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Association Between Genetic Polymorphism of the *MIF* Gene and Colorectal Cancer in Taiwan

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Background: Colorectal cancer (CRC) is the highest leading cause of cancer-related mortality in Taiwan. Macrophage migration inhibitory factor (MIF) has recently been defined as a novel protumorigenic factor that promotes cell proliferation, migration, and invasion. The aim of the present study is to identify the association between MIF gene polymorphism and CRC. *Methods:* A case–control study was designed to test the hypothesis. A total of 192 biopsy-diagnosed CRC patients (CRC) and 256 healthy subjects (control) were recruited. Genotyping of four single nucleotide polymorphism (SNPs; rs755662, rs11548059, rs1049829, rs1803976) at chromosome positions 755662 (5′ UTR), 11548059 (exon2), 1049829 (exon2), 1803976 (exon3) was performed using a Taqman SNP genotyping assay. *Results:* There is a significant difference in genotype frequency distribution of rs755662 polymorphism between CRC patients and controls ($P = 0.011$). No significant difference was found in the frequency distribution of rs11548059, rs1049829, rs1803976 polymorphism in CRC patients and controls ($P = 0.660$, $P = 0.700$, and $P = 0.959$, respectively). Moreover, the MIF-173 SNP was also significantly associated with young patients (age < 50 years, $P = 0.026$) late stage (Stage IV, $P = 0.038$) and poor differentiation group ($P = 0.040$). Compared to the control group, the MIF-173 SNP also significantly associated with patients with stages III and IV ($P = 0.034$ and 0.003, respectively). *Conclusion:* The presence of *MIF*-173 (G/C) gene polymorphism (rs755662) was associated with susceptibility, patient age, and stages of CRC in Taiwanese. J. Clin. Lab. Anal. 29:268–274, 2015.

Key words: colorectal cancer; genetic polymorphisms; macrophage migration inhibitory factor; susceptibility

Abbreviations

CRC	=	colorectal cancer
CT	=	computed tomography
FAP	=	Familial adenomatous polyposis
HNPCC	=	Hereditary nonpolyposis colorectal carcinoma
MIF	=	macrophage migration inhibitory factor
PCR	=	polymerase chain reaction
SNP	=	single nucleotide polymorphism
TMN stage	=	tumor node and metastasis stages

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INTRODUCTION

Colorectal cancer (CRC) is one of the most common forms of neoplasia and the third leading cause of cancer-related death worldwide (1) and is of highest prevalence in Taiwan. The risk factors of CRC include age; family history; inflammatory bowel diseases (IBD), including ulcerative colitis and Cohn's disease; and environmental and dietary procarcinogens. The epidemiological study showed that up to 40% patients with colitis developed colitis-associated CRC (2).

Macrophage migration inhibitory factor (MIF) is a member of the transferring growth factor-β (TGF-β) super family, which plays an important role in the pro- and anti-inflammatory response to infection. MIF is a potent promoter of the expression of cytokines, such as tumor necrosis factor (TNF-α), interleukin (IL)-1 β, IL-6, IL-8, and prostaglandin E2 (PGE2), in LPS-driven (where LPS is lipopolysaccharide) response (3), some of which play a vital role in the pathogenesis of rectal cancer and is known to contribute to the development and promotion of malignant tumors (4) and autoimmune disease (5–7). MIF overexpression has been observed in several human neoplasms (8), including prostate, breast, and lung cancer (9–11). The previous reports have demonstrated that the overexpression of MIF increases tumor differentiation and lymph node invasion in CRC (12).

Several studies have shown that single nucleotide polymorphism (SNP) of MIF gene has been linked to the risk of CRC (13, 14). The *MIF* gene is located on chromosome 22q11.2 and has a SNP (G to C transition) in the 50-flanking region at position 173G/C (rs755622), which has been reported to associate with susceptibility to adult inflammation (8, 15) and cancer (16–18). A number of studies have been focused on the association between *MIF* gene polymorphism and susceptibility to cancer (19–22). Interestingly, little information concerning the role of MIF on colon cancer has been obtained until now as no data have been reported especially on MIF polymorphism on CRC.

