

MTUS1 is Correlated with Immune Infiltration and Acts as a Promising Diagnostic and Prognostic Biomarker for Colorectal Cancer.

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Abstract

Background Colorectal cancer morbidity and mortality remain high, posing a serious threat to human life and health. Early diagnosis and prognostic evaluation are two major challenges addressed in clinical practice. MTUS1, is considered a tumor suppressor, plays important roles in inhibiting cell proliferation, migration and tumor growth. MTUS1 expression is decreased in a wide variety of human cancers, including CRC. However, the biological functions and molecular mechanisms of MTUS1 in CRC remain still ambiguous.

Methods: In this study, the data from The Cancer Genome Atlas (TCGA) database was analyzed using R statistical software (version 3.6.3.) to elucidate the diagnostic and prognostic value of MTUS1. In addition, we detected MTUS1 expression in colorectal tumor tissue and adjacent normal tissue using the GEO, TIMER and ONCOMINE and investigated the relationship between MTUS1 expression and clinicopathological characteristics. The correlation between MTUS1 expression and immune infiltrating level was identified via TIMER and GEPIA database. Furthermore, we constructed and analyzed PPI network and co-expression modules of MTUS1 to explore molecular functions and mechanisms.

Results: The CRC tissues exhibited higher MTUS1 mRNA expression levels than the normal tissues. The logistic regression analysis suggested that MTUS1 mRNA expression was associated with N stage, TNM stage, and neoplasm type. Patients with CRC exhibiting low MTUS1 mRNA expression were correlated with poor overall survival (OS). The multivariate analysis revealed that down-regulate expression of MTUS1 was an independent prognostic factor and was correlated with poor OS in patients with CRC. MTUS1 expression had a good diagnostic value based on ROC analysis. A group of potential MTSU1 interacting proteins and co-expressed genes was identified. GO and KEGG analyses showed that MTUS1 was involved in multiple cancer-related signaling pathways. Most importantly, MTUS1 expression was significantly related to the degree of multiple immune-cell infiltration. Moreover, MTUS1 expression strongly correlated with a variety of immune marker sets.

Conclusions: Our results suggested that MTUS1 may serves a promising biomarker for predicting diagnosis and prognosis of CRC patients and is expected to become a new molecular target for tumor immunotherapy.

Introduction

Colorectal cancer (CRC) is one of the principal causes of cancer-related morbidity and mortality worldwide (1). The most of colorectal cancer deaths arise from the primary tumor invading and metastasizing to other tissues (2). In recent years, with the continuous development of the treatment paradigms, especially clinical applications of targeted therapies and immunotherapy, the prognosis for CRC patients has greatly improved (3-5). However, not all patients have benefited equally. Resistance has developed in not a few patients to targeted anticancer drug in clinical applications, which has recently become a grand challenge (6). Low response rate and immune-related adverse effects have become two

major challenges addressed during the course of cancer Immunotherapy (7). The exact immune-related mechanisms of colorectal cancer remain to be elucidated. More worryingly, the incidence rates of colorectal cancers is increasing year by year and age distribution of patients with CRC tended to be young (8). When detected in early stages, patients with CRC usually have a good prognosis. However, most of the patients are in the mid-late stage of the disease at the time of diagnosis. The most important reason are the lack of both specific clinical symptoms and effective early diagnostic method at an early stage. Therefore, seeking a reliable diagnostic marker and immune-related therapeutic targets for patients with colorectal cancer has been a top priority.

MTUS1 (Microtubule Associated Scaffold Protein 1), a Protein Coding gene, is located on 8p22 chromosomal region and consists of 17 exons. MTUS1 encodes several of proteins with different functional properties, including ATIP1, ATIP3 (ATIP2, ATIP3a, and ATIP3b), and ATIP4 (9). Expression of ATIP1 and ATIP3 mediate cellular apoptotic mechanisms and interfere the growth promoting signals, and thereby affect the occurrence and progression of cancers (10-12). In addition, MTUS1 also is involved in the pathological process of cardiac hypertrophy, and SLE-like lymphoproliferative diseases (13). Previous studies have suggested that expression of MTUS1 was decreased in multiple cancers, including colorectal cancer and associated with poor prognosis lung adenocarcinoma, gastric cancer, renal cell carcinoma, gallbladder Carcinoma, salivary adenoid cystic carcinoma, and oral tongue squamous cell carcinoma (10, 14-17).

Regrettably, the mRNA expression level of MTUS1 and prognostic potential of MTUS1 in CRC remains largely unexplored. The connection between MTUS1 and tumor-infiltrating lymphocytes (TILs) in CRC had yet to be elucidated. Our study is the first to systematically analyzed MTUS1 expression and correlation with clinicopathological characteristics and prognosis of patients with CRC. The protein interaction network and enrichment analysis for MTUS1 are performed and plotted to reveal the molecular function of MTUS1 and its underlying regulation mechanism. More particularly, the relationship between MTUS1 and tumor infiltrating immune cells and markers was emphatically investigated. Our results demonstrate the potential prognostic and diagnostic value of MTUS1 and clarify tumor suppressor effects of MTUS1 in colorectal cancer. Thus, MTUS1 has the potential to become a novel diagnostic and prognostic marker and a predictor to assess the infiltration of immune cells for CRC patients, which will provides new ideas for clinical diagnosis and immunotherapy.

Materials And Methods

Data acquisition

The patient datasets, with gene expression profiles and paired clinical information, were downloaded from the TCGA data portal, and included 698 tissues (51 normal, 647 tumor). Subsequent processing excluded cases with missing data on age, overall survival time, TNM stage and distant metastasis. Finally, 643 cases with complete clinical information were devoted into univariate and multivariate regression analysis and analysis of immune infiltration. In addition, the colorectal microarray dataset

GSE23878 were downloaded from the public bioinformatics databases GEO (www.ncbi.nlm.nih.gov/geo) to validate the expression of MTUS1.

