

Relationship Between MUC4 Variants and Metastatic Recurrence in Colorectal Cancer

Jieqiong Liu¹, Rongge Xing², Jiakang Shao¹, Shunchang Jiao^{1,3}

¹Medical School of Chinese PLA, Beijing, 100853, People's Republic of China; ²Cangzhou Central Hospital, Cangzhou, Hebei Province, 061000, People's Republic of China; ³Department of Oncology, the First Medical Center, Chinese PLA General Hospital, Beijing, 100853, People's Republic of China

Correspondence: Shunchang Jiao, Department of Oncology, the First Medical Center, Chinese PLA General Hospital, 28 Fuxing Road, Haidian District, Beijing, 100853, People's Republic of China, Email jiaosc301@163.com

Background: Recurrent metastasis after radical resection in patients of colorectal cancer (CRC) is a great challenge for the world, in which genomic alterations play a major role in tumorigenesis. MUC4 plays a significant role in recurrence and metastasis in tumor. This study is aimed at exploring the association between MUC4 variants and metastatic recurrence of CRC.

Methods: Forty-seven patients relapsing with metastasis and 37 patients remaining disease-free postoperatively were enrolled. Next-generation sequencing (NGS) detected mutations. Mutation and mRNA expression data were downloaded from TCGA and cBioPortal databases. We analyzed the relationship between MUC4 variants and clinical parameters, as well as possible molecular mechanisms.

Results: MUC4 variants rs56359992 and rs781124621 were associated with survival in patients with CRC. Rs56359992 was more common in patients with metastatic recurrence. MAPK pathway, PI3K-Akt pathway, JAK-STAT pathway, cell cycle, WNT pathway and mTOR pathway were found to correlate with MUC4 mutation by GO/KEGG analysis, as well as resting and activated mast cell related to MUC4 mutation by CIBERSORT analysis.

Conclusion: Genetic variants of MUC4 with CRC may constitute a molecular signature of metastatic recurrence. MUC4 may become a new target for the treatment of CRC recurrence.

Keywords: colorectal cancer, MUC4, recurrence, next-generation sequencing

Introduction

Colorectal cancer (CRC) is a common malignant tumor that seriously threatens human life. Colorectal cancer is the third most common malignancy in cancer according to existing studies.¹ In 2020, CRC accounted for 10% of global cancer incidence and 9.4% of cancer deaths, just less than breast cancer and lung cancer. Based on population aging, population growth and human development, new CRC cases worldwide are expected to reach 3,200,000 in 2040.² According to data evaluation, in the next 20 years, the number of new CRC patients in China will increase from 560,000 in 2020 to 910,000 in 2040.³

Mucins are high molecular weight glycoproteins responsible for protecting, repairing, and surviving epithelial tissue in the gut.^{4,5} Mucins have a surface protective effect on epithelial cells by trapping pathogens and are also involved in cell signaling pathways.^{6,7} Several mucins have been found to be involved in the tumorigenesis of various solid tumors. In addition, limited studies are published about MUCs in CRC.⁸

MUC4 ordinarily lines the goblet cells and epithelial cells of the normal human small and large intestine.⁵ MUC4 has an effect on tumor proliferation, recurrence and metastasis. MUC4 promoted aggressive activity in triple negative breast cancer cells by altering the expression of EGFR, ErbB2, and ErbB3 molecules and their downstream signaling.⁹ In two pancreatic cancer cell lines, knocking down MUC4 downregulated lysosomal degradation of E-cadherin, resulting in downregulation of Wnt/ β -catenin signaling pathway. The above process eventually led to the proliferation and metastasis of the tumor.¹⁰ In stage I non-small cell lung cancer, MUC4 mutation was related to early recurrence.¹¹ Conflicting results associated with MUC4's role in colon tumor have been reported.¹² It was reported that overexpression of MUC4

in tissues obtained from colon cancer was related to a poorer prognosis.¹² However, others reported a loss of MUC4 in tissue samples from patients diagnosed with CRC.¹³

Recurrent metastasis after radical resection in CRC patients is a great challenge.¹⁴ About 60–80% of recurrences of CRC occurred 2 years after radical resection and 90% in the first 4 years.¹⁵ Metastasis is the main driver of CRC-related mortality, with liver, lung and peritoneal metastasis being the most commonly affected.^{16–18} CRC with peritoneal metastasis had a poor prognosis and was generally considered a terminal illness.¹⁹ A comparison of isolated site metastases among peritoneal, liver and lung metastasis reported median overall survival (OS): 16.3 months in peritoneal metastasis, 19.1 months in liver metastasis, and 24.6 months in lung metastasis.²⁰

Although many studies have shown abnormal expression of MUC4 in CRC,²¹ the relationship between genetic variants of MUC4 and metastatic recurrence in CRC is not clear. We analyzed genetic mutations of MUC4 in Chinese based on next-generation sequencing (NGS). Our study assessed the association of MUC4 variants with metastatic recurrence in CRC.

Materials and Methods

Study Population

This study included patients diagnosed with CRC who underwent radical surgery at Cangzhou Central Hospital from January 2018 to March 2021. A total of 84 patients with CRC stage I–III were enrolled: 47 patients in case group subsequently relapsed with metastasis; 37 age-matched patients in control group remained disease-free postoperatively. Clinicopathological data and follow-up data were collected from the hospital's case database and follow-up office respectively. We obtained information on registered patients including age, gender, time to diagnosis, TNM stage, nodules, lymphovascular invasion, perineural invasion, tumour localization, and their follow-up data on recurrence, metastasis, and survival. The study's inclusion criteria included: (1) Patients were not less than 18 years old; (2) Patients were pathologically diagnosed with CRC; (3) Patients had radical surgery. Patients would be excluded if they: (1) had metastasis; (2) comorbid other malignancies; (3) received neoadjuvant chemotherapy or targeted therapy or radiation therapy; (4) had no follow-up. This retrospective study was approved by the ethics committee of the Chinese PLA General Hospital and Cangzhou Central Hospital (S2022-305-01) and conformed to the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Genomic Data Analysis

DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) of tumor tissue specimens for NGS. We used noncancer Genome Aggregation Database (gnomAD) as representative of normal population controls. Genetic alterations were subsequently analyzed according to standard principles. Clinical and genetic data were downloaded from cBioPortal for Cancer Genomics (<https://www.cbioportal.org>).

mRNA Expression Profile Analysis

Corresponding mRNA expressions were downloaded from TCGA database. The expression data were divided into two groups including MUC4 mutation group and MUC4 wild type (MUC4 WT) group based on mutation data. R package was used for GO, KEGG, GSEA, ESTIMATE and CIBERSORT analysis and visualization.

