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. According to the Cancer Statistics, 2020, it was estimated that 43,340 new RC cases would occur in United States in 2020. Although diet, environmental exposures, and lifestyle factors were considered as the risk factors for RC carcinogenesis. 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. Macrophage migration inhibitory factor (MIF), also known as glycosylation-inhibiting factor (GIF), encodes a lymphokine involved in cell-mediated immunity, immunoregulation, and inflammation. It was implicated in the pathogenesis of many cancers, sepsis, and inflammatory and autoimmune diseases. 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. However,
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 Haplovlew 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’-TGACGGAACCAATACCATCAT AGG-3’) and a tetraclorofluorescein (TET) - labeled fluorescent reverse primer (TET-5’-AATGGTAAACTCGGGGAG-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 χ² 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-χ² 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.

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

| Genotype | Cases | Controls | Adjusted OR (95% CI)* | P value |
|----------|-------|----------|-----------------------|---------|
| rs5844572| 27    | 53       | 1.00 (reference)      |         |
| CATT 5/5 |       |          | 1.18 (0.64–2.17)     | 0.392   |
| CATT 5/6 | 97    | 166      | 2.57 (1.36–4.85)     | 0.003   |
| CATT 5/7 | 42    | 33       | 1.86 (1.11–3.11)     | 0.018   |
| CATT 6/6 | 138   | 150      | 2.34 (1.37–4.00)     | 0.002   |
| CATT 6/7 | 96    | 83       | 8.09 (3.09–21.17)    | <0.001  |
| CATT 7/7 | 20    | 5        | 1.28 (1.16–1.41)     | <0.001  |
| Per CATT  |       |          |                      |         |
| 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)      |         |
| AG       | 240   | 249      | 1.56 (1.16–2.10)     | 0.004   |
| AA       | 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)      |         |
| AG       | 144   | 137      | 1.44 (1.08–1.92)     | 0.013   |
| CG       | 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)      |         |
| AG       | 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.

### 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).

### 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...
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. Meanwhile, genetic variants of many cytokine genes, including TNF-α, TGF-β, tumor necrosis factor-α (TNF-α), Interleukin 1β (IL1β), have been evaluated for their associations with cancer susceptibility. 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.

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. Elevated serum level of MIF has been associated with higher risk of RC, autism, alopecia areata, and active pulmonary tuberculosis. 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. 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.