Oncomine database analysis

The Oncomine database (<http://www.oncomine.org>), which contains 715 datasets and 86,733 86733 tumor and normal samples, was used to probe dissimilarities of the MTUS1 mRNA expression between cancers and normal tissues. The thresholds were restricted as followed: P-value<1E-4; fold change: 2; gene rank: all; and data type: mRNA.

TIMER database analysis

TIMER (<https://cistrome.shinyapps.io/timer/>), a comprehensive web tool, could performed from analysis of immune infiltrates multiple perspective in human cancers. The correlation between the expression of MTUS1 and the abundance of tumor-infiltrating immune cells, including B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages and dendritic cells immune invasion was determined. We also compared tumor infiltration levels among tumors with different somatic copy number alterations for MTUS1. Additionally, the different expression of MTUS1 levels between a human patient's tumor and the adjacent normal tissues was analyzed by the TIMER.

GEPIA database analysis

GEPIA (<http://gepia.cancer-pku.cn/index.html>) is an online analysis tool of the RNA sequencing expression data based on TCGA and GTEx data. In the present study, the correlation between MTUS1 and gene markers of diverse tumor-infiltrating immune cells were analyzed through GEPIA. Spearman's rank correlation coefficient was used to determine the significance of correlations.

STRING database analysis

STRING (<https://string-db.org/>) can predicts protein-protein interactions and builds the network of functionally related proteins which we have leveraged.

Data processing and statistical analysis

The differences in MTUS1 mRNA expression levels in non-paired samples were compared using Wilcoxon rank-sum tests and Wilcoxon signed-rank tests were used to estimate expression differences in cases of paired samples. The median value of MTUS1 mRNA expression was set as the cut off value to stratify all cases of the patient datasets into MTUS1 mRNA high and low expression groups. The Kruskal-Wallis Test test and the chi-square test were used to assess correlation between MTUS1 expression and clinicopathological features. The Kaplan-Meier method were used to assess the distribution of OS and DSS between high MTUS1 and low MTUS1 groups, and differences between survival curves were compared by using the log-rank test. The univariate and multivariate Cox proportional hazard regression models were utilized to evaluate the effects of MTUS1 mRNA expression and clinicopathologic

characteristics on OS, and then we constructed the nomogram based on Cox proportional hazard regression models to determine independent prognostic factors. Moreover, the calibration curves was used to assess the predictive efficacy of the model. To investigate the potential diagnostic value of MTUS1, the receiver operating characteristic (ROC) curve was used to determine the area under the ROC curve. To better understand the function of MTUS1, we identified 50 co-expressed genes with the highest positive and negative correlation. Subsequently, the Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed to annotate their functions. In order to explore the role of MTUS1 in colorectal cancer further, we performed single-sample gene set enrichment analysis (ssGSEA) to investigate the impact of MTUS1 on fractions of various tumor-infiltrating immune cells. Associations between MTUS1 and the infiltration levels of different immune cells were examined using Spearman's correlation test and P-value. The R 3.6.3 software was used for statistical analyses. The p-value <0.05 was considered to indicate a statistically significant difference.

Results

The mRNA expression levels of MTUS1 in different types of human tumors.

In order to interrogate the expression level of MTUS1 in different human tumors and healthy tissues, we primarily analyzed the mRNA level of MTUS1 in multiple tumors and normal tissues by using the Oncomine database. The results showed that the expression of MTUS1 in head and neck cancer, kidney cancer, lymphoma, myeloma and other cancers was higher than that in normal tissues, while in brain and CNS cancer, breast cancer, colorectal cancer, head and neck cancer, kidney cancer, ovarian cancer, sarcoma, and lung cancer, MTUS1 mRNA expression was lower compared to their corresponding adjacent normal controls (Figure 1A). In order to further examine which cancers have differences in MTUS1 mRNA expression level, we detected the MTUS1 expression using the TIMER database. We found that MTUS1 expression in CHOL, KICH, STAD and THCA was found to be significantly higher compared to normal tissues. In contrast, MTUS1 was expressed at lower levels in 9 types of cancer (BLCA, BRCA, COAD, KIRC, KIRP, LUSC, PRAD, READ, UCEC) than in the corresponding normal controls (Figure 1B). Immediately following, we analyzed MTUS1 RNA expression data of the CRC tissues and normal tissues from TCGA (September 2021) using the Wilcoxon rank-sum test. The CRC tissues exhibited significantly lower MTUS1 mRNA expression levels than the normal tissues (Figure 1C). Additionally, the MTUS1 mRNA expression levels in 50 paired CRC and adjacent non-tumorous tissues were compared using Wilcoxon matched-pairs signed-rank test. Similarly, the result showed that MTUS1 mRNA was down-regulated expression in the CRC tissues.

Relationship between MTUS1 expression and clinicopathological parameters of colorectal cancer.

Because expression of MTUS1 was markedly up-regulated in CRC tissues, we further explored analyze the mRNA expression profiles of MTUS1 based on clinicopathological parameters. The Clinical characteristic data of 643 patients with CRC were downloaded from TCGA database in September 2021 (Table 1). The

expression of MTUS1 was significantly decreased in CRC compared with normal tissues according to the analysis results of the clinicopathological factors including gender, age, pathologic stage, T stage, N stage, M stage, CEA level, neoplasm type and survival status (Fig 2A-I). It is noteworthy that the expression of MTUS1 was apparently decreased in grade IV than in grade I and grade II in the tumor grade regard (Fig 2C). For the nodal metastasis status, MTUS1 expression was significantly downregulated in N2 compared to N0 (Fig 2E). Logistic regression was performed to further investigate the connection between MTUS1 expression and the clinicopathologic features of patients with cancer. All patients were divided into MTUS1 mRNA high and low expression groups using median expression (50%) as the cut-off. The logistic regression analysis demonstrated that decreased MTUS1 expression in CRC strongly associated with Pathologic stage (stage III/IV vs. stage I/II, OR = 0.626, p= 0.004), N stage (N1/N2 vs. N0, OR = 1.67, p= 0.008), and Neoplasm type (READ vs. COAD, OR = 0.699, p= 0.048) (Table 2). These results revealed that low expression of MTUS1 promoted tumor progression, the lymph node metastasis and distant metastasis of patients with CRC and MTUS1 may also have diagnostic and prognostic implications.