Statistical Analysis

Data analyses were performed by using R software version 4.2.2 for Windows and SPSS 23.0. The Kaplan–Meier method and Log rank test were applied to determine survival function. Chi-squared test or Fisher's exact test was used to evaluate statistical heterogeneity. Differences between two groups were determined by Student's *t* test when data were normally distributed. The *p*-value obtained was considered statistically significant at a threshold of *p* < 0.05.

Results

Comparison of Baseline Characteristics

We consulted NCBI database and selected three genetic variants of MUC4 with mutation frequency $\geq 10\%$ in our study, including rs56359992, rs1560322790, and rs781124621. We assessed baseline characteristics of 84 patients (Table 1). Colon cancer, N stage (N1-N2), lymphovascular invasion and rs56359992 were more in the case group than control group, with $P=0.013$, $P<0.001$, $P=0.039$, $P=0.019$ respectively (Table 1). Other demographic and clinicopathological characteristics were not statistically significant in the comparison between the two groups.

Relationship Between MUC4 Mutations and CRC Metastasis Recurrence

Kaplan–Meier analysis was used for survival analyses (Figure 1). We found potential relations between genetic variants of MUC4 and patient survival. Variants rs56359992 (Figure 1A) and rs781124621 (Figure 1C) were related to prognosis. Variants rs1560322790 (Figure 1B) were not related to prognosis. We further analyzed the association of genetic mutations with specific metastasis of CRC, including liver, lung, peritoneal and other organs. 70.3% of rs56359992,

Table 1 Baseline Characteristics of Case and Control Group (n,%)

Parameter	Total (n=84)	Case Group (n=47)	Control Group (n=37)	P
Gender				0.759
Female	37 (44.0%)	20 (42.6%)	17 (45.9%)	
Male	47 (56.0%)	27 (57.4%)	20 (54.1%)	
Age				0.922
<60years	30 (35.7%)	17 (36.2%)	13 (35.1%)	
≥ 60 years	54 (64.3%)	30 (63.8%)	24 (64.9%)	
Tumour Localization				0.013
Colon	20 (23.8%)	16 (34.0%)	4 (10.8%)	
Rectum	64 (76.2%)	31 (66.0%)	33 (89.2%)	
T Stage				0.623
T1-T2	14 (16.7%)	7 (14.9%)	7 (18.9%)	
T3-T4	70 (83.3%)	40 (85.1%)	30 (81.1%)	
N Stage				<0.001
N0	32 (38.1%)	10 (21.3%)	22 (59.5%)	
N1-N2	52 (61.9%)	37 (78.7%)	15 (40.5%)	
Nodules				0.117
Yes	18 (21.4%)	13 (27.7%)	5 (13.5%)	
No	66 (78.6%)	34 (72.3%)	32 (86.5%)	
Perineural invasion				0.443
Yes	8 (9.5%)	6 (12.8%)	2 (5.4%)	
No	76 (90.5%)	41 (87.2%)	35 (94.6%)	
Lymphovascular invasion				0.039
Yes	12 (14.3%)	10 (21.3%)	2 (5.4%)	
No	72 (85.7%)	37 (78.7%)	35 (94.6%)	
MUC4 Mutations				
rs56359992				0.019
Yes	37 (44.0%)	26 (55.3%)	11 (29.7%)	
No	47 (56.0%)	21 (44.7%)	26 (70.3%)	
rs1560322790				0.267
Yes	18 (21.4%)	8 (17.0%)	10 (27.0%)	
No	66 (78.6%)	39 (83.0%)	27 (73.0%)	
rs781124621				0.381
Yes	11 (13.1%)	8 (17.0%)	3 (8.1%)	
No	73 (86.9%)	39 (83.0%)	34 (91.9%)	

