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Effect of *MPP2* and its DNA methylation levels on prognosis of colorectal cancer patients

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Abstract

Colorectal cancer is one of the common malignant tumors with poor prognosis, which is partly due to the lack of an effective biomarker. The purpose of this study is to explore the impact of membrane palmitoylated protein (MPP2) on the prognosis of colorectal cancer patients. We obtained transcriptome data and DNA methylation data of 380 colorectal cancer patients from the Cancer Genome Atlas (TCGA). Then we used a series of bioinformatics analysis methods to reveal the relationship between MPP2 expression, DNA methylation sites, prognosis, immune checkpoint and clinical characteristics of patients. Finally, in vitro experiment and the meta-analysis of thousands of patients further confirmed the impact of MPP2 on the prognosis of colorectal cancer patients and cell migration and proliferation. The expression level of MPP2 is negatively regulated by its DNA methylation sites, which leads to its low expression in colorectal cancer. High expression of MPP2 is an independent prognostic risk factor, which may be a biomarker for colorectal cancer. Moreover, the expression of MPP2 shows a close relationship with immune checkpoint or immune cells infiltration, especially $CD4^+$T cells. In addition, meta-analysis involving 1584 patients from four different data further confirmed that MPP2 was a risk factor for colorectal cancer. Finally, knockdown of MPP2 could significantly inhibit the proliferation of SW480 cells via mTOR signaling pathway. This study was the first to suggest that MPP2 may become a promising biomarker, and has an important role in immune checkpoint or immune cell infiltration in colorectal cancer.

Keywords Colorectal cancer, MPP2, Biomarker, DNA methylation, Immune cells

Introduction

Colorectal adenocarcinoma was a fatal malignant tumor originated from polyps, which has become the fourth leading cause of cancer-related death about 900,000 patients deaths annually [1]. Although surgical resection, chemoradiotherapy and even more advanced immunotherapy have improved the prognosis of patients, the prognosis of patients with metastasis is still unsatisfactory [2]. The main reason for this depressing situation is that the mechanism of colorectal pathology has not been fully elucidated until now. Current studies have confirmed that the incidence of colorectal cancer was related to factors such as eating habits [3] and bodyweight [4], but the most important factor is caused by genetic and epigenetic abnormalities [5]. Therefore, a more

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comprehensive understanding of the regulatory mechanism of genetics and epigenetics in the pathological process of colorectal cancer may provide a theoretical basis for individualized diagnosis and treatment of colorectal cancer patients, which may improve the prognosis of patients.

With the development of sequencing technology, countries around the world are trying to build professional databases of various types of diseases, so as to promote the research of basic medicine and reveal the pathological mechanism of disease process more quickly and accurately [6]. TCGA, GEO and TIMER database have been established under this background, and the research of using these databases to reveal the pathogenesis of diseases is gradually improving. For example, previous studies combined TCGA database and GEO database to reveal the effect of LncRNA related ceRNA network on the prognosis of patients with glioblastoma [7]. Similar studies also revealed that HOXA2 activation of JAK-STAT-signaling pathway leads to a decrease in the overall survival time of patients through the joint analysis of CGGA database and TCGA data [8]. It has to be emphasized that there are similar studies in colorectal studies, which confirm that LncRNA MIR4435-2HG regulates VEGF pathway and leads to poor prognosis [9]. Based on the above introduction, this study attempts to use these public databases to fully reveal the functional mechanism of MPP2 in colorectal cancer patients.

The official website of the National Center for Biotechnology Information (NCBI) search found that MPP2 was one of the main members family of membrane-associated guanylate kinase homologs (MAGUKs) family, which has interaction with cytoskeleton, and can regulate cell proliferation and intracellular junctions. MPP2 contains a conserved sequence, called the SH3, which was suspected to play important roles in signal transduction. Through literature search, only studies have found that the expression level of MPP2 was down-regulated in glioblastoma, which is a key oncogene in the pathological process of gliomas [10]. This study attempts to reveal the relationship between MPP2 mRNA expression and its DNA methylation, immune cell infiltration, immune checkpoint, prognosis, or clinical characteristics of patients based on the big data comprehensive analysis of hundreds of tissue samples. This study first proposed that MPP2 is a novel oncogene in colorectal cancer patients, and revealed the regulatory mechanism of its biological function, which will provide a potential valuable biomarker for clinical diagnosis and treatment of patients.

Methods

Data sources

The Cancer Genome Atlas (TCGA) is an international public database for oncology research, which contains various tumor related data types, such as RNA sequencing data, DNA methylation data and mutation data (https://portal.gdc.cancer.gov/). In this study, transcr iptome sequencing of 488 cases of colorectal adenocarcinoma and DNA methylation data of 339 cases of colorectal cancer were obtained from TCGA database to analyze the influence of MPP2 mRNA expression level and its DNA methylation sites changes on the prognosis of colorectal patients and the relationship between MPP2 mRNA expression level and clinical characteristics of patients. The detailed clinical features of all colorectal patients are shown in Table S1. Moreover, we further downloaded three different data sets(GSE40967:573 patients; GSE39582:579 patients; GSE71187:52 patients;) from the GEO database including 1204 colorectal cancer samples (https://www.ncbi.nlm.nih.gov/geo/). These data, together with the data obtained from TCGA database, were used for meta-analysis to further verify the impact of MPP2 on the prognosis of patients. Finally, The Human Protein Atlas (HPA) database was used to compare the abnormal changes of MPP2 protein expression levels in normal control tissues and colorectal cancer (https://www.proteinatlas.org/).

Meta-analysis

In this study, we searched a variety of internationally renowned databases(The PubMed; Web of Science; Embase databases) for the impact of MPP2 on the prognosis of patients with colon adenocarcinoma, rectal adenocarcinoma and colorectal cancer. However, up to now, there is no report about MPP2 in colorectal adenocarcinoma. Therefore, this study can only use the existing public database data to do meta-analysis to further evaluate the impact of MPP2 on the overall survival of colorectal patients. HR value and 95% CI are important indicators to evaluate the prognosis of patients with colorectal cancer. The heterogeneity of various data was analyzed by the Q test (I^2 statistics). A fixed effects model or a random effects model was used to complete the meta-analysis based on the cut-off criterion was (I^2=50%) in R 3.4 software.

TIMER database analysis

TIMER is an international public data analysis platform, which can automatically analyze the infiltration relationship between target genes and immune cells after inputting target genes into the official website (https://cistrome.shinyapps.io/timer/) [11]. This study systematically evaluated the relationship between MPP2 mRNA expression and invasion of several immune cells (CD4+T cells, CD8+T cells, B cells, neutrophils, dendritic cells and macrophages) in colorectal cancer [12]. In addition, we also identified the relationship between MPP2 mRNA expression and the coding gene encoding the immune

checkpoints(PD-1 (PDCD1), PD-L1 (CD274) and PD-L2 (PDCD1LG2)).