Previously, we found that MIF acts as a biomarker to improve clinical predication of primary CRC with low preoperative serum carcinoembryonic antigen (CEA) (23). Many studies have been extensively conducted and have shown that the *MIF* gene polymorphism is associated with many diseases (15–22). Thus, the aim of the present study is to examine the association between *MIF* polymorphism and the risk of CRC in the Taiwanese population. We determined that the likelihood of the gene variants rs755662 (G/C), rs11548059 (C/T), rs1049829 (C/T), and rs1803976 (A/G) of MIF may increase an individual's susceptibility of CRC. The mutation analysis of *MIF* gene among CRC patients might be valuable for therapeutic study in the Taiwanese population.

TABLE 1. Clinical Characteristics of the CRC Patients (CRC, $n = 192$) and Healthy Subjects (control, $n = 256$)

Clinical parameters	CRC group ($N = 192$)	Control group ($N = 256$)	*P*-value
Mean age (years)	62.1	55.8	0.370
(Range)	36–82	40–84	
Sex			0.524
Male	116	147	
Female	76	109	
BMI			<0.001
<18.5	16	53	
18.5 ≦ BMI < 24	108	82	
24≦	68	121	
Smoking			0.442
Yes	55	82	
No	137	174	
Alcohol intake			0.137
Yes	47	79	
No	145	177	

PATIENTS AND METHOD

Study Population

In this study, we recruited 192 patients with primary sporadic CRC, who had been treated by resection at the Department of Surgery, China Medical University Hospital, from January to December 2007. Hereditary nonpolyposis colorectal carcinoma (HNPCC) and familial adenomatous polyposis (FAP) patients were identified by screening patient histories and immunohistochemical staining. Samples from the control group (256 healthy volunteers) were collected from our Health Examination Center. CRC patients who had been treated with preopchemoradiation and diagnosed based on the Amsterdam criteria were excluded from the study. Initial staging workup included history and physical examination, routine biochemistry, determination of CEA levels, and X-ray and abdominal computed tomography (CT) scans. The CRC diagnosis was performed by histological evaluation. Use of the tissues and blood samples for the research purposes complied with the regulations set by the Institutional Review Board (DMR-IRB 96–0704). The study was approved by the ethical committee of the China Medical University Hospital. The clinical characteristics of the CRC and control groups are reported in Table 1.

Genomic DNA Extraction and Genotyping of MIF Gene Genetic Polymorphisms

Genomic DNA was extracted from peripheral blood leukocytes according to standard protocols (Genomic DNA kit, Roche, Nutley, NJ). Four SNPs (rs755662, rs11548059, rs1049829, and rs1803976) at chromosome positions 755662 (5′UTR), 11548059 (exon 2), 1049829

(exon 2), and 1803976 (exon 3) in the MIF gene were genotyped by the Taqman SNP genotyping assay method (ABI: Applied Biosystems, Inc., Foster City, CA) as previously described (16, 17, 24). The primers and probes of SNPs were obtained from the ABI assay on demand (AOD) kit. Reactions were carried out according to the manufacturer's protocol. Briefly, polymerase chain reaction (PCR) was performed in the presence of 2× TaqMan® Universal PCR Master Mix (ABI). The total number of cycles was limited to 40 cycles. Real-time detection of fluorescence signals was performed using the ABI Prism 7900 Real-Time PCR System.

Statistical Analysis

The χ^2-test was used to determine significant differences in allele/genotype frequencies between patients and controls by comparing the different percentages. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were obtained using logistic regressions to determine associations between MIF alleles/genotypes and susceptibility. The alleles/genotypes have the individual OR value, we choose the minimum (OR > 0) as reference group (Ref, OR = 1). Genotype distributions were tested in Hardy–Weinberg equilibrium. Continuous variables as age and BMI were performed with Mann–Whitney *U*-test. All statistical tests were performed on SPSS software Version 15.0 (SPSS, Inc., Chicago, IL). *P*-value < 0.05 was considered as statistically significant.