Association between MTUS1 expression and survival prognosis in patients with colorectal cancer.

To further identify the prognostic potential of MTUS1 in colorectal cancer, Kaplan-Meier survival analysis was performed using data from the TCGA. As shown in Fig 3, lower expression of MTUS1 is positively correlated with poor overall survival (HR=0.62, P = 0.009; Fig 3A) and disease specific survival (Fig 3B, HR=0.55, P = 0.012). As shown in Table 3, the univariate Cox regression analysis demonstrated that low MTUS1 expression was observably correlated with poor OS [P=0.009, HR= 0.625, 95% CI (0.439-0.890)]. Among other clinicopathologic factors, age, pathologic stage, T stage, N stage, M stage and CEA level is also strongly associated with OS (Table 3). In multivariate Cox regression analysis, the down-regulated MTUS1 expression, higher pathological stage are independent prognostic factors of worse outcome (Table 3). From this COX model, we additionally constructed nomogram to predict the survival probability at 1, 3, and 5 years (Fig 4A). Figure 4B demonstrates the calibration plots to verify the reliability of this prognostic model.

Diagnostic Value of MTUS1 in colorectal cancer.

The results and the analysis presented above suggest that a significant difference in MTUS1 expression between tumor and non-tumor tissue was observed and MTUS1 is an independent prognostic factor. Given that low MTUS1 expression correlates with poor outcomes, ROC curves were plotted and the areas under the ROC curves (AUC) were computed to further analyze the diagnostic value of MTUS1 for CRC and the larger AUC, the higher the diagnostic value. The results show that MTUS1 expression had a modest diagnostic value for in patients (AUC=0.880; Fig 5A) and for patients with stage I-II of cancer (AUC =0.857; Fig 5B), while MTUS1 has demonstrated a high diagnostic value for patients with stage III-IV of cancer (AUC =0.915; Fig 5C). Unfortunately, MTUS1 has low diagnostic value in the assessment of pathological stages (AUC =0.587; Fig 5D). These results fully demonstrate that MTUS1 exhibit the

diagnostic ability to identify CRC from the general population and is expected to become a promising diagnostic markers.

Identification and enrichment analysis of key MTUS1-interacting genes and proteins.

To investigate mechanism of MTUS1 in CRC, we identified key genes related to MTUS1 and performed a series of pathway enrichment analyses on these molecule. As shown in Figure 6A, the protein-protein interaction (PPI) network containing 51 nodes and 280 edges for MTUS1 was constructed using the STRING database. The ten genes most significantly associated with MTUS1 were AGTR2, CEP170B, ANKRD28, PMFBP1, CWH43, ANKRD52, PPP6R2, UPP2, BDKRB2 and LGI3. Subsequently, we exhumed the top 100 genes that correlated with MTUS1 expression in the CRC cohort and the first 50 positively and negatively correlated genes are shown in the heat map (Figure 6B-C). Based on these two sets of data we performed cross analysis and obtained a common gene, namely, SEC24A (Figure 6D). We further assessed the relationship between MTUS1 and SEC24A and the Spearman correlation coefficients were calculated, the result is shown in Figure 6E. Gene ontology analyses and KEGG pathway analysis of 150 genes from these two data sets revealed that these gene are involved in different pathways and biological process associated with cancer, as shown in Figure 6F-G. KEGG pathways include the HIF-1 signaling pathway, the leukocyte transendothelial migration, the cGMP-PKG signaling pathway, the chemokine signaling pathway and the sphingolipid signaling pathway. GO_BP (biological process) was mainly associated with cell growth, TRAIL-activated apoptotic signaling pathway, positive regulation of epithelial cell migration, regulation of apoptotic signaling pathway and adenylate cyclase-modulating G protein-coupled receptor signaling pathway. GO_MF (molecular function) was mainly related to exonuclease activity, 3'-5' exonuclease activity, G protein-coupled receptor binding, nucleobase-containing compound kinase activity and protein phosphatase binding. GO_CC (cell component) terms were cytosolic large ribosomal subunit, ESCRT complex, cytosolic part, cytosolic ribosome and heterotrimeric G-protein complex. These results revealed that it is highly likely that MTUS1 and MTUS1-related genes were involved in biological processes associated with the onset and progression of tumors, such as immune cell infiltration, tumor cell proliferation, cell migration and apoptosis of cells.

MTUS1 expression is correlated with immune Infiltration Level in colorectal cancer.

Previous research has demonstrated that the density of TILs within a tumour were an independent predictor of favorable disease free and overall survival (18-20). Therefore, the relationship between MTUS1 expression and the degree of various immune cell infiltration were assessed by ssGSEA algorithm (Fig 7A). As shown in Fig7B, MTUS1 expression was significantly positively correlated with T cells, CD8 T cells, activated dendritic cells, macrophages, T helper cells, Th1 cells, Th2 cells, central memory T cell (Tcm), effector memory T cell (Tem) and T follicular helper cell (TFH) infiltration and can negatively regulates infiltration of natural killer (NK) cells and regulatory T cells (Tregs). Next, to better understand the role of MTUS1 in CRC, we investigate the relationship between MTUS1 expression and immune infiltration and whether the copy number variation of MTUS1 is related to the infiltration levels of immune cells using TIMER. We found that MTUS1 expression correlates significantly with tumor purity, infiltrating

levels of CD8 + T cells and neutrophils both COAD and READ. MTUS1 also was related to immune infiltration of B cells, CD4 T cells, macrophages and dendritic cells in COAD. The results are shown in Figure 8A. Moreover, FIG 8B-C showed that the copy number variation of MTUS1 had different degrees of correlation with the infiltration levels of 6 kinds of immune cells. Results shown above suggest that MTUS1 be involved in the recruitment of immune cells. To further confirm the correlation between MTUS1 expression and immune infiltrating cells in colorectal cancer, we analyzed the immune markers of T cells, CD8+T cells, B cells, monocytes, TAMs, M1 and M2 macrophages, neutrophils, NK cells, dendritic cells, Th1 cells, Th2 cells, Th17 cells, TFH cells, Tregs cells, and T cell exhaustion by using the GEPIA web tool. The results showed that the expression level of MTUS1 in tumor tissues (particularly colon cancer tissues) and most of the immune marker sets of immune cells are closely related (Table 4).