GSEA analysis

GSEA is one of the most commonly used biological analysis tools to predict the function of target genes [13]. In the study, we separately divided the data information of the TCGA RNA-seq 339 patient samples into high expression group and low expression group according to the expression level of MPP2. The GSEA 4.0.jar software was used to complete the analysis of cell signaling pathways involved in MPP2. the number of permutations was set to 1000 times, and 'KEGG cell signaling pathways' was selected as the gene sets database. Finally, based on the nominal *p*-value and normalized enrichment score (NES) as the cutting standard, we decide whether the analysis results are meaningful or not.

Gene ontology enrichment analysis

The cluster profiler package of R software was adopted to complete gene ontology analysis. Patients with colorectal cancer from TCGA database were artificially divided into MPP2 high expression group and MPP2 low expression group. The analysis of differential genes between the two groups was based on the false discovery rate less than 0.05 [12]. GO analysis is intentionally divided into biological process (BP), cellular component (CC) and molecular function (MF). The results of gene ontology were based on | log-FC | ≥ 1 combined with P value less than 0.05 was considered to be a significant. Finally, the most significant top 10 results of each group were graphically processed by the ggplot2 package of R software.

Cell treatment and RT-PCR

We purchased FHC, normal colorectal mucosal cell line, and HCT116 and SW480, colorectal cancer cell lines, from American Type Culture Collection (ATCC), cultured in complete medium consisting of 89% RPMI 1640 medium, 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin, and in a incubator with a cell constant temperature of 37 ℃ and 5% CO2. Total RNA was extracted from cells and reverse transcribed into cDNA. RT-PCR experiment was performed to verify expression level of MPP2 with specific primers: 5′ -TGGGGCAGC CGCTAAGAATA- 3′ (sense) and 5′ -GCTGCATGGCA GTTTCAGAG- 3′ (antisense). SW480 cell was chosen to be transfected with specific shRNA targeting on 5′ -TGG AAGATTTGATGTGGGTCGCTAT- 3′ for 24 h and prepared for for experiments.

Western blotting

The total protein of the transfected cells existed in the supernatant after cell lysis and centrifugation. After the protein concentration was determined by BCA assay, an appropriate amount of loading was added into protein sample, which then was analyzed by electrophoresis to be further transferred onto PVDF membrane. The membrane was sealed with 5% skimmed milk, the specific primary antibody was incubated with for 4℃ overnight. After PBS washing, the secondary antibody was incubated in the dark for 1 h at room temperature. Finally, the protein expression was detected by chemiluminescence system.

MTT and colony-forming experiment

After 24 h of transfection, cells were added to 96 well plates at the density of 2000 cells per well. When cultured to the detection time point, cells were added with 20ul MTT solution, followed by continuous culture at 37℃ for 4 h to form crystals. And crystals were then shaken and dissolved by 150ul DMSO solution under dark condition for 10 min. Finally, the absorbance value was measured at 490 nm.

24 h after transfection, the cells were spread on 6-well plates at a density of 1000 cells per well and cultured at 37℃ and 5% CO_2 for 14 days. After discarding the culture medium, the cells were washed with PBS, fixed with 4% paraformaldehyde, and then stained with crystal violet solution. Finally, the photos were taken, colony formations were counted by three different experimental personnel, and finally statistically analyzed.

Statistical analysis

The data analysis and processing of this study mainly rely on R software (version 3.4). According to the median expression level of MPP2 in all samples, they were divided into high expression group and low expression group, and then survival analysis was performed by KM method. The DNA methylation data of MPP2 also used a similar method to detect the impact of different DNA methylation sites on the prognosis of colorectal cancer patients. Moreover, the difference of MPP2 mRNA expression level and its DNA methylation sites for clinical characteristics of colorectal cancer was compared by student's t-test. The co-expression relationship between MPP2 mRNA level and its related its DNA methylation sites was established by Spearman method. Finally, univariate analysis and multivariate analysis based on Cox regression models were used to detect whether MPP2 mRNA level can be used as an independent risk factor for the prognosis of colorectal patients. The P-values of all statistical analysis results was less than 0.05, which was considered as a significant result in this study.

Results

The expression level of MPP2 was significantly decreased in tumors

Firstly, this study found that MPP2 plays an important regulatory role in the pathological process of human malignant tumors. Therefore, in order to further detect the expression level of MPP2 in malignant tumors, GEPIA, as an online data analysis platform, was used to detect the expression level of MPP2 in tumors. The results showed that the expression level of MPP2 was significantly lower in various malignant tumors than in normal tissues (Fig. 1A). It must be emphasized that the expression level of MPP2 was decreased not only in colorectal cancer, but also in rectal cancer. Since the pathological types of rectal cancer and colorectal cancer are adenocarcinoma, this study combined the two tumors to study the biological function of MPP2. Secondly, this study further downloaded 42 cases of adenocarcinoma tissues and 488 cases of colorectal cancer tissues from TCGA database to explore the expression level of MPP2. The results showed that the expression level of MPP2 was significantly lower than that in tumor tissue (Fig. 1B), which was further confirmed by paired analysis (Fig. 1C). Finally, in order to explore the protein expression of MPP2 in colorectal cancer, this study obtained the immunohistochemical data of MPP2 in HPA database, and found that the expression level of MPP2 in colorectal cancer did not increase or decrease significantly (Figure S1).

The increased mRNA level of MPP2 is associated with poor prognosis of colorectal cancer

Firstly, in order to explore the relationship between MPP2 and prognosis related clinical features of colorectal