RESULTS

MIF Gene Polymorphism in the CRC Patients and Controls

The genotypic and allelic frequencies of rs755622, rs1049829, rs1803976, and rs11548059 are shown in Table 2. The frequency distribution of rs755622 between CRC patients and control group has the significant difference ($P = 0.011$). However, there was no significant difference in the genotypic and allelic frequencies of rs11548059 ($P = 0.660$), rs1049829 ($P = 0.7$), and rs1803976 ($P = 0.959$) polymorphisms between the two groups. We found that the number of G carrier (GG + GC) of the rs755622 polymorphism was higher in the CRC group (97.9%) than the control group (94.5%), but statistically no significant difference was observed ($P = 0.071$). The similar trend was observed in the C carrier (CC + GC) and no significance difference was detected ($P = 0.086$). The frequency of the G allele of rs755622 was 81.5% in the patient group and 83.6% in the control group. The frequency of the C allele was 18.5% in the patient group and 16.4% in the control group. When the G allele frequency was compared

TABLE 2. Genotypic and Allelic Frequencies of MIF Genetic Polymorphism in Patients With CRC and Controls. The Values Within Brackets are Percentage and the "Ref" Stands for Each Compared Template

db SNP ID	CRC ($n = 192$)	Control ($n = 256$)	OR (95%CI)	*P*-value
rs755622				
GG	125	186	2.35 (0.76–7.31)	0.011*
GC	63	56	3.94 (1.22–12.66)	
CC	4	14	Ref	
Genotype(carrier)				
GG+GC	188 (97.9)	242 (94.5)	2.72 (0.88–8.40)	0.071
CC	4 (2.1)	14 (5.5)	Ref	
GC+CC	67 (34.9)	70 (27.3)	1.42 (0.95–2.13)	0.086
GG	125 (65.1)	186 (72.7)	Ref	
Allele frequency				
G	313 (81.5)	428 (83.6)	0.87 (0.61–1.63)	0.415
C	71 (18.5)	84 (16.40	Ref	
rs11548059				
CC	3	3	0.500 (0.03–8.95)	0.660
CT	187	252	0.37 (0.03–4.12)	
TT	2	1	Ref	
Genotype(carrier)				
CC+CT	190	255	0.37 (0.03–4.14)	0.403
TT	2	1	Ref	
CT+TT	189	253	0.75 (0.15–3.74)	0.722
CC	3	3	Ref	
Allele frequency				
C	193	258	0.99 (0.76–1.30)	0.969
T	191	254	Ref	
rs1049829				
CC	188	252	0.50 (0.08–3.01)	0.700
CT	1	2	0.33 (0.02–6.65)	
TT	3	2	Ref	
Genotype(carrier)				
CC+CT	189	254	0.50 (0.08–3.00)	0.436
TT	3	2	Ref	
CT+TT	4	4	1.34 (0.33–5.43)	0.680
CC	188	252	Ref	
Allele frequency				
C	377	506	0.64 (0.21–1.92)	0.420
T	7	6	Ref	
rs1803976				
AA	190	254	0.75 (0.05–12.04)	0.959
AG	1	1	1.00 (0.02–50.40)	
GG	1	1	Ref	
Genotype (carrier)				
AA+AG	191	255	0.75 (0.05–12.05)	0.838
GG	1	1	Ref	
AG+GG	2	2	1.34 (0.19–9.58)	0.772
AA	190	254	Ref	
Allele frequency				
A	381	509	0.75 (0.15–3.73)	0.723
G	3	3	Ref	

*$P < 0.05$.
CI, confidence interval; OR, odds ratio.