Discussion

MTUS1 down-regulation in a variety of cancers has previously been reported. A significant down-regulation of the MTUS1 expression in colorectal cancer has also been reported. However, its diagnostic and prognostic value in CRC has not yet been investigated. In this study, we performed a comprehensive analysis of CRC data from public databases to explore the expression, survival, prognosis, co-expression network and immune infiltration of MTUS1. The expression of MTUS1 was low in CRC tissues compared with normal tissues, which is consistent with the results of previous study. MTUS1 expression was also associated with gender, age, pathologic grade, TNM stage, CEA level, neoplasm type and survival status. We explored whether the expression of MTUS1 was correlated with the prognosis of patients with CRC. Low expression levels of MTUS1 were significantly correlated with worse OS and DSS, which indicates CRC patients with low MTUS1 expression levels showed a trend of high survival risk. Results of the logistic regression showed that MTUS1 expression in CRC was associated with advanced pathologic grade and N stage, which suggested that MTUS1 may play a critical role invasion and lymph node metastasis. Metastatic dissemination of the primary disease is responsible for most cancer-associated mortality. This could perhaps explain why low MTUS1 mRNA expression can lead to poor prognosis in CRC patients. Moreover, the results of our Cox model analysis also confirmed low mRNA expression of *mtus1* was an independent indicator of poor prognosis. The nomogram we constructed to predict the survival probability at 1, 3, and 5 years shows good predictive performance. In addition, the results of ROC curve analysis demonstrate potential diagnostic values of MTUS1. In order to explore the molecular mechanism and function of MTUS1 in the pathogenesis of CRC, we performed KEGG and GO term gene set enrichment analysis based the interacting proteins and co-expressed genes with MTUS1, proving the MTUS1 protein were involved in multiple pathways related to the development of cancer, including the Leukocyte transendothelial migration, the cGMP-PKG signaling pathway and the Chemokine signaling pathway. Furthermore, our study first provide evidence that immune infiltration levels and diverse immune marker sets are associated with levels of MTUS1 expression, which will help us to better understand the role of MTUS1 in tumor immunology.

Previous studies have shown that the mRNA expression level MTUS1 correlated with cell phenotypes such as cell proliferation, differentiation, apoptosis and ubiquitination and can serve as a prognostic

indicator for multiple cancers. MTUS1 can regulate the process of cell division through disturbing microtubule cytoskeleton and has also demonstrated utility to predict treatment response to paclitaxel-based chemotherapy in breast cancer (21-23). In lung adenocarcinoma tissues, low MTUS1 expression is related to various clinical pathological parameters such as tumor size, Ki-67 proliferation index, lymphovascular invasion and lymph node metastasis, which leads to a correspondingly poor prognosis of patients (14). The expression level of MTUS1 is synergistically inhibited by miR-19a and miR-19b, thereby contributing to lung cancer cell proliferation and migration (24). The interaction between lncRNA LIFR-AS1 with MTUS1 is considered to block the MEK/ERK pathway, thereby inhibiting gastric carcinoma cell proliferation, migration and invasion (16, 25). One of the major isoforms produced by the MTUS1 gene, ATIP3a, can exert obvious anti-proliferative and anti-migration effects on SACC (salivary adenoid cystic carcinoma) cells by regulating the ERK-Slug signaling pathway (11). Furthermore, the functional effect and the prognostic value of MTUS1 low expression in other cancers as well were successively confirmed (10, 15, 17, 26, 27). Although down-regulated expression and tumor-suppressor function of MTUS1 have also been reported in colorectal cancer, the specific molecular mechanism underlying MTUS1 expression remains to be elucidated (28, 29). In particular, research on the relationship between MTUS1 expression and tumor-infiltrating immune cells has not been reported. As we know, the status and density of tumor-infiltrating lymphocytes can predict prognosis of cancer and relates to tumorigenesis and tumor progression and play the critical role in anti-tumor immune therapy (30-32).

Thus, the unique feature of this study is we comprehensively explored the relationship between MTUS1 expression and typical markers of different types of immune cells, the potential impact for the recruitment of immune cells to the tumor microenvironment, along with the potential immune-related mechanism mediated by MTUS1. Results of immune infiltration analysis by using ssGSEA algorithm show a significant positive correlation between MTUS1 expression and infiltrating levels of T cells, Th1 cells, Th2 cells, central memory T cell (Tcm), T helper cells, and CD8 T cells and a certain level of negative correlation between MTUS1 expression and Tregs and NK cells infiltration. We similarly find that MTUS1 expression level correlates with degrees of B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages and dendritic cells infiltrations in TIMER. These correlations may suggest MTUS1 has the potential to recruit immune T cells, Th1, Th2, Tcm, T helper cells, CD4+ T cells and CD8 T cells into the tumor microenvironment and prevents recruitment of Tregs that promote immune tolerance and angiogenesis. CD8+ T cells can seek out tumor antigens and directly kill tumor cells which plays a crucial role in the adaptive immune response against cancer (33-35). Th1 cells and dendritic cells also mediate cellular immune responses against tumor (36-38). Moreover, NK cells and neutrophils also participate in the anti-tumor immune response (39, 40). In contrast, Tregs suppress anti-tumor immune responses contributing to tumor immune evasion, although they also are required to control immune responses and maintain the homeostasis of tumor microenvironment (41, 42). Results from expression correlation analysis by using GEPIA show that MTUS1 expression significantly correlates with the expression of CD8A, CD8B of CD8+ T cell, CD3D, CD3E, CD2 of T cell, CD79A of B cell, CD86, CSF1R of Monocyte, CD68 of TAM, NOS2 of M1 Macrophage, KIR2DL1, KIR2DL3, KIR2DL4, KIR3DL1, KIR3DL2, KIR3DL3 of NK cell, TBX21, STAT4, STAT1, IFNG of Th1, GATA3, STAT6, STAT5A, IL13 of Th2, FOXP3,

CCR8, TGFB1 of Treg, and PDCD1, CTLA4, LAG3, HAVCR2 of T cell exhaustion ($P < 0.05$). The results are consistent with both of these previous studies.