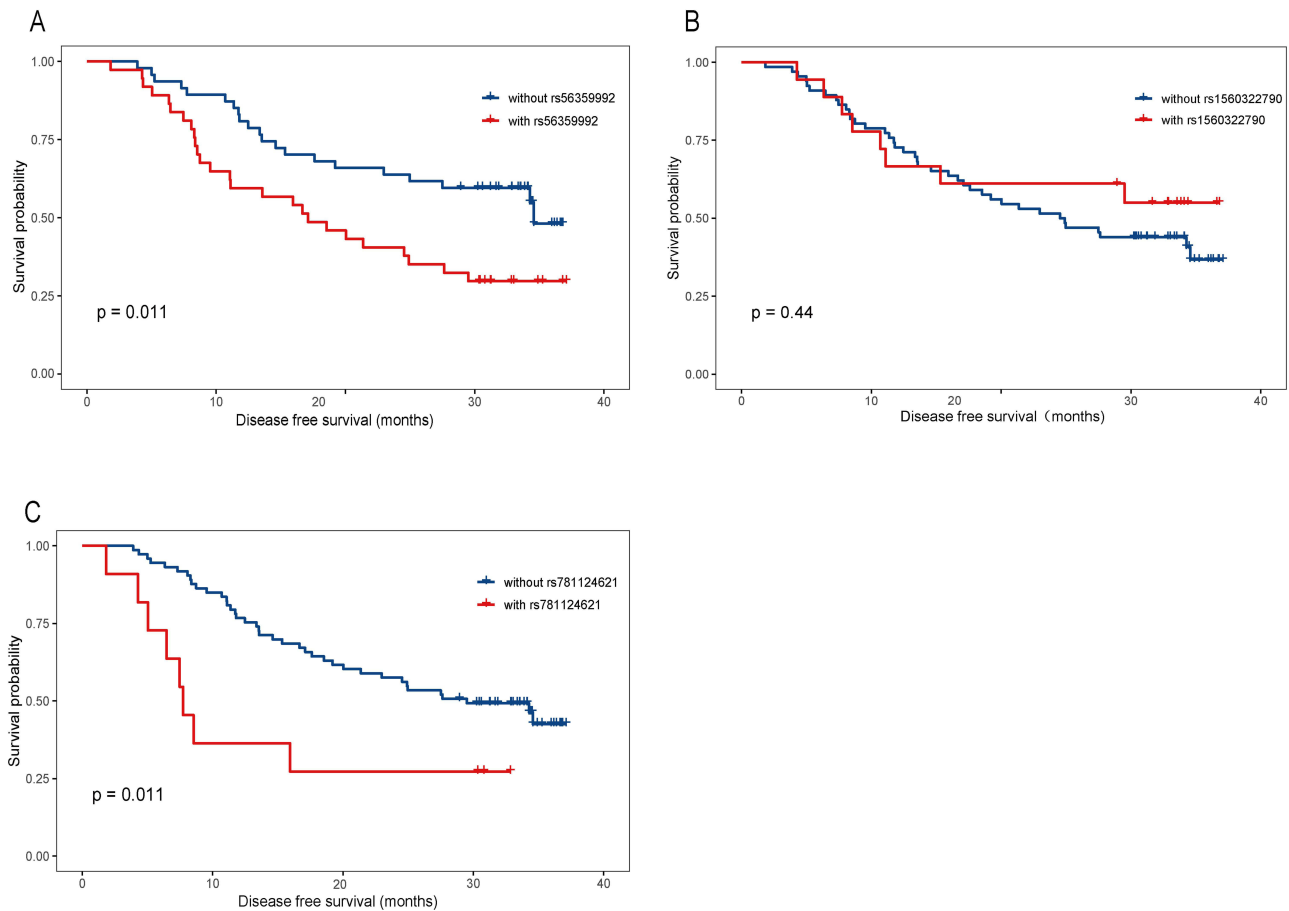


Figure 1 Correlation between MUC4 variants and prognosis in CRC patients. **(A)** Kaplan–Meier DFS curves for CRC patients with rs5635992 and without rs5635992. **(B)** Kaplan–Meier DFS curves for CRC patients with rs1560322790 and without rs1560322790. **(C)** Kaplan–Meier DFS curves for CRC patients with rs781124621 and without rs781124621.

44.4% of rs1560322790 and 72.7% of rs781124621 were metastasis (Figure 2). The analysis showed that different metastases were distributed differently in rs781124621, while no statistical significance in rs5635992 and rs1560322790 (Figure 2). We analyzed that patients with rs781124621 were more likely to have recurrent peritoneal metastasis (Figure 3).

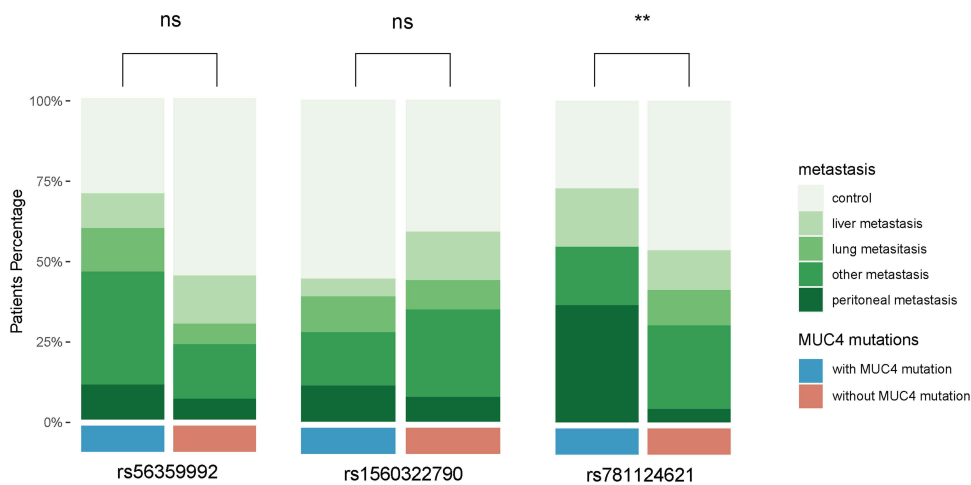


Figure 2 Relationship between MUC4 variants and CRC metastasis after radical surgery. The barplot showed the proportion of CRC patients with metastasis. **p<0.01.

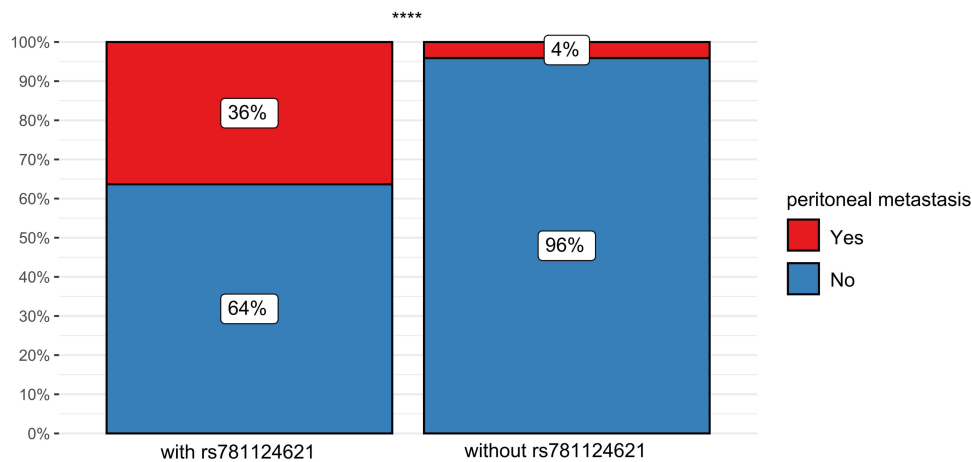


Figure 3 Relationship between variant rs781124621 and peritoneal metastasis. **** $p < 0.0001$.

