Original Paper

MEGF6 Promotes the Epithelial-to-Mesenchymal Transition via the TGFβ/SMAD Signaling Pathway in Colorectal Cancer Metastasis

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Key Words

Megf6 • EMT • TGFβ/SMAD signaling pathway • Metastasis • Colorectal cancer

Abstract

Background/Aims: Colorectal cancer (CRC) is a malignancy that has high morbidity and mortality and is initiated from accumulative genetic events. Although much effort has been made to elucidate the genetic mechanism underlying this disease, it still remains unknown. Here, we discovered a novel role for multiple epidermal growth factor-like domains protein 6 (MEGF6) in CRC, namely, that it induces the epithelial-to-mesenchymal transition (EMT) to promote CRC metastasis via the transforming growth factor beta (TGFβ)/SMAD signaling pathway. **Methods:** RNA sequencing data from the Gene Expression Omnibus database were analyzed using R software. Based on The Cancer Genome Atlas Colon Adenocarcinoma (TCGA-COAD) cohort, the clinical significance of MEGF6 was investigated. HCT8R, HCT116, and LoVo CRC cells were transfected with small interfering RNA against MEGF6, and their proliferation and sensitivity to fluorouracil were evaluated with the MTT cell proliferation and colony formation assays. Proteins associated with cell growth were detected by western blot analysis. The apoptosis of cells was evaluated by Annexin V/propidium iodide staining, and transwell assays were performed to assess the involvement of MEGF6 in cell migration. Markers of EMT and TGFβ/SMAD signaling were evaluated by quantitative PCR and western blotting, and the correlation between MEGF6 and these markers was assessed in the TCGA colon and renal adenocarcinoma cohort. **Results:** The results showed that MEGF6 was upregulated in HCT8R cells. In addition, MEGF6 was significantly overexpressed in tumor tissue and predicted a poor survival in the TCGA-COAD cohort. Moreover, MEGF6 accelerated CRC cell growth and inhibited apoptosis, and promoted CRC metastasis by inducing the EMT. Finally, we found that

TGF β /SMAD signaling triggered the expression of Slug, which regulates the MEGF6-mediated EMT. **Conclusions:** MEGF6 may serve as an oncogene to promote cell proliferation and inhibit apoptosis. MEGF6 can also accelerate cell migration via TGF β /SMAD signaling-mediated EMT.

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Introduction

Colorectal cancer (CRC) is a serious malignancy that is common worldwide [1] and arises from accumulative genetic events [2]. Genomic instability, loss of tumor suppressor genes, and activation of oncogenic pathways all contribute to the complex nature of this disease [3]. It is widely accepted that transforming growth factor (TGF- β), Wnt/ β -catenin, neurogenic locus notch homolog protein (NOTCH), and the p53 signaling pathway play key roles in cancer development and progression [4]. Adjuvant chemotherapy for CRC usually includes fluorouracil (5-FU), which is the most widely used chemotherapeutic agent for the treatment of this disease. Specifically, combination therapy of 5-FU with oxaliplatin, irinotecan, or capecitabine has been extensively used to treat CRC [5], and has been successful in the majority of patients with advanced disease [6]. However, these patients often experience recurrence caused by drug resistance, and the 5-year survival rate is lower than 10% in patients with advanced CRC [7]. Interestingly, a minority of patients with CRC have been responsive to innovative immune checkpoint inhibitors [8]. Clinical trials have shown that 5-FU is the cornerstone of CRC therapy [9, 10]; thus, it is essential to elucidate the mechanisms underlying resistance to this drug.

TGF- β is a secreted protein that activates canonical pathways, including SMAD2, SMAD3, and SMAD4, and non-canonical pathways, including phosphoinositide 3-kinase/Akt, mitogen-activated protein kinase-extracellular signal-regulated kinase, and c-Jun N-terminal kinase [11]. The role of TGF- β is paradoxical due to its tumor suppressor and oncogenic activities [12]. TGF- β can activate the epithelial-to-mesenchymal transition (EMT), which has been implicated in tumorigenesis and is mediated by transcription factors such as Twist, Snail, and Slug, leading to tumor invasion and metastasis [13]. Multiple epidermal growth factor (MEGF)-like domains have similar characteristics as epidermal growth factor (EGF) [14], and MEGF plays a critical role in cell adhesion and receptor–ligand interactions in neural system development [14]. Recessive mutations in MEGF10 reportedly cause congenital myopathy by inducing neuron apoptosis [15]. MEGF6, another member of the MEGF family, is involved in many disorders of the neural system [16]. However, there have been few studies on the role of MEGF6 in CRC.