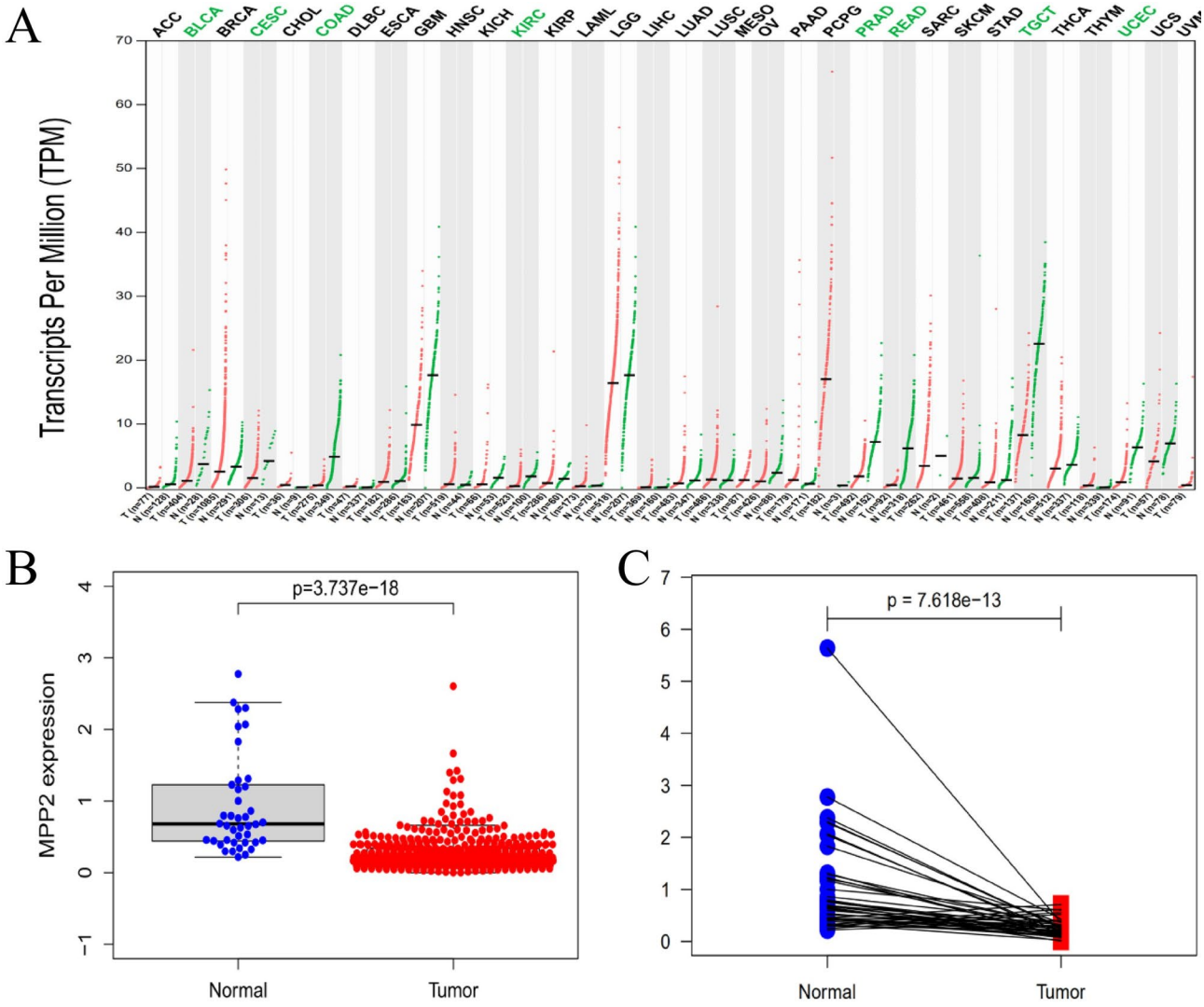

Fig. 1 The RNA expression level of MPP2 in multiple tumors. (**A**) The expression level of MPP2 in malignant tumors with GEPIA. (**B**) The expression level of MPP2 was significantly lower in normal tissues than that in tumor tissues from TCGA database. (**C**) The expression level of MPP2 with paired analysis

cancer patients, the results showed that there was a relationship between N and T in TNM staging (Fig. 2A, B). These results suggest that MPP2 may be related to the prognosis of colorectal cancer patients. Therefore, we further used KM method to explore the relationship between MPP2 and overall survival. The results showed that the increased expression of MPP2 can significantly reduce the overall survival of colorectal cancer patients (Fig. 2C).

The increased mRNA level of MPP2 is an independent risk factor for colorectal cancer patients

In order to explore whether the high expression of MPP2 leads to the decrease of overall survival is an accidental factor or an inevitable condition, we used Cox method to do univariate analysis and multivariate analysis of MPP2. Univariate analysis showed that the higher expression level of MPP2, lymphatic invasion, pathologic M, pathologic N, pathologic T and tumor stage was a risk factor for the prognosis of patients with colorectal cancer($p<0.05$, [HR] > 1) (Fig. 3A). Subsequence multivariate analysis showed that not only MPP2 ($p=0.007$, hazard ratio [HR]=2.876(95%CI [1.334–6.201])) can be used as an independent prognostic risk factor, but also pathological T ($p=0.018$, hazard ratio [HR]=2.033(95%CI[1.130–3.660])) can be used in colorectal patients (Fig. 3B). Therefore, it is not difficult to infer that MPP2 can be used as an independent prognostic factor to reduce the overall survival of colorectal cancer patients.

MPP2 mRNA expression is negatively regulated by DNA methylation sites of MPP2

In order to understand the role of DNA methylation sites in the regulation of MPP2 mRNA expression, we obtained DNA methylation data and RNA-seq data of 380 cases of colorectal cancer in TCGA database to explore the relationship between them. Firstly, we found that MPP2 mRNA expression was negatively correlated with its DNA methylation sites (Fig. 4B). The distribution of 17 CpG sites of MPP2 was shown in Fig. 4A. Secondly, Pearson correlation analysis was used to identify the MPP2 CpG sites at which degree of methylation was related to MPP2 mRNA expression. The results showed that 4 CpG sites (cg22377486, cg21989213, cg01370063 and cg00794378) were negatively correlated with the expression of MPP2 mRNA (Fig. 4C-F). However, cg18439195 was positively correlated with the expression of MPP2 mRNA (Fig. 4G). The remaining 12 CpG sites were not associated with the expression of MPP2 mRNA. Thirdly, this study further investigated the effect of 17 CpG sites of MPP2 on the overall survival of colorectal cancer patients. The results showed that cg17552029 and cg18439195 DNA methylation sites can affect the prognosis of colorectal cancer patients (Figure S2A, B). Finally, the study also investigated the relationship between MPP2 DNA methylation sites and clinical features of colorectal cancer patients. The results showed that there was a close relationship between MPP2 DNA methylation sites and age, pathological M and tumor stage (Figure S2C-E). In conclusion, MPP2 DNA methylation sites not only regulates MPP2 mRNA expression, but also has an important impact on the prognosis of colorectal cancer patients.

Relationships of MPP2 with immune cells and immune checkpoints

In this study, the timer database was selected to determine the relationship between MPP2 mRNA expression with immune cell infiltration. As shown in the scatter diagram (Figure S3), the expression level of MPP2 mRNA was highly positively correlated with a variety of immune infiltrating cells, such as CD4+T cells, neutrophils, macrophage and dendritic cell infiltration in colon cancer, but also in rectal cancer, except for neutrophils. There was no significant correlation between MPP2 expression and B cells or CD8+T cells in colorectal cancer and colorectal