Functional Enrichment Analysis

We downloaded data from public databases to further analyze possible biological processes involved in MUC4 mutation in CRC. We divided data into MUC4-mutated and MUC4-WT groups and performed GO, KEGG and GSEA analysis (Figure 4). The volcano map showed that 268 of differentially expressed genes (DEGs) were upregulated and 451 were downregulated (Figure 4A). Enrichment analysis showed that DEGs were mainly involved in some biological functions (Figure 4B) like cell–substrate junction, focal adhesion, and signaling pathways (Figure 4C) such as MAPK pathway, PI3K–Akt pathway, JAK–STAT pathway, cell cycle, WNT pathway and mTOR pathway. GSEA analysis also found immune activity like natural killer cell-mediated cytotoxicity (Figure 4D).

Immunological Correlation of MUC4 Mutation

MUC4 mutations were found to be associated with immune-related biological activity in our study. To obtain more specific results, we performed ESTIMATE and CIBERSORT analyses. The immunescore of the MUC4 mutated group was significantly higher than that of the MUC4 WT group (Figure 5). Analysis of infiltrated immune cells of two groups showed that activated mast cell was higher in MUC4-mutated group, while mast cell resting was higher in MUC4-WT group (Figure 6).

Discussion

CRC is a worldwide problem, and surgery is still the first-line treatment for early-stage CRC.²² Despite the improved initial cure rates for CRC, 20%–30% of stage I–III patients still experienced recurrences, including liver metastasis, lung metastasis, peritoneal metastasis and other types of recurrence.²³ Metastatic recurrence of tumors involved a series of complex processes. For example, epithelial–mesenchymal transition (EMT) can promote tumor recurrence and metastasis.²⁴ Early detection and treatment of disease recurrence were proved beneficial.²³ Patients were detected metastases by regular surveillance to improve prognosis,²⁵ but the results were not satisfactory. Therefore, scholars tried to find suitable predictors for CRC recurrence and prognosis.^{26–28} For this purpose, we investigated the relationship between CRC recurrence and MUC4 mutations.

First, we compared the baseline characteristics of patients and found that people with metastatic recurrences had more colon cancer, N stage (N1–N2) and lymphovascular invasion. Researchers found that anatomical location of primary tumor and N stage were related to tumor recurrence.^{29,30} Lymphovascular invasion was also detected to be associated with a high recurrence rate.²⁵ Therefore, recurrence of CRC was associated with clinicopathological features, such as tumor localization, N stage and lymphovascular invasion.

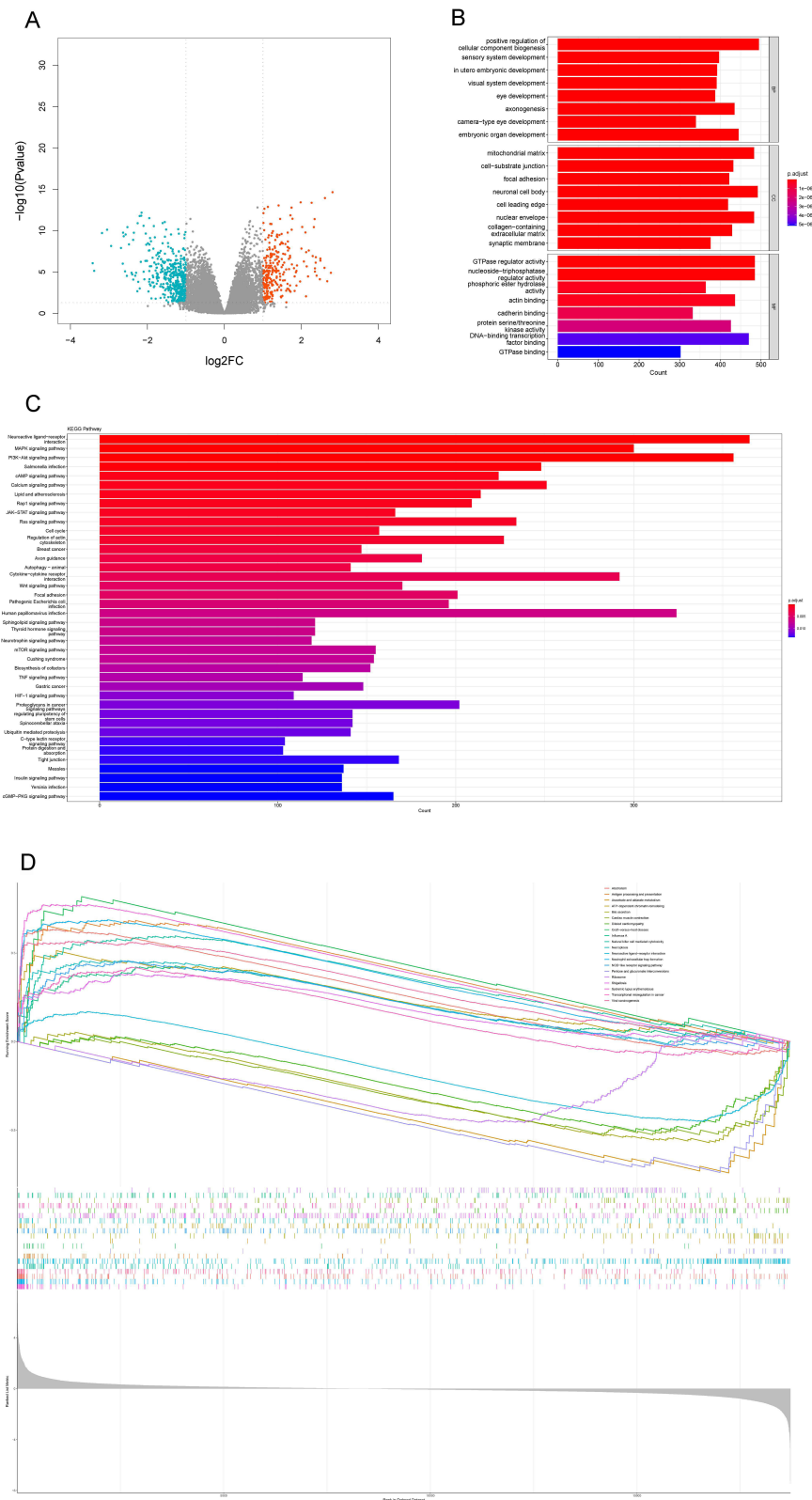


Figure 4 Analysis of biological activity associated with MUC4 mutations. **(A)** The volcano plot of the DEGs. **(B)** Gene ontology enrichment analysis. **(C)** Kyoto Encyclopedia of Genes and Genome pathway enrichment analysis. **(D)** Gene set enrichment analysis.