The above findings would seem to suggest that MTUS1 may play a key role in regulating immune cell infiltration as an immune modulating factor in CRC. Nevertheless, further in-depth exploration is needed to understand the precise functions and mechanism of MTUS1 in the tumor immune microenvironment. Our study provides new insights into cancer immunotherapy. However, this current study has certain limitations. We only performed a bioinformatics analysis using patient's data from the TCGA portal. Therefore, it is difficult to judge the true impact of MTUS1 on development-related signaling pathways and biological behavior. More experiments need to be performed to further verify the expression and related mechanisms of MTUS1 and elucidate the true associations between MTUS1 expression and tumor-infiltrating immune cell.

In summary, MTUS1 expression is significantly decreased and clearly correlated with the clinicopathologic stages and prognosis of CRC patients. MTUS1 and associated pathway changes are involved in colorectal cancer development and progression. Moreover, MTUS1 expression may affect the level of immune cell infiltration. Therefore, MTUS1 is a meaningful diagnostic and sensitive prognostic marker and is involved in immune cell infiltration in the immune microenvironment of colorectal cancer. This study is expected to provide a promising new direction for clinical diagnosis and treatment.

Declarations

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Authors' contributions

Lin-Yao Cheng: Writing-Original Draft Preparation. Hai-Ming Ru: Writing-review and editing. Mao-sen Huang: Data Analysis and Interpretation. Si-Si Mo: Data Analysis and Interpretation. Chun-Yin Wei: Data Analysis and Interpretation. Hua-Ge Zhong: Writing-review and editing. Zi-Jie Su: Writing-review and editing. Xian-Wei Mo: Writing-review and editing. Lin-Hai Yan: conceived and designed this project. Wei-Zhong Tang: Supervision. All authors participated in writing or revising the manuscript.

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Availability of data and materials

The datasets supporting the conclusion of this article are included within the article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Due to technical limitations, tables are only available as a download in the Supplemental Files section.

Figures

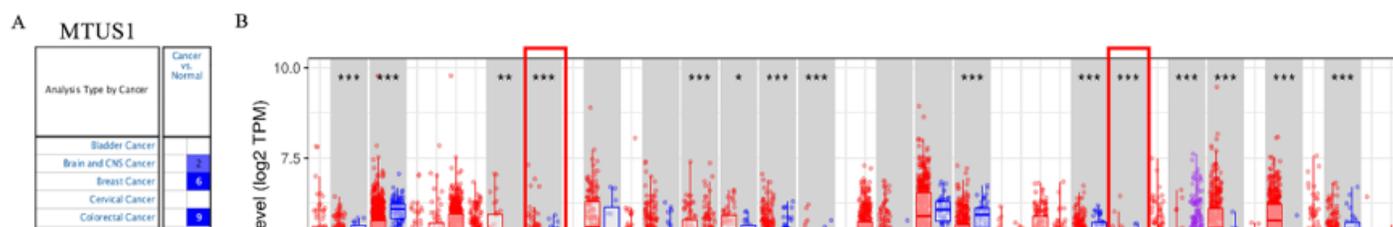


Figure 1

Expression of MTUS1 in different types of cancer. (A) MTUS1 expression levels in data sets of different cancers compared with normal tissues in the Oncomine database. 9 studies have confirmed that MTUS1

expression is downregulated in colorectal cancer. (B) MTUS1 expression levels in different types of cancer in TIMER database (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). The result is consistent with Oncomine database. (C) MTUS1 mRNA expression in the tumor and normal tissues from TCGA data. (D) Comparison of MTUS1 expression in 50 pairs of tumor and adjacent tissues. (E) The expressions of MTUS1 from GSE13507 array data of the GEO database.