Here, we found that MEGF6 was highly expressed in HCT8 5-FU-resistant cells, which was associated with tumor aggressiveness and patient survival in The Cancer Genome Atlas Colon Adenocarcinoma (TCGA-COAD) cohort. In addition, MEGF6 promoted cell proliferation and repressed the apoptotic response of CRC cells. Moreover, MEGF6 promoted CRC cell metastasis by inducing the EMT via the TGF β /SMAD signaling pathway.

Materials and Methods

High-throughput data analysis

RNA sequencing (RNA-seq) data were acquired from the Gene Expression Omnibus database (GEO, Accession No. GSE81005; <http://www.ncbi.nlm.nih.gov/geo/>). This dataset comprised RNA-seq data from human HCT8 5-FU-sensitive cells (HCT8S) and HCT8 5-FU-resistant cells (HCT8R). GSM2140576, GSM2140577, and GSM2140578 represented RNA-seq data from HCT8S cells; and GSM2140585, GSM2140586, and GSM2140587 represented data from HCT8R cells. The read count file for each sequenced sample was combined into a count file for further differential expression analysis. Differential expression analysis was performed using the limma package, and hierarchical clustering was performed with R software (version 3.3.3).

Cell lines

SW480, SW620, HCT116, HT29, and LoVo CRC cell lines were acquired from the Colorectal Cancer Institute of Harbin Medical University (Hunan, China). HCT8S and HCT8R cells were purchased from Shanghai Meixuan Corporation (Shanghai, China). LoVo, SW480, and SW620 cells were cultured in RPMI-1640 medium (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS). HCT8R was cultured in RPMI-1640 medium (Gibco) supplemented with 10% FBS and 15 µg/mL 5-FU according to the manufacturer's protocol. HCT116, HT29, and HCT8S cells were cultured in Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% FBS. All cells were cultured at 37°C in a humidified 5% CO₂ atmosphere.

Cell proliferation, colony formation, and migration assays

The cell proliferation assay was performed using a standard procedure [17]. For the colony formation assay, 1000 cells/well were seeded in a 6-well plate in 2 mL complete media for 10 days. Then cells were fixed in 4% paraformaldehyde and stained with 0.5% crystal violet, after which the number of cells was counted. The cell migration assay was performed using the Transwell Chamber from BD Biosciences (San Jose, CA, USA). Briefly, cells (2×10^5) were seeded in the upper chamber in serum-free media. The lower chamber was filled with corresponding media with 10% FBS as a chemoattractant. After 24 h, cells that invaded through the membrane were stained with 0.5% crystal violet and counted.

The Cancer Genome Atlas Analysis

TCGA colon and renal adenocarcinoma (COAD, READ) cohort consisted of 380 primary CRC and 51 normal samples, and TCGA-COAD cohort consisted of 282 primary CRC and 41 normal samples. All data on expression and clinical features were obtained from the USUC Xena Cancer Genomics Browser (<http://xena.usuc.com>).

Oligonucleotide transfection

Small interfering RNA (siRNA) against MEGF6 was purchased from Genepharma (Shanghai, China). Oligonucleotide transfection was performed using the Lipofectamine 2000 Transfection Reagent (Thermo Fisher Scientific, West Palm Beach, FL, USA) according to the manufacturer's protocol, while nonspecific siRNA was used as a negative control.

RNA extraction and quantitative PCR

Total RNA was extracted from CRC cell lines with Trizol reagent (Invitrogen, Carlsbad, CA, USA) and 1 µg total RNA was reverse-transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Quantitative PCR (qPCR) was performed in triplicate using the Applied Biosystem 7500 qPCR system (Applied Biosystems) according to a previous report [18]. Relative expression was compared by the $2^{-\Delta\Delta Ct}$ method, and β-actin served as the internal reference gene. The sequences of all the primers were obtained from PrimerBank (<https://pga.mgh.harvard.edu/primerbank/>).