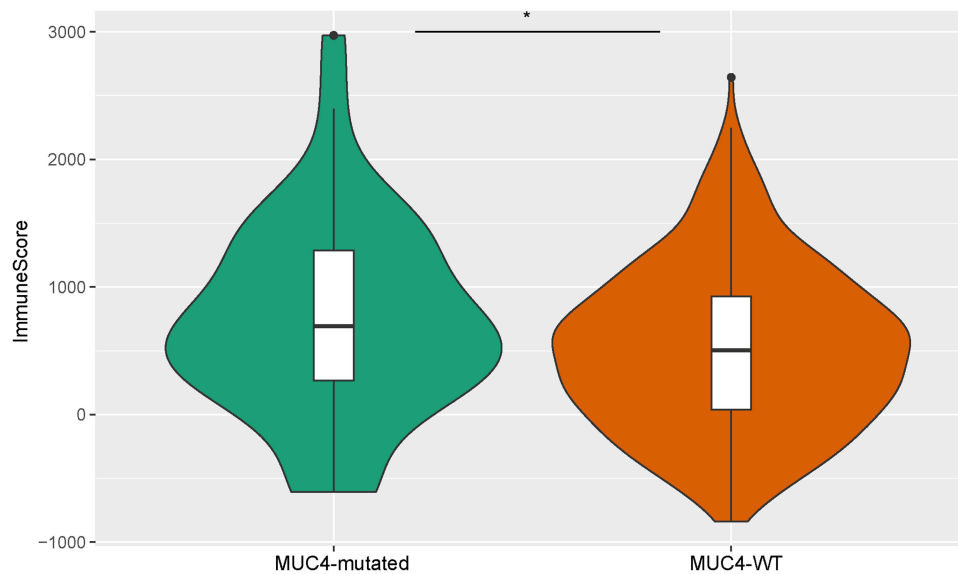


Figure 5 Immunescore of MUC4 mutated group and MUC4 WT group. * $p < 0.05$.

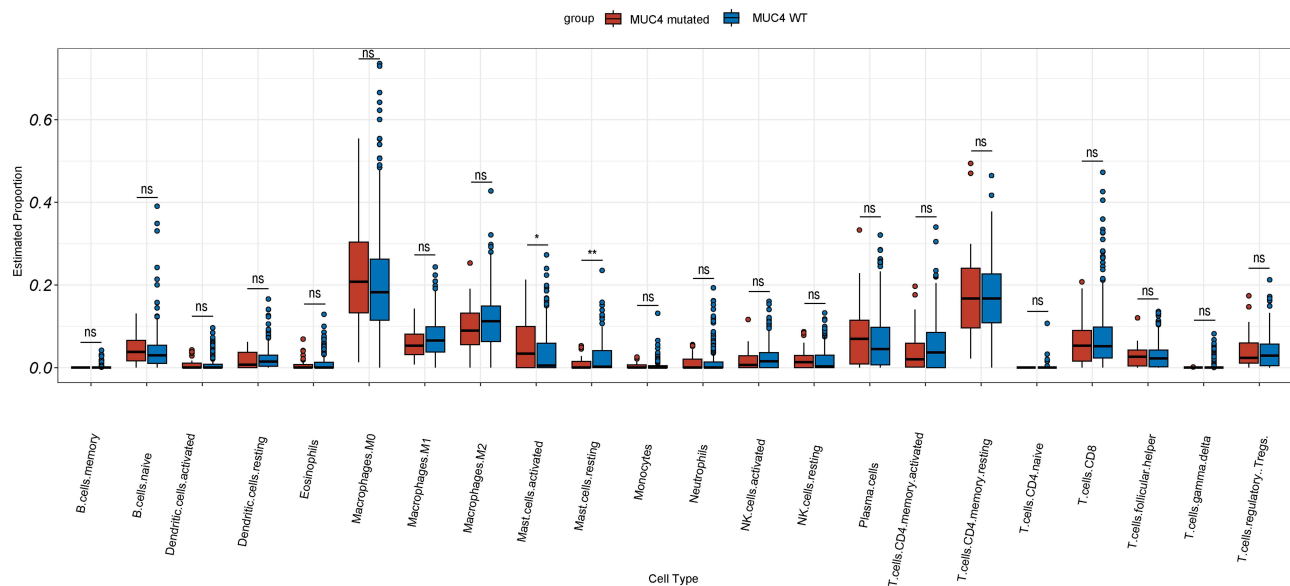


Figure 6 Distributions of immune cells in MUC4 mutated group and MUC4 WT group. * $p < 0.05$; ** $p < 0.01$.

In addition, we analyzed the relationship between MUC4 mutations and CRC prognosis and found that MUC4 variants rs56359992 and rs781124621 were associated with CRC prognosis. Meanwhile, MUC4 mutation rs56359992 was found associated with CRC metastatic recurrence.

Scholars looked for molecular markers to predict CRC recurrence and mucins had great exploration value. Mucins contain a large amount of clustered oligosaccharides O-glycosidically.³¹ Mucins play a role in cell adhesion, metastasis, and signal transduction.³² In the early stages of metastasis, mucins reduced cell adhesion, and changed matrix interaction, which gave cancer cells access to cell motility and invasion.³³ MUC4 was found to be involved in both tumor metastasis and progression.³⁴ Loss of MUC4 expression in gastric adenocarcinoma was significantly related to recurrence.³⁵ Previous studies showed that MUC4 was related to proliferation, invasion and distant metastasis of pancreatic cancer cells.³⁶ Mutations were found to be associated with recurrent metastasis of CRC.³⁷ MUC4 genetic variants were

associated with mortality and susceptibility to CRC.³⁸ These findings showed that MUC4 mutations were closely related to CRC metastasis and prognosis and could be novel biomarkers for the prediction of CRC patients' prognosis.