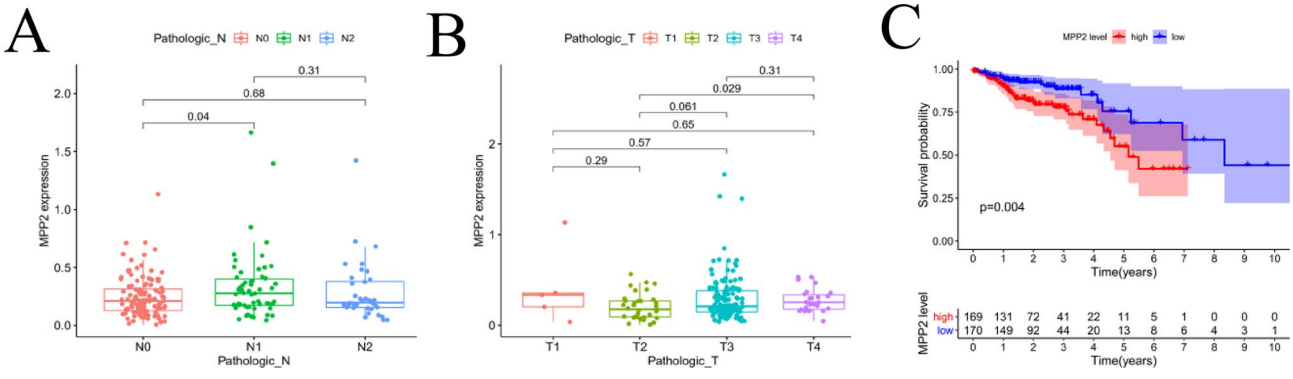

Fig. 2 The relationship between MPP2 and prognosis related clinical features. (**A**) The expression level of MPP2 in malignant tumors with GEPIA. (**B**) The expression level of MPP2 was significantly lower in normal tissues than that in tumor tissues from TCGA database. (**C**) The expression level of MPP2 with paired analysis

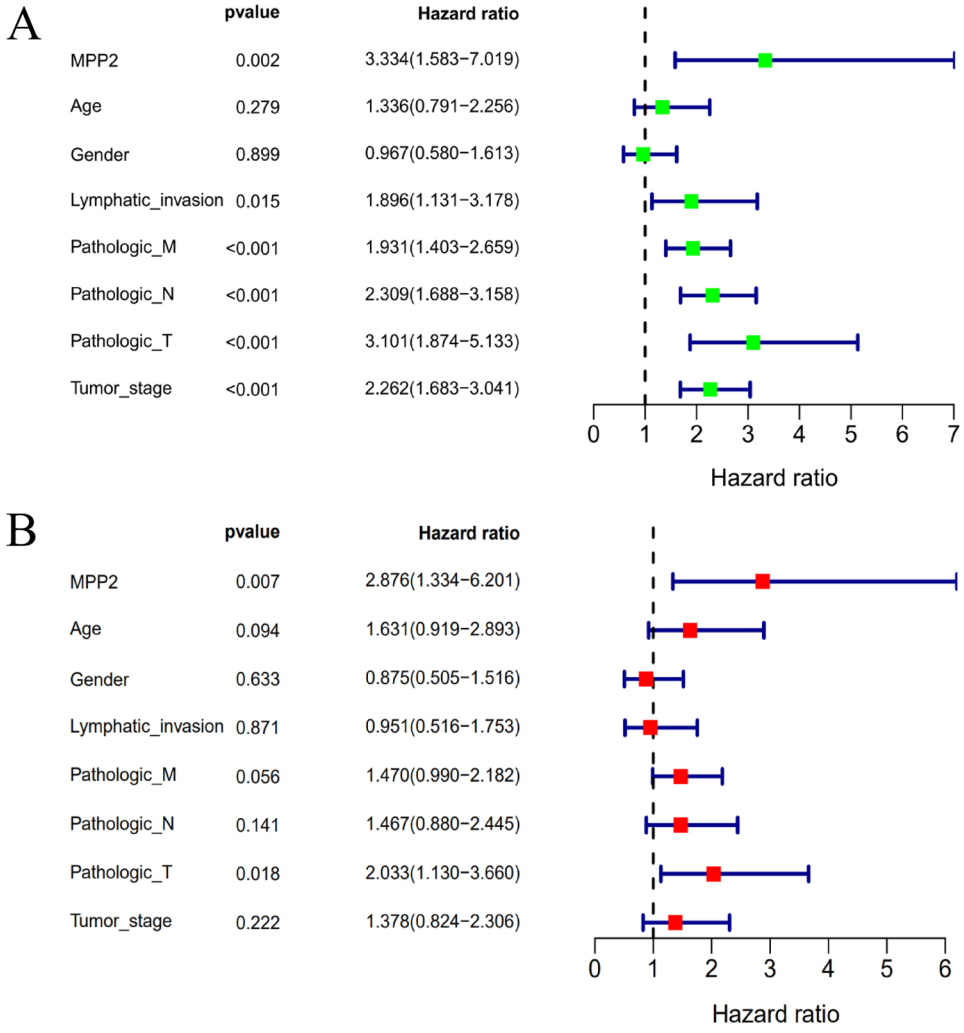

Fig. 3 Cox regression analysis of MPP2 expression and clinical related factors in CRC. (**A**) Univariate regression analysis of prognostic in patients with CRC. (**B**) Multivariate regression analysis of prognostic in patients with CRC

cancer. Besides, due to the progress in immunotherapy of PD-L1 in tumor, we further established the relationship between MPP2 expression and the coding genes of PD-1, PD-L1 and PD-L2. We also noticed that the expression of MPP2 was positively correlated with the expression of PD-1(PDCD1), PD-L1(CD274) and PD-L2(PDCD1LG2). But PD-L1 was not statistically significant in rectal cancer (Fig. 5). Since the expression of MPP2 is highly correlated with the infiltration of immune cells, and the high expression of MPP2 is also correlated with the poor prognosis of colorectal cancer patients, we speculate that the expression of MPP2 may affect the prognosis of colorectal cancer patients, which may be partly due to immune infiltration.

MPP2-related signaling pathways in colorectal cancer

In order to explore the functional role of MPP2 in colorectal cancer, we first annotated MPP2 through gene ontology and found that MPP2 was related to cell adhesion molecule binding, growth factor binding, extracellular matrix organization and focal adhesion (Figure S4). Subsequently, KEGG analysis of GSEA showed that the high expression of MPP2 could activate MAPK signaling pathway, Wnt signaling pathway, mTOR signaling pathway and toll like receptor signaling pathway, which were the cellular signaling pathways of carcinogenesis and development (Fig. 6; Table 1).