References
1. Siegel RL, Miller KD, Goding Sauer A, et al. Colorectal cancer statistics, 2020. CA Cancer J Clin. 2020.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7–30. doi:10.3322/caac.21590
3. Jeon J, Du M, Schoen RE, et al. Determining risk of colorectal cancer and starting age of screening based on lifestyle, environmental, and genetic factors. Gastroenterology. 2018;154:2152–2164.e19. doi:10.1053/j.gastro.2018.02.021
4. Vitiello GAF, Losi Guembarovski R, Amarante MK, Ceribelli JR. Interleukin 7 receptor alpha Th244Ile genetic polymorphism is associated with susceptibility and prognostic markers in breast cancer subgroups. Cytokine. 2018;103:121–126. doi:10.1016/j.cyt.2017.09.019
5. Stephens KE, Levine JD, Ouzuner BE, Paul SM, Abrams G. Associations between genetic and epigenetic variations in cytokine genes and mild persistent breast pain in women following breast cancer surgery. Cytokine. 2017;99:203–213. doi:10.1016/j.cyt.2017.07.006
6. Zidi S, Gazouani E, Stayoussf M, Mezlini A, Ahmed SK, Yacoubi-Loueslati B. IL-10 gene promoter and intron polymorphisms as genetic biomarkers of cervical cancer susceptibility among Tunisians. Cytokine. 2015;76(2):343–347. doi:10.1016/j.cyt.2015.05.028
7. Dwivedi S, Goel A, Khatri S, Mandhani A, Sharma P. Genetic variability at promoters of IL-18 (pro-) and IL-10 (anti-) inflammatory gene affects susceptibility and their circulating serum levels: an explorative study of prostate cancer patients in North Indian populations. Cytokine. 2015;74(1):117–122. doi:10.1016/j.cyt.2015.04.001
8. Zhou L, Xie J, Gu EL, Huang Y, Qu Y, Xu AP. Common genetic variant on BMP4 contributes to colorectal adenoma and cancer: A meta-analysis based on 15 studies. Cytokine. 2015;72(2):154–159. doi:10.1016/j.cyt.2014.12.021
9. Kiyohara C, Horiuchi T. Genetic polymorphisms involved in the inflammatory response and lung cancer risk: a case-control study in Japan. Cytokine. 2014;65(1):88–94. doi:10.1016/j.cyt.2013.09.015
10. Qiu LX, He J, Wang MY, et al. The association between common genetic variant of microRNA-146a and cancer susceptibility. Cytokine. 2011;56(3):695–698. doi:10.1016/j.cyt.2011.09.001
11. Khashtr SY, Saha A, Gries P, Tiziani S, Stone E. Enzyme-mediated depletion of l-lys(e) ine synergizes with thioredoxin reductase inhibition for suppression of pancreatic tumor growth. NJP Precis Oncol. 2019;3:16. doi:10.1038/s41609-019-0088-z
12. Morand EF, Leech M, Bernhagen J. MIF: a new cytokine link between rheumatoid arthritis and atherosclerosis. Nat Rev Drug Discov. 2006;5(5):399–410. doi:10.1038/nrd2029
13. Calandra T, Roger T. Macrophage migration inhibitory factor: a regulator of innate immunity. Nat Rev Immunol. 2003;3 (10):791–800. doi:10.1038/nnri1200
14. Bin Lim S, Chua MLK, Yeong JPS, Tan SJ. Pan-cancer analysis connects tumor matrisome to immune response. NJP Precis Oncol. 2019;3:15. doi:10.1038/s41609-019-0087-0
15. Lippitz BE. Cytokine patterns in patients with cancer: a systematic review. Lancet Oncol. 2013;14(6):e218–228. doi:10.1016/S1470-2245(12)70582-X
16. Han Z, Qu J, Zhao J, Zou X. Genetic variant rs755622 regulates expression of the multiple sclerosis severity modifier d-dopachrome tautomerase in a sex-specific way. Biomed Res Int. 2018;2018:8285653. doi:10.1155/2018/8285653
17. Sharaf-Eldien M, Elghannam D, Abdel-Malak C. MIF-173G/C (rs755622) polymorphism as a risk factor for acute lymphoblastic leukemia development in children. J Gene Med. 2018;20(9):e3044. doi:10.1002/jgm.3044
18. Areeshi MY, Mandal RK, Dar SA, et al. MIF −173 G > C (rs755622) gene polymorphism modulates tuberculosis risk: evidence from a meta-analysis and trial sequential analysis. Sci Rep. 2017;7(1):17003. doi:10.1038/s41598-017-17308-y
19. Ma G, Yuan Q, Wang Q, et al. Association between MIF-as rs755622 and nephrolithiasis risk in a chinese population. Med Sci Monit. 2016;22:563–568. doi:10.12659/MSM.895818
20. Luo JY, Xu R, Li XM, et al. Polymorphism rs755622 is associated with coronary artery disease and severity of coronary lesions in a chinese kazakh population: a case-control study. Medicine. 2016;95(4):e2617. doi:10.1097/MD.0000000000002617
21. Castaneda-Moreno VA, De la Cruz-mosso U, Torres-Carrillo N, Macias-Islas MA. MIF functional polymorphisms (−794 CATT5–8 and −173 G=C) are associated with MIF serum levels, severity and progression in male multiple sclerosis from western Mexican population. J Neuroimmunol. 2018;320:117–124. doi:10.1016/j.jneuroim.2018.04.006
22. Qian L, Wang XY, Thapa S, et al. Macrophage migration inhibitory factor promoter polymorphisms (−794 CATT5–8); relationship with soluble MIF levels in coronary atherosclerotic disease subjects. BMC Cardiovasc Disord. 2017;17(1):144. doi:10.1186/s12872-017-0570-x
23. Martinez-Guzman MA, Alvarado-Navarro A, Pereira-Suarez AL, Munoz-Valle JF, Fafutis-Morris M. Association between STR −794 CATT 5–8 and SNP −173 G/C polymorphisms in the MIF gene and Lepromatous Leprosy in Mestizo patients of western Mexico. Hum Immunol. 2016;77(10):985–989. doi:10.1016/j.humimm.2016.07.006
24. Morales-Zambrano R, Bautista-Herrera LA, De la Cruz-mosso U, et al. Macrophage migration inhibitory factor (MIF) promoter polymorphisms (−794 CATT5–8 and −173 G=C) association with MIF and TNFalpha in psoriatic arthritis. Int J Clin Exp Med. 2014;7(9):2605–2614.
25. De la Cruz-mosso U, Bucala R, Palafoux-Sanchez CA, et al. Macrophage migration inhibitory factor: association of −794 CATT5–8 and −173 G=C polymorphisms with TNF-alpha in systemic lupus erythematosus. Hum Immunol. 2014;75(5):433–439. doi:10.1016/j.humimm.2014.02.014
26. Tong X, Zheng B, Tong Q, et al. MIF −173G/C gene polymorphism increase gastrointestinal cancer and hematological malignancy risk: evidence from a meta-analysis and FPRP test. Int J Clin Exp Med. 2015;8(9):15949–15957.
27. Wang CD, Li TM, Ren ZJ. Contribution of macrophage migration inhibitory factor −173G/C gene polymorphism to the risk of cancer in chinese population. Asian Pac J Cancer Prev. 2015;16(11):4597–4601. doi:10.7314/APJCP.2015.16.11.4597
28. Zhang X, Weng W, Xu W, et al. The association between the migration inhibitory factor −173G/C polymorphism and cancer risk: a meta-analysis. Onco Targets Ther. 2015;8:601–613. doi:10.2147/OTT.s72795
29. Crunkhorn S. Designing cytokine mimics can optimize cancer therapy potential. Nat Rev Drug Discov. 2019;18(3):173. doi:10.1038/d41573-019-00020-z
30. Zagoda M, Sarnecka AK, Staszczak Z. Correlation of TNF-alpha and TGF-beta polymorphisms with protein levels in pancreatic ductal adenocarcinoma and colorectal cancer. Contemp Oncol. 2019;23 (4):214–219. doi:10.5114/wo.2019.91537
31. Karakaxas D, Gazouli M, Coker A, Agalinos C, Papanikolaou IS, Patapis P. Genetic polymorphisms of inflammatory response gene TNF-alpha and its influence on sporadic pancreatic neuroendocrine tumors predisposition risk. Med Oncol. 2014;31(10):241.
32. Cigovskii Berkovic M, Catela Ivkovic T, Marout J, Zjacek-Rotkiew V, Kapitanovic S. Interleukin 1beta gene single-nucleotide polymorphisms and susceptibility to pancreatic neuroendocrine tumors. DNA Cell Biol. 2012;31(4):531–536. doi:10.1089/dna.2011.1317
33. Loomans-Kropp HA, Umar A. Cancer prevention and screening: the next step in the era of precision medicine. NPJ Precis Oncol. 2019;3:3. doi:10.1038/s41698-018-0075-9