Moreover, we further analyzed the relationship between MUC4 variants and CRC metastasis, and found that CRC patients with rs781124621 were more likely to have peritoneal metastasis. Recurrent peritoneal metastasis in CRC patients was tricky. The first three sites of recurrent metastasis after radical CRC surgery were liver, lung and peritoneum.²⁵ The median overall survival of patients with peritoneal metastasis was found to be not optimistic and related to mucus histology.^{17,39} People were still not very clear about the genetic changes and mutations in peritoneal metastasis. Researchers found that mutations in KRAS and BRAF were associated with overall survival in peritoneal metastasis patients.⁴⁰ Peritoneal metastases were presumed to begin with the shedding of single cells or tumor masses by transmitting tumor cells through the adhesion molecule E-cadherin.⁴¹ Downregulation of E-cadherin was also involved in the EMT process, which could promote tumor metastasis.⁴² At the same time, high levels of E-cadherin were required for adhesion connections.⁴³ MUC4 loss in pancreatic cancer cells resulted in downregulation of lysosomal degradation of E-cadherin.¹⁰ MUC4 may promote peritoneal metastasis of CRC via E-cadherin. Thus, MUC4 mutation may play an important role in peritoneal metastasis in CRC patients.

Furthermore, we analyzed the biological activity associated with MUC4 mutations in CRC through GO, KEGG and GSEA analyses. We obtained some biological functions like cell–substrate junction and focal adhesion through GO analysis, and MAPK and PI3K–Akt signaling pathways through KEGG analysis, and natural killer cell-mediated cytotoxicity through GSEA analysis. Focal adhesion played a significant role in tumor cell survival, proliferation, differentiation and invasion.⁴⁴ MUC4 deletion led to a decrease in focal adhesion kinase which promoted tumor progression.^{45,46} MAPK and PI3K–Akt signaling pathways highly depended on calcium ion to activate key component proteins.⁴⁷ Regulating E-cadherin and β -Chain protein signaling promoted the progression of CRC through MAPK pathway.⁴⁸ Autophagy activated through PI3K/Akt/mTOR pathway could suppress cancer cell migration and invasion.⁴⁹ Enhancing the EMT process through PI3K/AKT/mTOR pathway can promote migration and invasion of CRC cells.⁵⁰ MUC4 was demonstrated to regulate the MAPK signaling pathway.⁵¹ These results suggested MUC4 may be involved in CRC recurrent metastasis by regulating MAPK pathway and PI3K-AKT pathway, but more experiments were needed to prove these conjectures.

Finally, we analyzed the relationship between MUC4 mutations and immune activity, and found immune score of MUC4 mutation group was higher than that of MUC4 WT group, and found that activated mast cells appeared higher in MUC4 mutation group. In our study, MUC4 mutations were also involved in inflammatory pathways in KEGG analysis, such as JAK-STAT signaling pathway. Meanwhile GSEA also found natural killer cell-mediated cytotoxicity. MUC4 protects disseminated tumor cells from immune recognition by masking various immunogenic cell surface antigens through its large glycosylated extracellular domain.⁴⁵ The physical interaction between MUC4 and platelets and immune cells enhanced the survival and extravasation of disseminated tumor cells.⁵² In CRC, mast cell was correlated with angiogenesis, migration and prognosis.⁵³ The relationship between MUC4 and mast cells was not clear. MUC4 and mast cells may influence CRC through interactions, which needed further verification. Our research suggested that MUC4 mutations were related to immune activity in CRC.

Several limitations in this study may affect the accuracy of experiment and further research is needed. Firstly, the DNA required for NGS was extracted from FFPE, resulting in more pseudo mutations than fresh or frozen tissue. Secondly, not enough patients were recruited to the point which may occur inaccurate results. Thirdly, no matching other omics data accompanied the NGS data for in-depth analysis. Finally, the potential targets found in this study required further biological validation.

Conclusion

Understanding molecular characteristics of metastatic recurrence of CRC was crucial to evolve future therapeutic strategies. NGS was performed on FFPE from CRC patients to show that MUC4 may play a key role in CRC metastatic recurrence especially peritoneal metastasis, and maybe a potential therapeutic target. This study investigated the molecular mechanism of CRC metastatic recurrence and provided some new insights for further genomic research.

Ethics Approval and Informed Consent

This retrospective study was approved by the ethics committee of the Chinese PLA General Hospital and Cangzhou Central Hospital (S2022-305-01) and conformed to the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Confidentiality and anonymity were assured as no personal identifiers were used, and the requirement for informed consent was therefore waived.