Meta analysis of MPP2 expression on the prognosis of colorectal cancer

In order to find more evidence to support the effect of MPP2 on the prognosis of colorectal patients, we used meta-analysis to complete the project included a total of 1584 patients in the study. Since there was no previous literature on MPP2 in colorectal patients, we can only collect data from multiple datasets in GEO database. The results of meta-analysis showed that MPP2 expression was a risk factor for colorectal cancer patients in

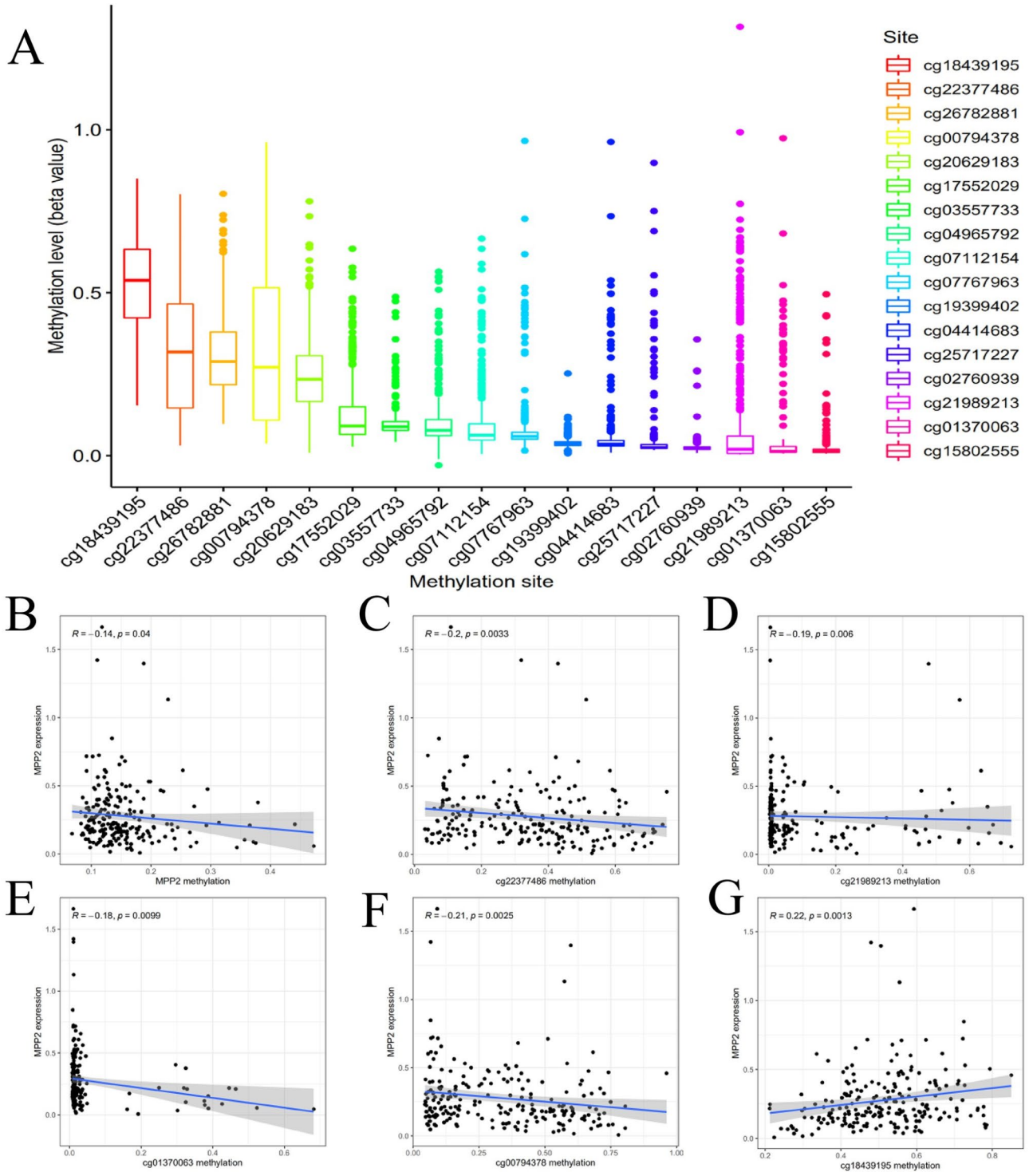

Fig. 4 The expression and methylation of MPP2. (**A**) The distribution of MPP2 DNA promoter CpG sites. (**B**) The expression of MPP2 is negatively related with its methylation. (**C**) Correlation between MPP2 expression level and cg22377486 methylation. (**D**) Correlation between MPP2 expression level and cg21989213 methylation. (**E**) Correlation between MPP2 expression level and cg01370063 methylation. (**F**) Correlation between MPP2 expression level and cg00794378 methylation. (**G**) Correlation between MPP2 expression level and cg18439195 methylation

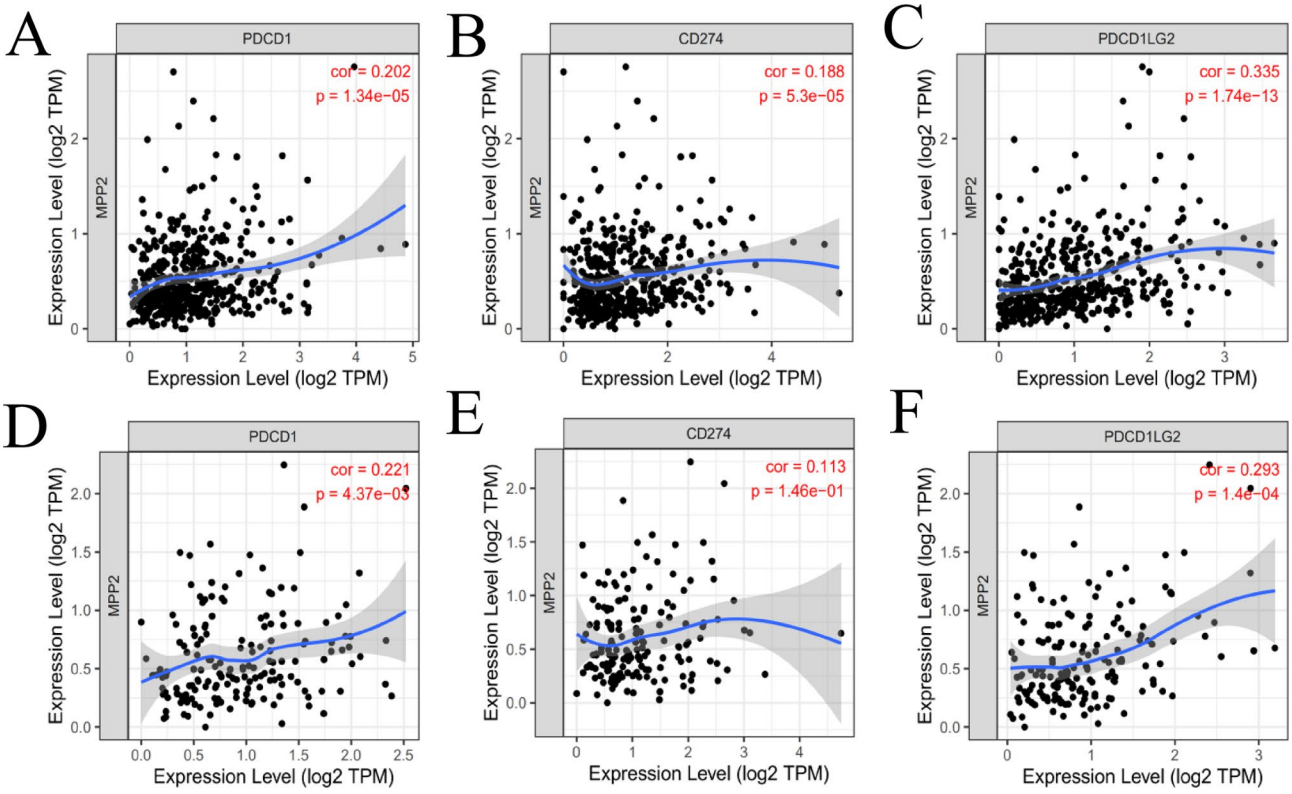

Fig. 5 Correlation of MPP2 with immune checkpoints. (**A**) The relationship between MPP2 and PD1 in COAD. (**B**) The relationship between MPP2 and PD-L1 in COAD. (**C**) The relationship between MPP2 and PD-L2 in COAD. (**E**) The relationship between MPP2 and PD1 in READ. (**F**) The relationship between MPP2 and PD-L1 in READ. (**G**) The relationship between MPP2 and PD-L2 in READ