TABLE 3. Stratified Analysis of Clinical and Pathological Characteristics of the 192 Sporadic CRC Patients According to MIF Polymorphism (rs 755662)

	MIF(rs755622)			
Clinical parameters	GG ($n = 125$)	GC ($n = 63$)	CC ($n = 4$)	P-value (OR [95%CI])
Age				0.026*
≧50 years ($n = 142$)	98 (69.0)	43 (30.2)	1 (0.7)	
<50 years ($n = 50$)	27 (54.0)	20 (40.0)	3 (6.0)	
	10.89 (1.09–108.93)	6.45 (0.63–65.93)	Ref	OR (95% CI)
Gender				0.250
Male ($n = 113$)	78 (69.0)	32 (28.3)	3 (2.7)	
Female ($n = 79$)	47 (59.5)	31 (39.2)	1 (1.3)	
	0.55 (0.06–5.47)	0.34 (0.03–3.49)	Ref	OR (95%CI)
Pre-OP CEA				0.963
<5 ng/ml ($n = 51$)	34 (66.7)	16 (31.4)	1 (1.9)	
>5 ng/ml ($n = 141$)	91 (64.5)	47 (33.4)	3 (2.1)	
	1.12 (0.11–11.15)	1.02 (0.10–10.53)	Ref	OR (95%CI)
Tumor sites				0.909
Colon ($n = 107$)	71 (66.4)	34 (31.8)	2 (1.8)	
Rectum ($n = 85$)	54 (63.5)	29 (34.1)	2 (2.4)	
	1.31(0.18–9.63)	1.17(0.16–8.85)	Ref	OR (95%CI)
TMN classification				0.056
Stage I + II ($n = 114$)	82 (71.9)	30 (26.3)	2 (1.8)	
Stage III + IV ($n = 78$)	43 (55.1)	33 (42.3)	2 (2.6)	
	1.92 (0.26–14.01)	0.91 (0.12–6.86)	Ref	OR (95%CI)
TMN classification				0.038*
Stage I + II + III	116 (68.2)	51 (30.0)	3 (1.8)	
IV	9 (40.9)	12 (54.5)	1 (4.6)	
	4.30 (0.40–45.62)	1.42 (0.14–14.84)	Ref	OR (95% CI)
Differentiation				0.040*
Well + moderate ($N = 172$)	117 (68.0)	52 (30.2)	3 (1.8)	
Poor ($N = 20$)	8 (40.0)	11 (55.0)	1 (5.0)	
	4.88 (0.45–52.35)	1.58 (0.15–16.60)	Ref	OR (95% CI)

*$P < 0.05$.

between CRC patients and control groups, the differences were not statistically significant ($P = 0.415$).

Relationship Clinical Features of rs755662 Polymorphism in CRC Patients

Furthermore, we investigated whether the MIF polymorphism was associated with certain clinical and pathological characteristics, such as age, gender, pre-OP CEA, tumor node and metastasis (TMN) stage, and differentiation. The frequency distribution of MIF SNP polymorphism with age (<50, <50), gender, preoperation carcinoembryonic antigen level (Pre-OP CEA), tumor sites (colon and rectum), TMN classification (stage I, II, III, and IV), and differentiations (well, moderate, and poor) are shown in Table 3. The result shows that the GC genotype is significantly higher in young group than in old group ($P = 0.026 < 0.05$), TMN stage IV ($P = 0.038 < 0.05$), and poor differentiations ($P = 0.04 < 0.05$). However, there is no significant difference in the gender, pre-OP CEA level, and tumor sites. Furthermore, we found that there was no significant association between rs755622 polymorphism and stages I and II (data not shown). Besides, we observed that stages III and IV have the significant difference in rs755662 polymorphism compared to healthy volunteers (control group; stage III: $P = 0.034 < 0.05$; stage IV: $P = 0.003 < 0.05$). Moreover, both the C carrier ($P = 0.002 < 0.05$) and C allele frequency ($P = 0.010 < 0.05$) are significantly higher in the stage IV patients, even the number is only 22. It must be noted that the number of GC and CC allele patients has increased (approximately) by 1.44-fold compared to GG allele patients in stage IV (Table 4).