Western blot assay

Western blot analysis was performed with reference to a standard protocol [19]. The cell extracts were collected and quantified with the BCA Protein Assay Kit (Wanlei Bio, Shenyang, China). A total of 50 µg protein was resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis and then electrotransferred to polyvinylidene fluoride membranes (Millipore, Stafford, VA, USA). Then, the membranes were blocked in 5% non-fat milk for 2 h and then incubated with primary antibodies at 4°C overnight. Secondary antibodies labeled with horseradish peroxidase (Cell Signaling Technology [CST], Danvers, MA, USA) were incubated with the membranes at room temperature for 2 h and proteins were detected using an ECL Kit (Wanlei Bio). β-actin served as the control for whole-cell lysates. MEGF6 primary antibody was purchased from Invitrogen, Ki-67 primary antibody was purchased from Abcam (Cambridge, UK), and other primary antibodies were purchased from CST using the recommended concentrations in accordance with the manufacturer's instructions.

Apoptosis detection

Cell apoptosis was examined by flow cytometry. Annexin V fluorescein (FITC)/propidium iodide (PI) double staining (BD Biosciences) was performed following the manufacturer's protocol. The samples were analyzed using the BD Accuri C6 Flow Cytometer, and subsequent analyses were performed with FlowJo software (Treestar Inc., San Carlos, CA, USA). All assays were performed in triplicate.

Liver metastasis in mice

We performed liver metastasis assays in mice according as previously described [20]. LOVO-pLKO and LOVO-shMEGF6 were washed with complete medium once and re-suspended in phosphate-buffered saline (PBS). The nude mice were anesthetized with pentobarbital sodium, a tiny left abdominal incision was made, and 1×10^7 cells in 100 μ L PBS were injected into the spleen parenchyma. The spleen was resected to avert intrasplenic tumor growth after 10 min, and the incision was closed. After 4 weeks, the mice were sacrificed and the livers were harvested.

Statistical analyses

The data are expressed as the mean $\pm$ standard deviation (SD). Statistical analyses were performed using the Student's *t*-test for independent groups with Graphpad 6.0 software (GraphPad, La Jolla, CA, USA). The association between MEGF6 expression and other genes was analyzed by the Pearson's correlation coefficient. *P* values less than 0.05 were considered statistically significant.

Results

MEGF6 was significantly upregulated in HCT8R cells

To investigate the potential mechanism of 5-FU resistance, we analyzed published data on the GEO website (GSE81005). We compared the differential expression of mRNA in HCT8S or HCT8R cells using the limma algorithm. The filtering criteria were defined as log fold change > 1 or < -1 , $p < 0.05$, and false discovery rate (FDR) $q < 0.05$. We found 492 upregulated and 245 downregulated genes (Fig. 1A), of which MEGF6 was the second most upregulated gene. To validate these data, we analyzed the expression in different cancer cell lines and found that MEGF6 was more highly expressed in HCT8R cells than in HCT8S cells (Fig. 1B). We used western blotting (Fig. 1C)

Fig. 1. MEGF6 was significantly expressed in the HCT8R colon cancer cell line. (A) The heat map presented differentially expressed mRNA using RNA-seq data from the GEO database (GSE81005), and hierarchical clustering was performed. Upregulated (red) and downregulated genes (green) are shown (log fold change < -1 or > 1 , $p < 0.05$, FDR $q < 0.05$). (B) The mRNA expression of MEGF6 in HCT8R, LoVo, HCT116, HT29, SW480, SW620, and HCT8S cell lines was detected by qPCR. (C) siRNA-mediated MEGF6 knockdown was detected by western blotting. (D) siRNA-mediated MEGF6 knockdown was determined by qPCR.

and qPCR (Fig. 1D) to determine the knockdown efficiency of MEGF6 siRNA in HCT8R cells. As shown, siMEGF6-1 most effectively knocked down the expression of MEGF6, thereby serving as a tool for subsequent studies (Fig. 1D).