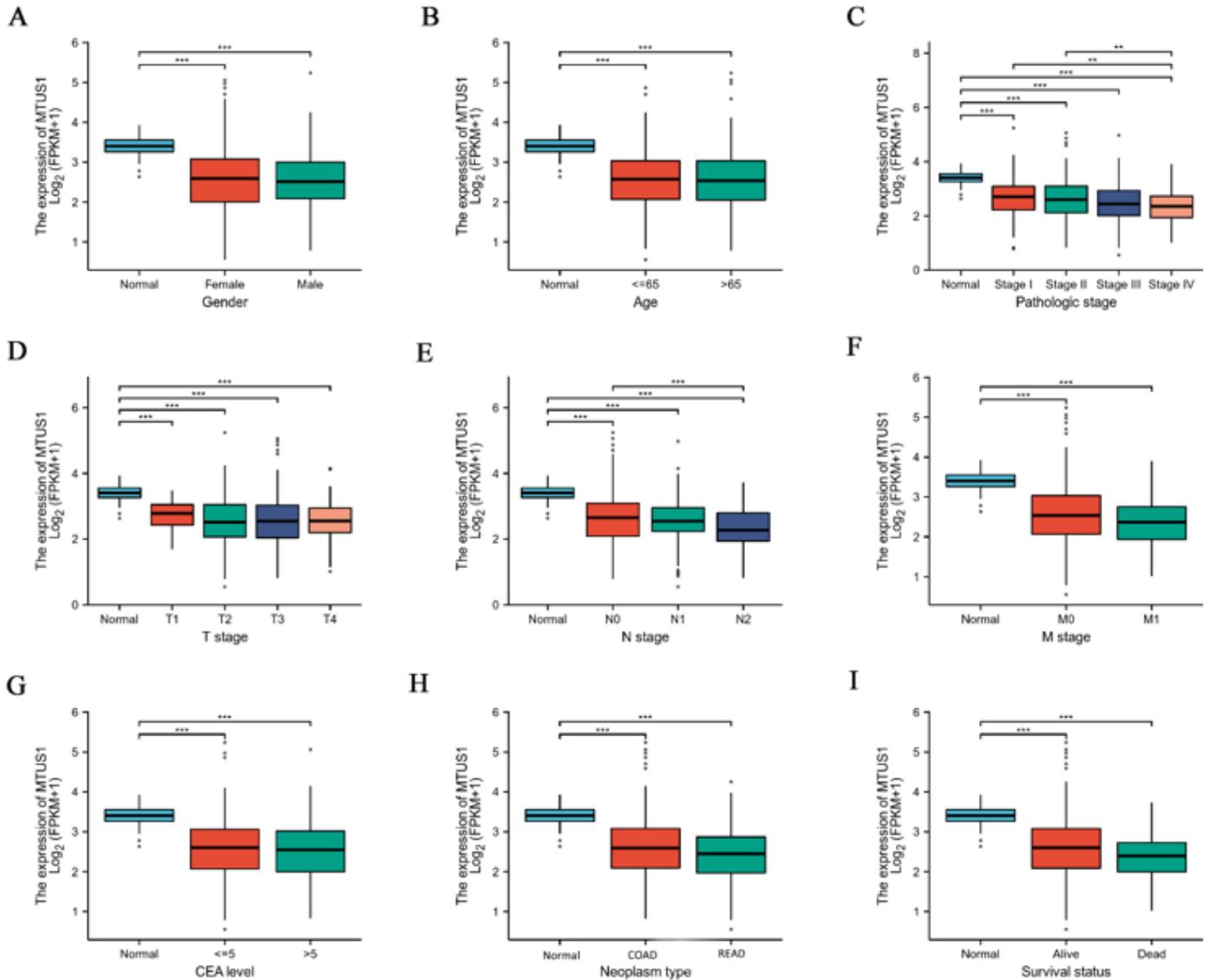


Figure 2

Differential expression analysis in different (A) gender, (B) age, (C) pathologic stage, (D) T stage, (E) N stage, (F) M stage, (G) CEA level, (H) neoplasm type and (I) survival status.

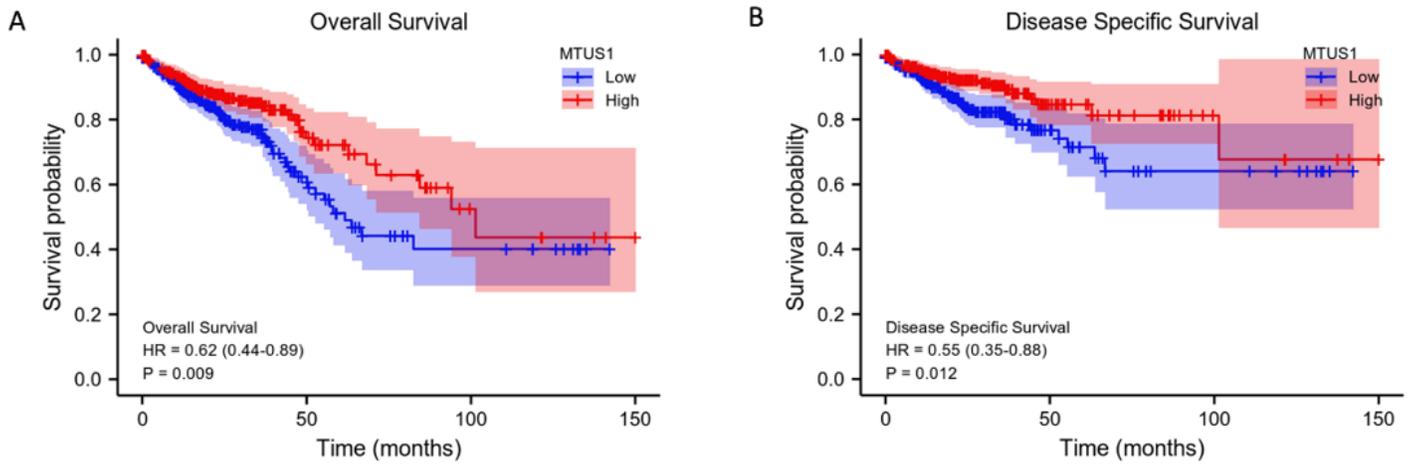


Figure 3

Kaplan–Meier survival curves comparing (A) OS and (B) PFI among MTUS1 high expression and low expression in CRC to determine the prognostic value of MTUS1.