each of the four data sets([HR] > 1). At the same time, the combined results of the four databases also suggested that MPP2 was a risk factor for the prognosis of colorectal cancer patients (Fig. 7). Because of the heterogeneity among the four data (I^2>50%), we must choose a random effects model in this study.

MPP2 knockdown inhibits the proliferation of SW480 cells via mTOR signaling pathway

To further explore the specific mechanism of MPP2 in colon cancer and verify our above speculation, we first detected the expression of MPP2 in colon cancer cell line through in vitro experiment. It was found that the expression of MPP2 was highest in SW480, which was about 5 times that of human normal colorectal mucosal cell line FHC (Fig. 8A). Next, we knocked down the expression of MPP2 by shRNA, and found decreased phosphorylation levels of Akt and mTOR (Fig. 8B), indicating that MPP2 was involved in the regulation of mTOR signaling pathway. For the purpose of exploring the effects on SW480 cell, we confirmed that knockdown of MPP2 would inhibit the proliferation of colon cancer through MTT assay and colony formation assay (Fig. 8C-D). In addition, we detected the expression of proliferation related markers Ki67 in transfected cells by immunofluorescence, which once again confirmed that MPP2 could affect the proliferation of colon cancer cells (Fig. 8E). In conclusion, we have verified that MPP2 does play a vital role in colon cancer through basic experiments.

Discussion

Some oncogenes have been identified and revealed in the pathological process of colorectal cancer [14, 15], but the relationship between MPP2 and prognosis of colorectal cancer has not been reported. In order to confirm the change of MPP2 expression level and its impact on the prognosis of colorectal cancer patients, we downloaded the MPP2 mRNA expression level of 488 cases of colorectal adenocarcinoma with the detailed clinical information and 42 cases of colorectal cancer adjacent tissues from TCGA database. Firstly, we found that MPP2 level in tumor tissue was significantly decreased. Moreover, in order to further determine whether there was statistical bias, similar results were obtained after further the paired difference analysis. Lastly, excluding the imbalance between normal tissue samples and tumor tissue samples, we found that MPP2 was indeed low expression in tumor tissues after expanding normal tissue samples in GEPIA database (Fig. 1). In order to investigate the prognostic significance of MPP2 in patients with

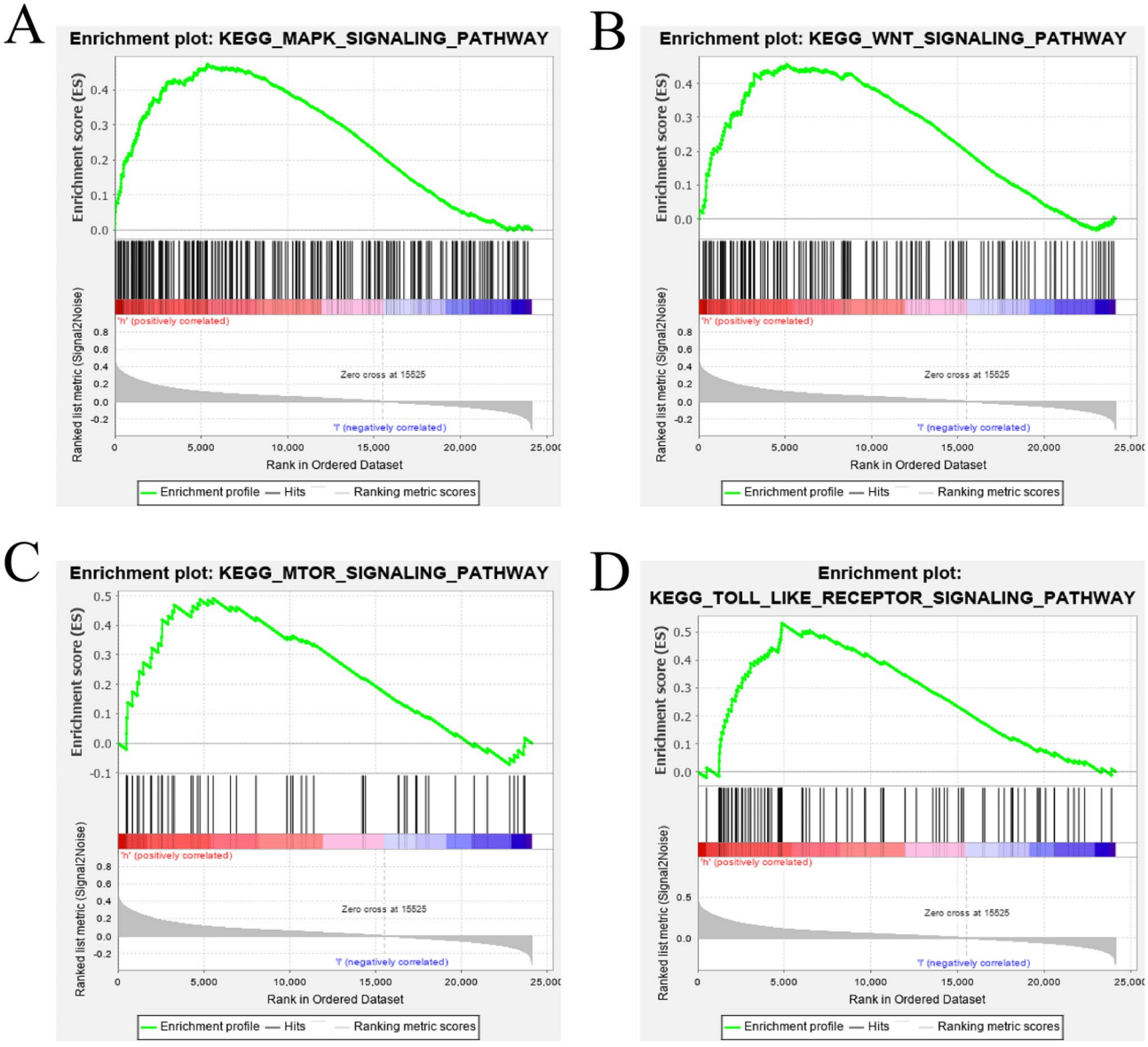

Fig. 6 GSEA analysis of MPP2 in CRC. (**A**) MAPK signaling pathway. (**B**) WNT signaling pathway. (**C**) mTOR signaling pathway. (**D**) Toll like receptor signaling pathway