MEGF6 was associated with CRC progression and patient outcome

To examine the potential relationship between MEGF6 and CRC, we analyzed data from the TCGA-COAD cohort, and found that MEGF6 was significantly upregulated in tumor tissues compared to normal tissues (Fig. 2A), indicating that MEGF6 may act as an oncogene in CRC. Then we evaluated MEGF6 expression based on American Joint Committee on Cancer stage. MEGF6 was more highly expressed in late-stage CRC (stages III and IV) than in early-stage CRC (stages I and II) (Fig. 2B). This suggests that MEGF6 may be associated with cancer aggressiveness. The Kaplan–Meier curve showed that MEGF6 predicted poor survival (Fig. 2C). These results show that MEGF6 is not only involved in CRC initiation and progression but is also correlated with patient outcome.

MEGF6 promoted CRC cell proliferation in vitro

Because MEGF6 is overexpressed in CRC, we determined whether it could increase the proliferation of CRC cells. We found that MEGF6 knockdown caused a significant decrease in CRC cell proliferation (Fig. 3A). Consistently, a reduced number of colonies was observed after MEGF6 depletion in CRC cells (Fig. 3B). The inhibitory effects on cell proliferation were confirmed by western blotting for proliferating cell nuclear antigen (PCNA) and Ki-67 (Fig. 3C). Next, we found that MEGF promoted resistance to 5-FU in CRC cells (Fig. 3D). We used flow cytometry to define apoptotic cells as Annexin V/PI double-positive cells. The percentage of apoptotic cells was significantly increased after MEGF6 knockdown in CRC cells (Fig. 4). These data suggest that MEGF6 may play an important role in CRC cell proliferation.

MEGF6 promoted metastasis in vitro and in vivo

To evaluate the effects of MEGF6 on the migration of CRC cells, transwell assays were conducted. The depletion of MEGF6 resulted in a decreased number of migratory cells (Fig. 5A). To examine the role of MEGF6 on metastasis in vivo, stably transfected LOVO cells (LOVO-pLKO and LOVO-shMEGF6) were injected into the spleen of nude mice. Less liver metastatic nodules were observed in mice injected with shMEGF6-transfected cells (Fig. 5B). Representative hematoxylin and eosin staining of liver tissues confirmed the results (Fig. 5C). We also analyzed a dataset in GEO (GSE81582) that compared MEGF6 mRNA expression in primary tumors with that in metastatic tumors. Consequently, we found that MEGF6 was highly expressed in metastatic tumors (Fig. 5D). These results suggest that MEGF6 may promote metastasis in CRC.

Fig. 2. MEGF6 was associated with CRC tumorigenesis and patient outcome in the TCGA-COAD cohort. (A) In the TCGA-COAD cohort, MEGF6 was more significantly expressed in cancer patients ($n=282$) than in healthy controls ($n=41$, **** $p<0.0001$). (B) In the TCGA-COAD cohort, MEGF6 was more significantly expressed in late-stage CRC (stages III and IV) patients than in early-stage CRC (stages I and II) patients (** $p<0.01$). (C) Overall survival (OS) was compared between patients with low and high MEGF6 expression in the TCG-COAD cohort.

MEGF6 induced the EMT of CRC cells

The EMT plays a critical role in tumor metastasis, induces the loss of connections between cells, and results in mesenchymal features [21, 22]. Consequently, we investigated if MEGF6 enhanced the EMT. After depletion of MEGF6, increased mRNA expression of the epithelial marker cadherin 1 (CDH1) and reduced expression of mesenchymal markers CDH2 and vimentin were observed (Fig. 6A). Western blotting provided further confirmation that knockdown of MEGF6 increased E-cadherin and reduced N-cadherin and vimentin expression in CRC cells (Fig. 6B). In TCGA (COAD, READ) cohort, MEGF6 mRNA expression was positively correlated with that of CDH2 and vimentin, but was negatively correlated with that of CDH1 (Fig. 6C). These data suggest that MEGF6 induces the EMT to promote CRC metastasis.

