleles have a progressively higher serum level of MIF. While, the minor allele C of rs2012133 and rs755622 could progressively increase the serum level of MIF, which then causes the carcinogenesis of RC. These findings proved that genetic variants of the MIF gene played an important role for susceptibility of RC.

A strength of the current study was the moderate sample size for genetic association studies of RC susceptibility, which gave enough power for such associations. Some limitations of this study also should be considered when interpreting the results. First, population stratification can still occur as self-reported race does not accurately reflect genetic ancestry; second, the hospital-based case–control study might bring potential selection bias; third, the biological mechanisms of the three variants are not clear, so further functional studies are needed to provide more evidence.

Conclusion

Our study found that the serum level of MIF was associated with an increased risk of RC, while MIF rs5844572, rs2012133 and rs755622 were associated with both increased serum level of MIF and risk of RC. These results suggest that MIF plays an important role in the carcinogenesis of RC, and could be used as a biomarker for early detection and prediction of RC. Studies involving diverse populations are warranted to confirm our results, and a functional assay should be carried out on the mechanism of MIF in RC carcinogenesis.

Disclosure

No competing financial interests exist. The authors report no conflicts of interest for this work.

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