Table 1 The gene set enriches the high MPP2 in TGGA RNA-seq database

Name	NES	NOM *p*-value	FDR q-value
MAPK SIGNALING PATHWAY	2.092	0	0.002
mTOR SIGNALING PATHWAY	1.972	0	0.008
WNT SIGNALING PATHWAY	1.955	0	0.008
TOLL LIKE RECEPTOR SIGNALING PATHWAY	1.886	0.012	0.0132

NES: normalized enrichment score; NOM: nominal; FDR: false discovery rate. Gene sets with NOM p-value<0.05 and FDR q-value<0.25 were considered as significantly enriched

colorectal cancer, we used survival analysis to find that MPP2 can reduce the overall survival time of patients with colorectal cancer, and has a significant relationship with a variety of clinical features related to prognosis of patients (Fig. 2). In order to determine whether MPP2 is an inevitable factor for the prognosis of colorectal cancer patients, we used multivariate analysis to confirm that MPP2 is an independent risk factor for the prognosis of colorectal cancer patients (Fig. 3). Although several literatures have confirmed that MPP2 plays an important

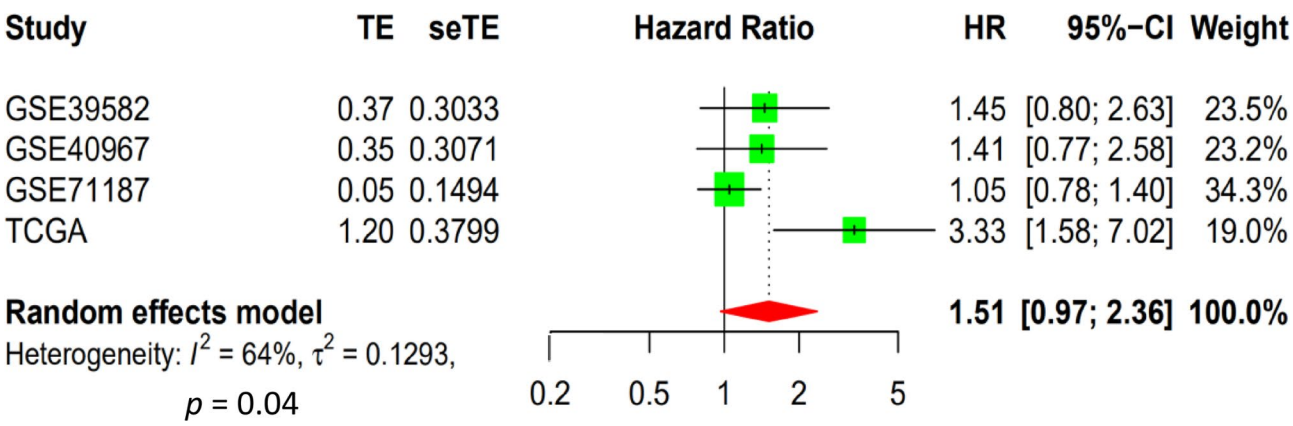

Fig. 7 Meta analysis of MPP2 expression on the prognosis of colorectal cancer

role in regulating the pathological process of malignant tumors [10, 16], but has never been studied in colorectal cancer. Therefore, we can only obtain the original data from the database for meta-analysis to collect more evidence to confirm its adverse impact on the prognosis of colorectal patients. From the above discussion, we have enough evidence to confirm that MPP2 is an oncogene in colorectal cancer. However, it is worth discussing why the expression level of MPP2 is decreased in colorectal cancer tissue samples.

Epigenetics is often used to describe the regulation process of DNA template, including DNA methylation, histone modification, nucleosome remodeling and noncoding RNA modification. The imbalance of these processes is very important for the formation and development of cancer, and the treatment of epigenetics has been paid more and more attention [17]. DNA methylation is one of the most abundant studies in epigenetic modification, and plays an important role in regulating various physiological and pathological states [18]. In this study, we found that the expression level of MPP2 was negatively correlated with the overall methylation sites level, which also explained why MPP2 as an oncogene was low expressed in tumors. Interestingly, the methylation of cg18439195 site in CpG island was positively correlated with the expression of MPP2(Figure 4). When we further analyzed the DNA methylation sites of MPP2 and survival time, we found that the degree of methylation of cg18439195 site was negatively correlated with survival time. The results also indirectly indicate that the higher the expression level of MPP2, the shorter the survival period of patients. Due to the fact that the core of this study is to explore whether MPP2 can be used as a prognostic factor for colorectal cancer patients, there has not been a more detailed mechanism study on its DNA methylation status.

With the in-depth understanding of the progress of cancer and the continuous exploration of the immune system, tumor immunity and immunotherapy have become the focus of cancer research, and are recognized as an important weapon against cancer [19, 20]. In colorectal cancer, immune checkpoint therapy is approved for highly mutated tumors, mismatch repair defects and microsatellite instability [21]. In order to further study the effect of MPP2 on the immune microenvironment of colorectal cancer, we analyzed the relationship between MPP2 and immune invasion in colorectal cancer using the time database. The results showed that MPP2 was positively correlated with the infiltration of CD4+T cells, macrophages and dendritic cells. These results suggest that the effect of MPP2 on the immune infiltration of colorectal cancer is similar to that of CRC. The discovery and clinical application of immune checkpoint is the biggest progress in the field of immunotherapy in the past decades [22, 23]. We observed a strong positive correlation between MPP2 and PD1, PDL1 and PDL2. These results suggest that the effect of MPP2 on CRC is partly mediated by immune regulation, and further suggest that MPP2 may be a good therapeutic target.