MEGF6 increased Slug expression to enhance the EMT

A large number of studies have reported that transcriptional repressors of CDH1 induce the EMT including the SNAI family (Snail and Slug), Zeb family (Zeb1 and Zeb2), and TWIST family (TWIST1 and TWIST2) [23–27]. Thus, we determined whether MEGF6 regulated the expression of these important transcriptional factors to induce the EMT. Zeb1 has mainly been studied in CRC [28, 29] and Zeb2 has mainly been studied in ovarian, gastric, and pancreatic tumors [30–32]; thus, the mRNA expression of SNAI1, SNAI2, Zeb1, TWIST1, and TWIST2 was detected using qPCR. As expected, MEGF6 knockdown

Fig. 3. MEGF6 promoted the proliferation of CRC cells. (A) The knockdown of MEGF6 inhibited the proliferation of HCT8R, HCT116, and LoVo CRC cells, according to the growth curve assay. (B) MEGF6 silencing inhibited colony formation in HCT8R, HCT116, and LoVo CRC cells. (C) The effects of MEGF6 on cell proliferation were demonstrated by using western blotting to evaluate PCNA and Ki-67 protein expression, β -actin served as the control. All samples were collected after 48 h post-transfection with siRNA. (D) The survival rate of cells was calculated by the MTT assay after treating with different concentrations of 5-FU. All the data are expressed as the mean $\pm$ SD from three independent experiments. Statistical significance was determined by the unpaired Student's t-test. ** $p < 0.01$.

Fig. 4. Depletion of MEGF6 caused apoptosis of CRC cells. Knockdown of MEGF6 induced apoptosis in CRC cell lines, as determined by Annexin V-FITC and PI double staining analysis by flow cytometry. Data are expressed as the mean $\pm$ SD from three independent experiments. Statistical significance was determined by the unpaired Student's t-test.

Fig. 5. MEGF6 promoted metastasis in vitro and in vivo. (A) MEGF6 knockdown inhibited the migration of HCT8R, HCT116, and LoVo cells, as determined by the transwell assay. (B) In vivo liver metastatic assay. Representative image of a mouse liver after injection of LOVO-pLKO and LOVO-shMEGF6 cells into the spleen. (C) Histological image of liver metastatic tumor by hematoxylin and eosin staining. (D) Analysis of GSE81582 dataset compared the mRNA expression of MEGF6 in primary tumors with that of metastatic tumors.

significantly repressed the mRNA expression of SNAI2 (Fig. 7A). The expression of protein Slug was detected by western blotting, and as expected, a reduction in Slug protein was found as well (Fig. 7B). In addition, MEGF6 mRNA expression was more positively correlated with that of SNAI2 in TCGA (COAD, READ) cohort (Fig. 7C).

MEGF6 induced the EMT via the TGF β /SMAD signaling pathway

Next, we investigated the mechanism underlying MEGF6 regulation of Slug expression. Increasing evidence has suggested that signaling pathways such as WNT/ β -catenin and

TGF β /SMAD pathways may be involved in modulating tumorigenesis [33, 34]. Among these pathways, activation of TGF β /SMAD plays an important role in enhancing the EMT to remodel the pro-metastasis phenotype [11], and SNAIL family members are regulated by the TGF β /SMAD signaling pathway [22]. Thus, we confirmed the expression of proteins critical in the TGF β /SMAD pathway. As expected, TGF β -1 and phosphorylation of SMAD2 and SMAD3 were reduced after depletion of MEGF6, which indicates that MEGF6 may induce the TGF β /SMAD signaling pathway (Fig. 8A). In addition, data from TCGA (COAD, READ) cohort showed that MEGF6 significantly positively correlated with TGF β 1, which is more evidence that MEGF6 may impact the TGF β /SMAD signaling pathway (Fig. 8B).