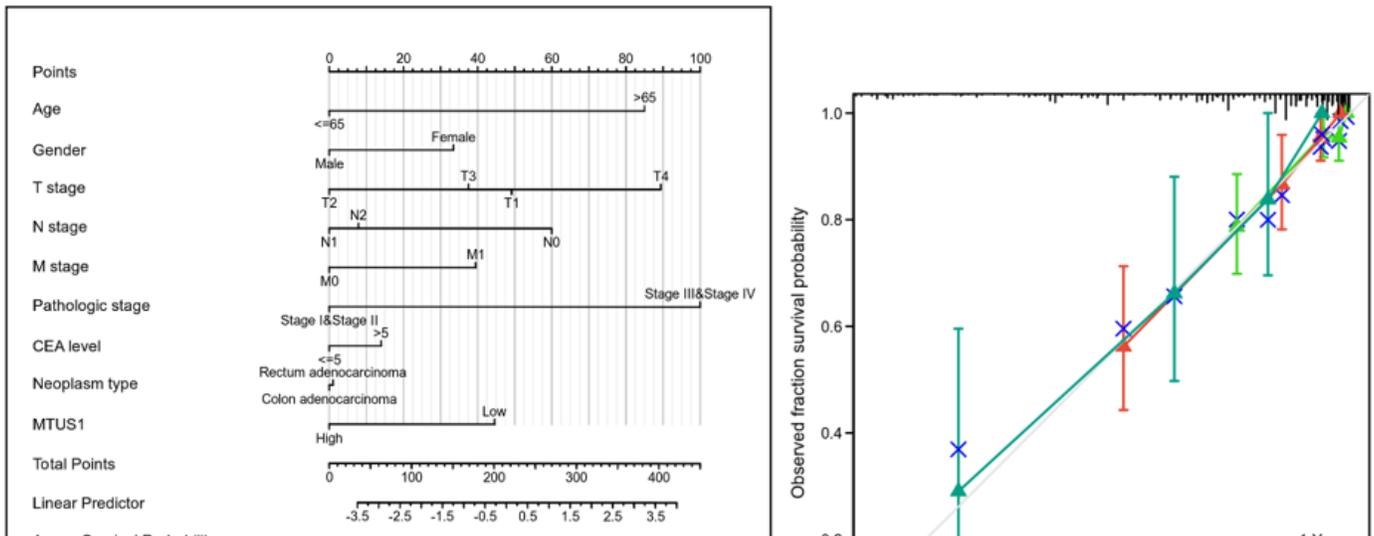


Figure 4

(A) Nomogram was established for predicting the survival probability at 1, 3, and 5 years of patients with CRC based on multi-factor regression analysis. (B) The calibration plots shows a good prediction performance of this prognostic model.

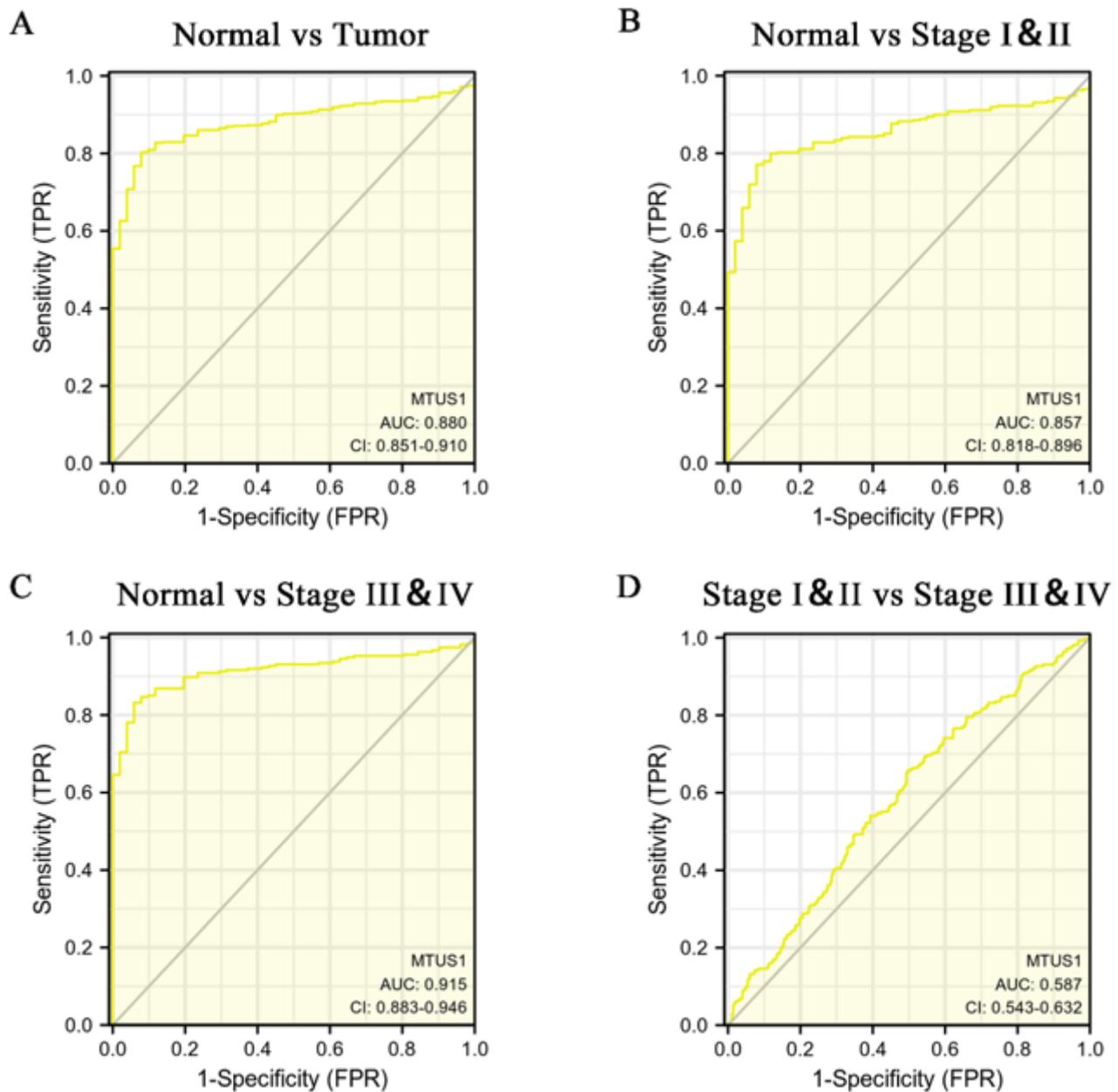


Figure 5

Receiver operating characteristic analysis of MTUS1 expression in normal vs. cancerous tissues overall (A), normal vs. stage I&II cancerous tissues (B), normal vs. stage III&IV cancerous tissues (C), stage I&II vs. stage III&IV cancerous tissues (D).