In order to identify the signal pathway of MPP2 in colorectal cancer, we used GSEA analysis and found MAPK, Wnt, mTOR and Toll like receptor are positively correlated with MPP2. Previous studies have shown that ERK-MAPK pathway is one of the important pathways of CRC cells proliferation. Overexpression and activation of ERK-MAPK play an important role in the progression of CRC, and may become a therapeutic target [24, 25]. Wnt signaling pathway is a key regulatory factor in maintaining tissue homeostasis and repair, and plays an important role in tumor development. Although the therapy targeting Wnt has not yet shown good results, we still believe that Wnt is a potential tumor therapeutic target, especially in colorectal cancer [26, 27]. PI3K-mTOR signaling pathway is very important for the development of CRC. In addition, the activation of this signaling pathway promotes the metastasis and chemoresistance of CRC. Inhibitors of PI3K/AKT/mTOR pathway, such as metformin and diclofenac, have been shown to inhibit the

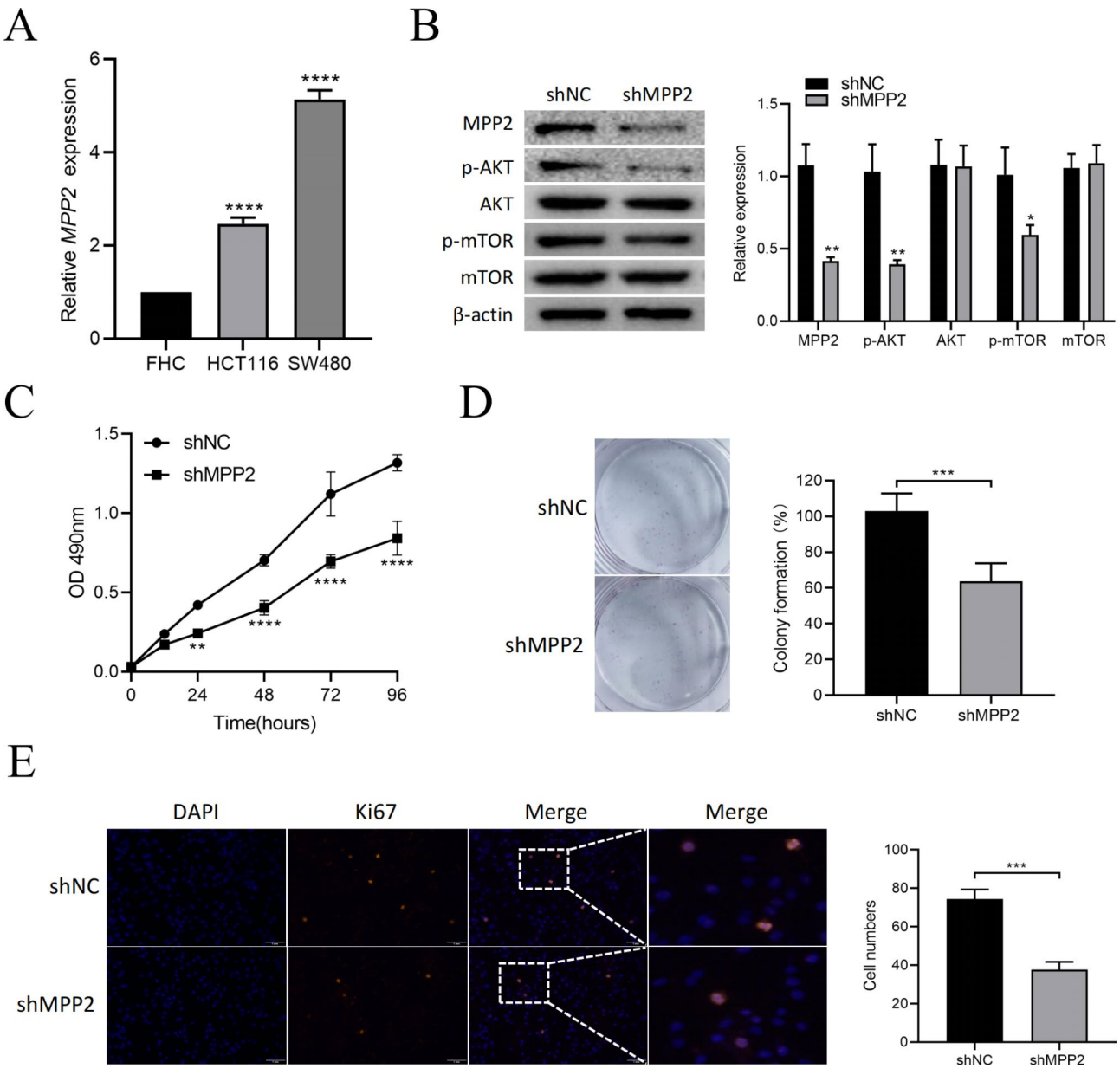

Fig. 8 Knockdown of MPP2 inhibits the proliferation of SW480 cells. (**A**) The expression levels of MPP2 in different cell lines. **** $P<0.0001$ vs. FHC. (**B**) The expression of related proteins in transfected cells. (**C**) MTT results of different treated cells. (**D**) Colony formation assay of shNC and shMPP2 treated cells. (**E**) Ki67 staining results in SW480 cells with treatment of shRNA. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$ vs. shNC. All experiments were repeated three times independently

survival of colorectal cancer cells [28]. It is not difficult to deduce that all cell signaling pathways have a regulatory relationship with the proliferation and migration of tumor cells based on the above analysis results. However, these results are only indirect evidence, so in order to analyze the accuracy and reliability of the results.Through further literature search, it was found that there was no literature reporting the relationship between MPP2 and mTOR pathway. Therefore, we selected mTOR pathway as the validation object. According to the instructions of GSEA results, we knocked down the expression of MPP2 with shRNA and found that the activation level of mTOR pathway related proteins also decreased significantly, and significantly inhibited cell proliferation, indicating that MPP regulated the proliferation of colorectal cancer through mTOR signaling pathway. In collection, our results indicate that MPP2 is an critical regulatory gene for the development of colorectal cancer, which provides a new theoretical basis for the treatment of colorectal cancer and may be used as a new drug target to improve the treatment effect and prognosis of patients.

Conclusions

The low expression level of MPP2 in colorectal cancer is due to its DNA methylation sites. High expression of MPP2 is an independent risk factor for poor prognosis in colorectal cancer patients. In addition, the expression of MPP2 is closely related to the infiltration of CD4+T cells, macrophages and dendritic cells and the immune checkpoint PD1, PDL1 and PDL2 in colorectal cancer. Therefore, MPP2 can not only be used as a prognostic marker of colorectal cancer, but also an effective therapeutic target.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12957-024-03567-3.

Supplementary Material 1

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Author contributions
Zhizhao Yang; Jiaxing Chen; Zhihao Fu: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper. Dongfeng Deng; Yongqiang Cui and Zhilei Zhao: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper. Xiao Zhang: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data availability
The Cancer Genome Atlas (TCGA)((https://portal.gdc.cancer.gov/))（GSE40967:573 patients; GSE39582:579 patients; GSE71187:52 patients;） from the GEO database including 1204 colorectal cancer samples (https://www.ncbi.nlm.nih.gov/geo/)The Human Protein Atlas (HPA) database was used to compare the abnormal changes of MPP2 protein expression levels in normal control tissues and colorectal cancer (https://www.proteinatlas.org/).TIMER is an international public data analysis platform, which can automatically analyze the infiltration relationship between target genes and immune cells after inputting target genes into the official website (https://cistrome.shinyapps.io/timer/).

Declarations

Ethics statement
All patients participating in this study signed written informed consent before participating.

Competing interests
The authors declare no competing interests.

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