Relationship rs755662 Polymorphism Between Young Patients and TMN Classification in CRC Patients

The frequency distribution of GC genotype was significantly higher in young patients (<50 years) and late TMN

TABLE 4. Comparison of the Genotypes and Allelic Frequencies of MIF Genetic Polymorphism Between the Stage III and IV CRC Patients and Control Group. The Values Within Brackets Are Percentage and the "Ref" Stands for Each Compared Template

dbSNP ID	Stage III ($n = 56$)	Control ($n = 256$)	OR (95% CI)	*P*-value
rs 755622				
GG	34 (60.7)	186 (72.7)	2.56 (0.33–20.11)	0.034*
GC	21 (37.5)	56 (21.9)	5.25 (0.65–42.44)	
CC	1 (1.8)	14 (5.4)	Ref	
Genotype(carrier)				
GG+GC	55 (98.2)	242 (94.5)	3.18 (0.41–24.71)	0.243
CC	1 (1.8)	14 (5.5)	Ref	
GC+CC	22 (39.3)	70 (27.3)	1.72 (0.94–3.14)	0.076
GG	34 (60.7)	186 (72.7)	Ref	
Allele frequency				
G	89 (79.5)	428 (83.6)	0.76 (0.45–1.27)	0.294
C	23 (20.5)	84 (16.4)	Ref	
dbSNP ID	Stage IV ($n = 22$)	Control ($n = 256$)	OR (95% CI)	*P*-value
rs 755622				
GG	9 (40.9)	186 (72.7)	0.68 (0.08–5.74)	0.003*
GC	12 (54.6)	56 (21.9)	3.00 (0.36–25.05)	
CC	1 (4.5)	14 (5.4)	Ref	
Genotype(carrier)				
GG+GC	21 (95.5)	242 (94.5)	1.21 (0.15–9.70)	0.854
CC	1 (4.5)	14 (5.5)	Ref	
GC+CC	13 (59.1)	70 (27.3)	3.84 (1.57–9.38)	0.002*
GG	9 (40.9)	186 (72.7)	Ref	
Allele frequency				
G	30 (68.2)	428 (83.6)	0.42 (0.21–0.83)	0.010*
C	14 (31.8)	84 (16.4)	Ref	

*$P < 0.05$.
CI, confidence interval; OR, odds ratio.

stages as described above. Thus, we focus on the young patients ($n = 50$) to analyze the rs755662 polymorphism among the TMN stages. The result shows that rs755662 polymorphism has no significant difference between the early (stage I + II) and late stage (stage III + IV; ($P = 0.139 > 0.05$). However, result shows difference between the stage I + II + III and stage IV groups ($P = 0.031 < 0.05$). The statistic distribution shows that GC allele is a major mutation in the stage IV within the young patients. It also must be noted that the number of GC and CC allele patients increased by (approx) 3.33-fold compared to GG allele patients in the stage IV young patients (Table 4).

DISCUSSION

In our study, we investigated the association between MIF-173 SNP and CRC. It has been documented that there is an association between MIF gene-173 polymorphism and CRC. Recent studies have suggested that the increase in MIF protein levels has been demonstrated in human sporadic CRC tissue compared to normal colorectal mucosa by immunohistochemical study, and the protein overexpression has been detected in several human and mouse primary culture CRC cell and LoVo CRC cell line (25). MIF is located on chromosome 22q11.2, which has been implicated in carcinogenesis, was identified in vitro and in vivo models (8, 26). MIF plays a role in p53-dependent apoptosis and promotes Rat sarcoma (RAS)-mediated transformation of fibroblasts (27, 28). MIF has also been implicated in lymphoma and melanoma cell tumor growth and angiogenesis in rodents (29).