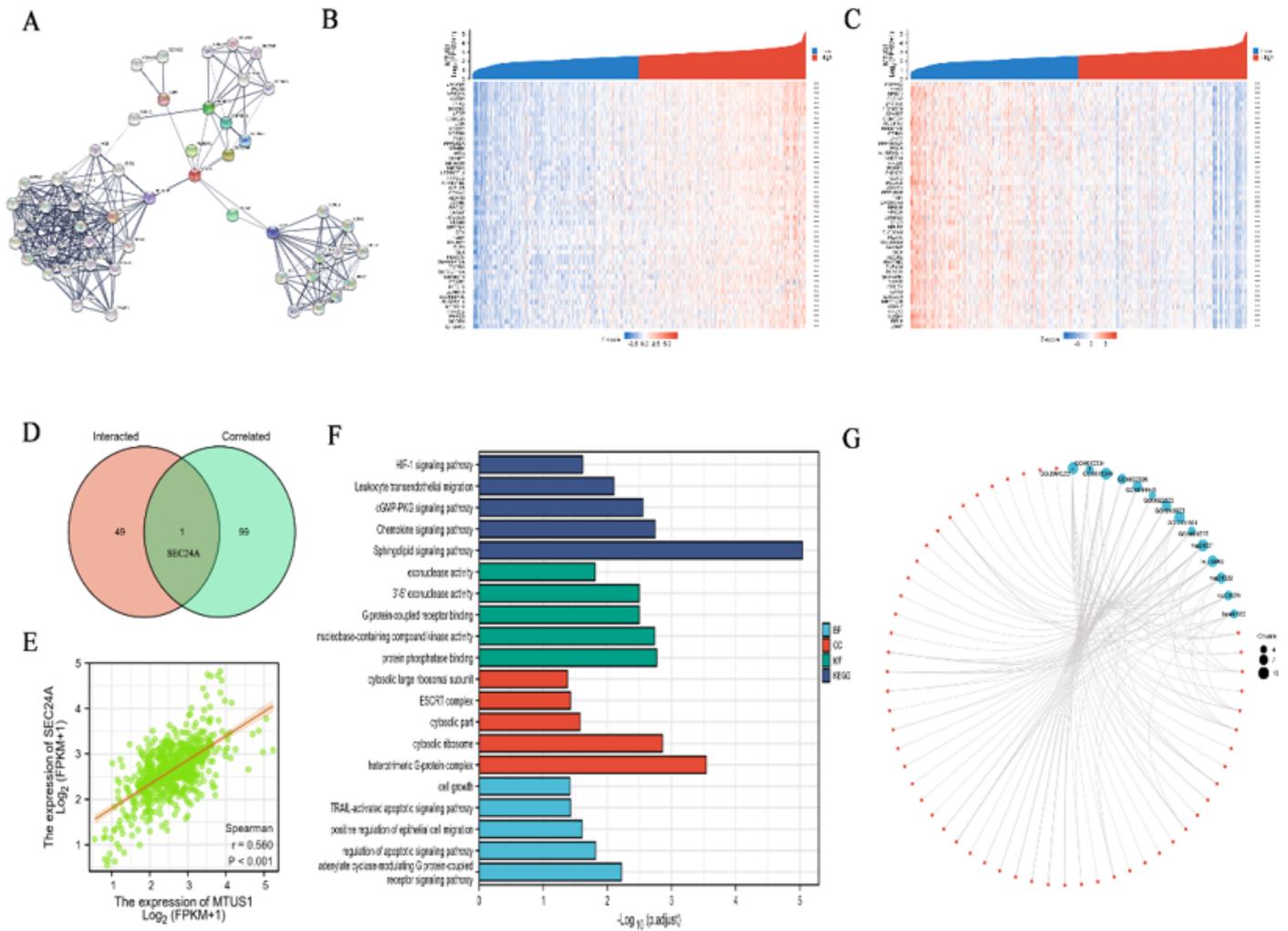


Figure 6

MTUS1 related gene enrichment analysis. (A) 50 MTUS1-binding proteins were determined by the STRING database. We obtained the first 100 positively (B) and negatively (C) co-expression genes with MTUS1 in TCGA projects. (D-E) An intersection analysis of the MTUS1-binding and correlated genes was performed. (F) The MTUS1-binding and interacted genes were subjected to GO and KEGG pathway analyses and (F) Molecular interaction networks were built according to functional clustering.

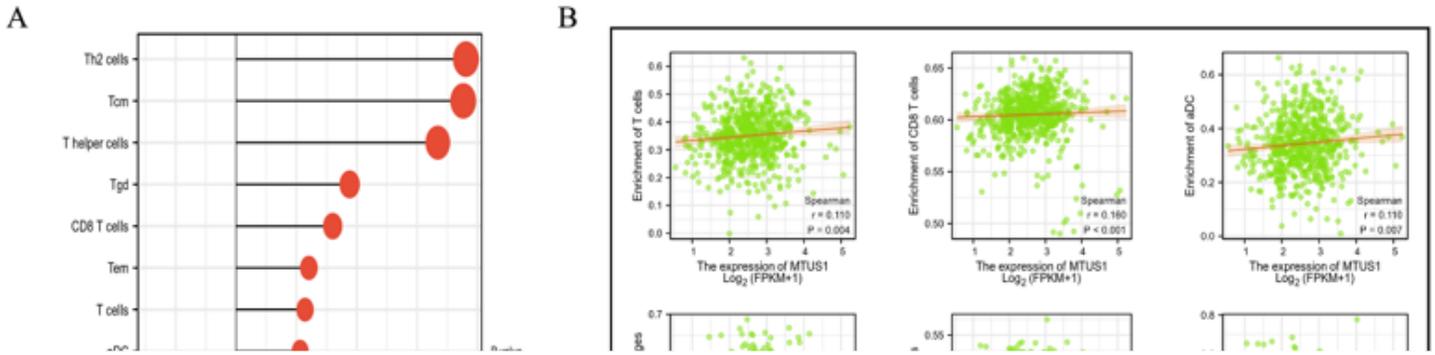


Figure 7

The association between MTUS1 expression and infiltration levels of immune cell was investigated using the ssGSEA algorithm. The results are presented in lollipop diagram (A) and scatter plot (B) form.

Figure 8

Correlations between MTUS1 expression with immune B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages and dendritic cells in COAD and READ was retested by TIMER (A) and we also identified the impact of the copy number variation of MTUS1 on the infiltration levels of 6 kinds of immune cells (B).

Supplementary Files

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