Acknowledgments

The authors wish to thank Lai Song for help of data analysis and the Beijing MyGenostics Co. Ltd. for technical support.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Yue X, Pengfei X. Global colorectal cancer burden in 2020 and projections to 2040. *Transl Oncol.* 2021;14(10):101174. doi:10.1016/j.tranon.2021.101174
2. Keum N, Giovannucci E. Global burden of colorectal cancer: emerging trends, risk factors and prevention strategies. *Nat Rev Gastroenterol Hepatol.* 2019;16(12):713–732. doi:10.1038/s41575-019-0189-8
3. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin.* 2021;71(3):209–249. doi:10.3322/caac.21660
4. Andrianifahanana M, Moniaux N, Batra SK. Regulation of mucin expression: mechanistic aspects and implications for cancer and inflammatory diseases. *Biochim Biophys Acta.* 2006;1765(2):189–222. doi:10.1016/j.bbcan.2006.01.002
5. Zeljkovic A, Vekic J, Mihajlovic M, et al. Revealing the Role of High-Density Lipoprotein in Colorectal Cancer. *Int J Mol Sci.* 2021;22(7):3352. doi:10.3390/ijms22073352
6. Kaur S, Kumar S, Momi N, et al. Mucins in pancreatic cancer and its microenvironment. *Nat Rev Gastroenterol Hepatol.* 2013;10(10):607–620. doi:10.1038/nrgastro.2013.120
7. Pai P, Rachagani S, Dhawan P, et al. MUC4 is negatively regulated through the Wnt/beta-catenin pathway via the Notch effector Hath1 in colorectal cancer. *Genes Cancer.* 2016;7(5–6):154–168. doi:10.18632/genesandcancer.108
8. Almasmoum H. The Roles of Transmembrane Mucins Located on Chromosome 7q22.1 in Colorectal Cancer. *Cancer Manag Res.* 2021;13:3271–3280. doi:10.2147/CMAR.S299089
9. Mukhopadhyay P, Lakshmanan I, Ponnusamy MP, et al. MUC4 overexpression augments cell migration and metastasis through EGFR family proteins in triple negative breast cancer cells. *PLoS One.* 2013;8(2):e54455. doi:10.1371/journal.pone.0054455
10. Zhi X, Tao J, Xie K, et al. MUC4-induced nuclear translocation of beta-catenin: a novel mechanism for growth, metastasis and angiogenesis in pancreatic cancer. *Cancer Lett.* 2014;346(1):104–113. doi:10.1016/j.canlet.2013.12.021
11. Hu C, Shu L, Chen C, et al. A prediction model integrated genomic alterations and immune signatures of tumor immune microenvironment for early recurrence of stage I NSCLC after curative resection. *Translational Lung Cancer Res.* 2022;11(1):24–42. doi:10.21037/tlcr-21-751
12. Shanmugam C, Jhala NC, Katkooi VR, et al. Prognostic value of mucin 4 expression in colorectal adenocarcinomas. *Cancer.* 2010;116(15):3577–3586. doi:10.1002/cncr.25095
13. Biemer-Hüttmann AE, Walsh MD, McGuckin MA, et al. Immunohistochemical staining patterns of MUC1, MUC2, MUC4, and MUC5AC mucins in hyperplastic polyps, serrated adenomas, and traditional adenomas of the colorectum. *J Histochem Cytochem.* 1999;47(8):1039–1048. doi:10.1177/002215549904700808
14. Kahlenberg MS, Sullivan JM, Witmer DD, et al. Molecular prognostics in colorectal cancer. *Surg Oncol.* 2003;12(3):173–186. doi:10.1016/S0960-7404(03)00006-9
15. Ergun Y, Bal O, Dogan M, et al. Does primary tumor resection contribute to overall survival in unresectable synchronous metastatic colorectal cancer? *J Res Med Sci.* 2020;25:14. doi:10.4103/jrms.JRMS_1056_18
16. Chandra R, Karalis JD, Liu C, et al. The Colorectal Cancer Tumor Microenvironment and Its Impact on Liver and Lung Metastasis. *Cancers.* 2021;13(24):6206. doi:10.3390/cancers13246206
17. Riihimäki M, Hemminki A, Sundquist J, et al. Patterns of metastasis in colon and rectal cancer. *Sci Rep.* 2016;6:29765. doi:10.1038/srep29765
18. Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. *CA Cancer J Clin.* 2015;65(2):87–108. doi:10.3322/caac.21262
19. Ceelen W, Ramsay RG, Narasimhan V, et al. Targeting the Tumor Microenvironment in Colorectal Peritoneal Metastases. *Trends Cancer.* 2020;6(3):236–246. doi:10.1016/j.trecan.2019.12.008
20. Franko J. Therapeutic efficacy of systemic therapy for colorectal peritoneal carcinomatosis: surgeon's perspective. *Pleura peritoneum.* 2018;3(1):20180102. doi:10.1515/pp-2018-0102