An SNP of the *MIF* gene promoter (MIF-173) has been recently identified in juvenile idiopathic arthritis and has been shown to be functionally relevant to the expression of MIF protein (15), especially MIF-173 G/C polymorphism recently was functionally identified both in vivo and in vitro in acute lymphoblastic leukemia (21). Therefore, we selected this SNP as one of the candidate SNP for this study. However, up to date, reports on the impact of specific *MIF* gene polymorphism on tumor cell biology and tumor growth is very limited (17, 20). MIF-173 C allele has been associated with an increased risk of developing various cancers, such as CRC, gastric cancer, and with higher incidences, more advanced clinical stages and higher tumor aggressiveness in gastric cancer (17).

However, no study focused on the *MIF* gene polymorphism and their relationship with CRC susceptibility or the related studies until now. To our knowledge this is the first report that tells the association of the MIF gene SNP with CRC. In our study, we investigated the possible role of the diverse *MIF* gene mutations in the CRC susceptibility. Four known polymorphisms (rs755662, rs11548059, rs1049829, and rs1803976) of the *MIF* gene were identified in samples of 192 patients and 256 controls. The rs755662 had smaller *P*-value ($P = 0.011$) than rs11548059 ($P = 0.66$), rs1049829 ($P = 0.7$), and rs1803976 ($P = 0.959$), therefore, we perform the rs755662-related analysis in the advance study. To further validate the results, we calculated some parameters as well. In our study, the MIF-173 G/C was found to be associated with the risk of CRC in young group, TMN stage IV, and poor differentiation (Table 2), although the MIF-173 G/C was not observed to be associated with stage III + IV (Table 2). In comparison to the MIF-173 G/C between the patients of different stage and healthy volunteers, the GC allele was shown to have significantly higher frequency in the stage III and IV, respectively. Moreover, both the C carrier and C allele frequency are significantly higher in the stage IV patients. The MIF-173 G/C with incident CRC assuming a dominant model stratified by ethnicity within Taiwanese population. The phenomena have not been observed in the stages I and II. The results indicated the MIF-173 G/C did associate with the CRC progress.

TABLE 5. Comparison of Young Group (<50 years) and TMN Stages in CRC Patients With Different Genotype Distributions of rs755662

		MIF (rs 755662)			
Age (years)		GG	GC	CC	*P*-value
	TMN stage				0.139
<50 ($n = 50$)	I + II	17	7	1	
	III + IV	10	13	2	
	TMN stage				0.031*
<50 ($n = 50$)	I + II + III	24	11	2	
	IV	3	9	1	

*$P < 0.05$.

Interestingly, the comparison of CRC in West, South, and South East Asia has been reported with greater frequency in young patients usually under 50 years (30). In general, CRC is a disease of middle age and elderly, with the majority diagnosed after the age of 55. Some 2–10% of all CRCs have been reported in young patients (<50 years; (31)). According to the previous study, we chose the cutoff of age as 50 in our study, the data show that MIF-173 G/C is significantly higher in young patients (Table 1). The frequency of the GC allele in the young group was shown to be 1.32-fold higher than the old group (<50 years). In order to clarify the role of MIF-173 G/C in young patients and TMN stage, we compared the frequencies of MIF-173 G/C in the viable TMN stage groups from the young patients (Table 5). The result indicated that the occurrence of MIF-173 G/C was also significantly higher in the stage IV group, and even in young patients ($P = 0.031$). As mentioned previously, we found a strong association between rs755622 and CRC. The result demonstrated MIF-173 G/C and C carrier did contribute to the late stage of CRC in Taiwan. According to stratification analysis, the results suggest that the effect of MIF SNP also influences the certain clinical features in CRC. It is also necessary to further elucidate how this polymorphism of MIF exactly exerts their role in the other regions. Based on the diverse genetic background, we need more complete evidences to verify the hypothesis in the other regions.

CONCLUSIONS

In conclusion, G to C transition has been reported for the first time at position MIF-173 mutation of the *MIF* gene related with the clinical TMN stages in CRC patients. *MIF*-173 (G/C) genotype may be used as a risk marker in late stage CRC, which may offer the approaches on diagnosis and treatments for the CRC in the Taiwanese population. Our findings also support the idea to develop the cancer research and the advance therapies of the CRC for Taiwanese population.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests related to this work.

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