34. Katayama H, Tsou P, Kobayashi M, Capello M, Wang H, Esteva F. A plasma protein derived TGFbeta signature is a prognostic indicator in triple negative breast cancer. NPJ Precis Oncol. 2019;3:10.

35. Renner P. Macrophage migration inhibitory factor: gene polymorphisms and susceptibility to inflammatory diseases. Clin Infect Dis. 2005;41(Suppl 7):S513–S519. doi:10.1086/432009

36. Xue N, Lin JH, Xing S, et al. Plasma macrophage migration inhibitory factor and cC3 as potential biomarkers for distinguishing patients with nasopharyngeal carcinoma from high-risk individuals who have positive Epstein-barr virus capsid antigen-specific IgA. Cancer Research Treatment. 2019;51(1):378–390. doi:10.4143/crt.2018.070

37. Li YS, Chen W, Liu S. Serum macrophage migration inhibitory factor levels are associated with infarct volumes and long-term outcomes in patients with acute ischemic stroke. Int J Neurosci. 2017;127(6):539–546. doi:10.1080/00207454.2016.1211648

38. Salem SA, Asaad MK. Evaluation of macrophage migration inhibitory factor (MIF) levels in serum and lesional skin of patients with alopecia areata. Int J Dermatol. 2016;55(12):1357–1361. doi:10.1111/ijd.13344

39. Shang ZB, Wang J, Kuai SG, Zhang YY, Ou QF. Serum macrophage migration inhibitory factor as a biomarker of active pulmonary tuberculosis. Ann Lab Med. 2018;38(1):9–16. doi:10.3343/alm.2018.38.1.9

40. Ning J, Xu L, Shen CQ. Increased serum levels of macrophage migration inhibitory factor in autism spectrum disorders. Neurotoxicology. 2019;71:1–5. doi:10.1016/j.neuro.2018.11.015

41. Xiang X, Wang Y, Zhang H, et al. Vasodilator-stimulated phosphoprotein promotes liver metastasis of gastrointestinal cancer by activating a beta1-integrin-FAK-YAP1/TAZ signaling pathway. NPJ Precis Oncol. 2018;2(1):2. doi:10.1038/s41698-017-0045-7

42. Witek MA, Aufforth RD, Wang H, et al. Discrete microfluidics for the isolation of circulating tumor cell subpopulations targeting fibroblast activation protein alpha and epithelial cell adhesion molecule. NPJ Precis Oncol;2017. 1. doi:10.1038/s41698-017-0028-8