21. Lu S, Catalano C, Huhn S, et al. Single nucleotide polymorphisms within MUC4 are associated with colorectal cancer survival. *PLoS One*. 2019;14(5):e0216666. doi:10.1371/journal.pone.0216666
22. Shinji S, Yamada T, Matsuda A, et al. Recent Advances in the Treatment of Colorectal Cancer: a Review. *J Nippon Med School*. 2022;89(3):246–254. doi:10.1272/jnms.JNMS.2022_89-310
23. van der Stok EP, Spaander MCW, Grünhagen DJ, et al. Surveillance after curative treatment for colorectal cancer. *Nat Rev Clin Oncol*. 2017;14(5):297–315. doi:10.1038/nrclinonc.2016.199
24. Aiello NM, Kang Y. Context-dependent EMT programs in cancer metastasis. *J Exp Med*. 2019;216(5):1016–1026. doi:10.1084/jem.20181827
25. Miyake H, Kawai K, Nozawa H, et al. Less intensive surveillance after radical surgery for stage I-III colorectal cancer by focusing on the doubling time of recurrence. *Surg Today*. 2021;51(4):550–560. doi:10.1007/s00595-020-02135-y
26. Mo S, Ye L, Wang D, et al. Early Detection of Molecular Residual Disease and Risk Stratification for Stage I to III Colorectal Cancer via Circulating Tumor DNA Methylation. *JAMA oncol*. 2023;9(6):770–778. doi:10.1001/jamaoncol.2023.0425
27. Chen G, Peng J, Xiao Q, et al. Postoperative circulating tumor DNA as markers of recurrence risk in stages II to III colorectal cancer. *J Hematol Oncol*. 2021;14(1):80. doi:10.1186/s13045-021-01089-z
28. Turgunov Y, Ogizbayeva A, Shakeyev K, et al. The dynamics of the lipopolysaccharide-binding protein (LBP) level in assessing the risk of adverse outcomes in operated colorectal cancer patients. *Asian J Surg*;2023. S1015-9584(23)01268-X. doi:10.1016/j.asjsur.2023.08.101
29. Qaderi SM, Galjart B, Verhoef C, et al. Disease recurrence after colorectal cancer surgery in the modern era: a population-based study. *Int J Colorectal Dis*. 2021;36(11):2399–2410. doi:10.1007/s00384-021-03914-w
30. Yamano T, Yamauchi S, Tsukamoto K, et al. Evaluation of appropriate follow-up after curative surgery for patients with colorectal cancer using time to recurrence and survival after recurrence: a retrospective multicenter study. *Oncotarget*. 2018;9(39):25474–25490. doi:10.18632/oncotarget.25312
31. Byrd JC, Bresalier RS. Mucins and mucin binding proteins in colorectal cancer. *Cancer Metastasis Rev*. 2004;23:77–99. doi:10.1023/A:1025815113599
32. Mizejewski GJ. The adenocarcinoma cell surface mucin receptor for alpha-fetoprotein: is the same receptor present on circulating monocytes and macrophages? A commentary. *Tumour Biol*. 2014;35(8):7397–7402. doi:10.1007/s13277-014-2183-7
33. Ganguly K, Rauth S, Marimuthu S, et al. Unraveling mucin domains in cancer and metastasis: when protectors become predators. *Cancer Metastasis Rev*. 2020;39(3):647–659. doi:10.1007/s10555-020-09896-5
34. Dhanisha SS, Guruvayoorappan C. Pathological Implications of Mucin Signaling in Metastasis. *Curr Cancer Drug Targets*. 2023;23(8):585–602. doi:10.2174/1568009623666230320121332
35. Hwang I, Kang YN, Kim JY, et al. Prognostic significance of membrane-associated mucins 1 and 4 in gastric adenocarcinoma. *Exp Ther Med*. 2012;4(2):311–316. doi:10.3892/etm.2012.598
36. Chaturvedi P, Singh AP, Moniaux N, et al. MUC4 mucin potentiates pancreatic tumor cell proliferation, survival, and invasive properties and interferes with its interaction to extracellular matrix proteins. *Mol Cancer Res*. 2007;5(4):309–320. doi:10.1158/1541-7786.MCR-06-0353
37. Lan YT, Chang SC, Lin PC, et al. Clinicopathological and Molecular Features of Patients with Early and Late Recurrence after Curative Surgery for Colorectal Cancer. *Cancers*. 2021;13(8). doi:10.3390/cancers13081883
38. Kwon MJ, Lee JY, Kim EJ, et al. Genetic variants of MUC4 are associated with susceptibility to and mortality of colorectal cancer and exhibit synergistic effects with LDL-C levels. *PLoS One*. 2023;18(6):e0287768. doi:10.1371/journal.pone.0287768
39. Sadeghi B, Arvieux C, Glehen O, et al. Peritoneal carcinomatosis from non-gynecologic malignancies: results of the EVOCAPE 1 multicentric prospective study. *Cancer*. 2000;88(2):358–363. doi:10.1002/(SICI)1097-0142(20000115)88:2<358::AID-CNCR16>3.0.CO;2-O
40. Schneider MA, Eden J, Pache B, et al. Mutations of RAS/RAF Proto-oncogenes Impair Survival After Cytoreductive Surgery and HIPEC for Peritoneal Metastasis of Colorectal Origin. *Ann Surg*. 2018;268(5):845–853. doi:10.1097/SLA.0000000000002899
41. Roth L, Russo L, Ulugoel S, et al. Peritoneal Metastasis: current Status and Treatment Options. *Cancers*. 2021;14(1):60. doi:10.3390/cancers14010060
42. Loh CY, Chai JY, Tang TF, et al. The E-Cadherin and N-Cadherin Switch in Epithelial-to-Mesenchymal Transition: signaling, Therapeutic Implications, and Challenges. *Cells*. 2019;8(10):1118. doi:10.3390/cells8101118
43. Conacci-Sorrell M, Simcha I, Ben-Yedidia T, et al. Autoregulation of E-cadherin expression by cadherin-cadherin interactions: the roles of beta-catenin signaling, Slug, and MAPK. *J Cell Biol*. 2003;163(4):847–857. doi:10.1083/jcb.200308162
44. Duperret EK, Ridky TW. Focal adhesion complex proteins in epidermis and squamous cell carcinoma. *Cell Cycle*. 2013;12(20):3272–3285. doi:10.4161/cc.26385
45. Chaturvedi P, Singh AP, Chakraborty S, et al. MUC4 mucin interacts with and stabilizes the HER2 oncoprotein in human pancreatic cancer cells. *Cancer Res*. 2008;68(7):2065–2070. doi:10.1158/0008-5472.CAN-07-6041
46. Zhang P, Cao X, Guan M, et al. CPNE8 Promotes Gastric Cancer Metastasis by Modulating Focal Adhesion Pathway and Tumor Microenvironment. *Int J Biol Sci*. 2022;18(13):4932–4949. doi:10.7150/ijbs.76425
47. Liu L, Wu N, Wang Y, et al. TRPM7 promotes the epithelial-mesenchymal transition in ovarian cancer through the calcium-related PI3K / AKT oncogenic signaling. *J Exp Clin Cancer Res*. 2019;38(1):106. doi:10.1186/s13046-019-1061-y
48. Shen L, Zhang J, Xu M, et al. DDX3 acts as a tumor suppressor in colorectal cancer as loss of DDX3 in advanced cancer promotes tumor progression by activating the MAPK pathway. *Int J Biol Sci*. 2022;18(10):3918–3933. doi:10.7150/ijbs.73491
49. Zhang M, Liu S, Chua MS, et al. SOCS5 inhibition induces autophagy to impair metastasis in hepatocellular carcinoma cells via the PI3K/Akt/mTOR pathway. *Cell Death Dis*. 2019;10(8):612. doi:10.1038/s41419-019-1856-y
50. Liao H, Zhang L, Lu S, et al. viaKIFC3 Promotes Proliferation, Migration, and Invasion in Colorectal Cancer PI3K/AKT/mTOR Signaling Pathway. *Front Genet*. 2022;13:848926. doi:10.3389/fgene.2022.848926
51. Jonckheere N, Skrypek N, Merlin J, et al. The mucin MUC4 and its membrane partner ErbB2 regulate biological properties of human CAPAN-2 pancreatic cancer cells via different signalling pathways. *PLoS One*. 2012;7(2):e32232. doi:10.1371/journal.pone.0032232
52. Rowson-Hodel AR, Wald JH, Hatakeyama J, et al. Membrane Mucin Muc4 promotes blood cell association with tumor cells and mediates efficient metastasis in a mouse model of breast cancer. *Oncogene*. 2018;37(2):197–207. doi:10.1038/onc.2017.327
53. Liu X, Li X, Wei H, et al. Mast cells in colorectal cancer tumour progression, angiogenesis, and lymphangiogenesis. *Front Immunol*. 2023;14:1209056. doi:10.3389/fimmu.2023.1209056

International Journal of General Medicine

Dovepress

Publish your work in this journal

The International Journal of General Medicine is an international, peer-reviewed open-access journal that focuses on general and internal medicine, pathogenesis, epidemiology, diagnosis, monitoring and treatment protocols. The journal is characterized by the rapid reporting of reviews, original research and clinical studies across all disease areas. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/international-journal-of-general-medicine-journal>