Discussion

Extensive metastasis is a major obstacle in effectively treating cancer [1]. The EMT, in which epithelial cells lose polarity and gain the stromal phenotype, has been invoked as a critical component of the metastatic process [35–38]. Different studies have shown that the suppression of E-cadherin is a vital molecular event involving specific E-boxes located in the proximal elements of promoters [24, 39]. The pattern of E-cadherin loss is currently thought to be transcriptional repression of CDH1 in different malignancies [22]. Indeed, several transcriptional factors strongly repress CDH1 transcription such as members of Snail, Zeb, and basic-helix-loop-helix (bHLH) families [22]. The TGF β /SMAD signaling pathway plays an important role in a complicated signaling network, which coordinates the expression of Slug, Zeb, and bHLH families to promote tumor progression and enhance the EMT [11]. However, the detailed mechanisms underlying the EMT are not fully understood. Here, we present novel data showing that MEGF6 promotes the EMT via the TGF β /SMAD signaling pathway.

The MEGF family plays an important role in nervous system development. MEGF10 induces clearance of apoptotic astrocytes in the brain [40] and its deficiency is associated with NOTCH signaling to regulate cell proliferation and migration [41, 42]. Furthermore, methylation of MEGF10 reduces relapse-free survival in neuroblastoma [43]. MEGF6 is

Fig. 6. MEGF6 promoted cell migration by inducing the EMT. (A) qPCR was used to show that depletion of MEGF6 significantly increased the mRNA expression of the epithelial marker (CDH1) and decreased expression of the mesenchymal markers (CDH2 and vimentin). (B) Depletion of MEGF6 induced E-cadherin expression and reduced N-cadherin and vimentin expression, as determined by western blotting. (C) Correlation between EMT markers and MEGF6 in TCGA (COAD, READ) cohort. MEGF6 expression was positively correlated with CDH2 ($r = 0.49$, $p < 0.0001$) and vimentin expression ($r = 0.47$, $p < 0.0001$), and was negatively correlated with CDH1 expression ($r = -0.26$, $p < 0.0001$).

Fig. 7. MEGF6 induced the expression of Slug to promote the EMT. (A) qPCR analysis showed that MEGF6 knockdown significantly reduced the mRNA expression of SNAI2. (B) Western blotting showed that MEGF6 knockdown specifically reduced the protein expression of Slug. (C) Correlation between MEGF6 and SNAI1 and SNAI2. The coefficient of SNAI2 ($r = 0.33$, $p < 0.0001$) was much larger than that of SNAI1 ($r = 0.17$, $p < 0.01$), and SNAI2 was positively correlated with MEGF6.

located at chromosome 1, which encodes a high-molecular-weight protein similar to that of MEGF10 with respect to amino acid sequence [44]. Proteomic analysis of the epidermal growth factor receptor interactome revealed that MEGF6 may regulate this protein, and modulate cell growth and migration in CRC [45]. In addition, it has been reported that MEGF6 regulates cell-to-cell adhesion and degeneration of Purkinje cells in human inherited ataxia [16]. These results suggest that MEGF6 may regulate the growth and migration of cells.

In this study, we found that MEGF6 was upregulated in HCT8R cells, which suggests that MEGF6 might be involved in CRC development. Moreover, we confirmed that MEGF6 was significantly overexpressed in tumor tissue and had potential clinical significance in TCGA-COAD cohort. Thus, the role of MEGF6 in CRC was investigated. We found that MEGF6 knockdown inhibited CRC cell proliferation, as determined by growth curve, colony formation, and western blot assays. Our results also showed that MEGF6 promoted metastasis by transwell assays *in vitro* and liver metastasis assays *in vivo*. Moreover, we found that MEGF6 induced SNAI2 expression to trigger the EMT via the TGF β /SMAD signaling pathway. These data indicate the role of TGF β /SMAD signaling in promoting metastasis in CRC.

Fig. 8. MEGF6 induced the EMT by activating the TGF β /SMAD signaling pathway. (A) The expression of TGF β -1, and phosphorylation of SMAD2, SMAD3 was assessed by western blotting in HCT8R, HCT116, and LoVo cells. Depletion of MEGF6 decreased the expression of TGF β -1, and phosphorylated SMAD2 and SMAD3. (B) MEGF6 was positively correlated with TGF β -1 in the TCGA (COAD, READ) ($r = 0.49$, $p < 0.0001$).

5-FU is widely used in CRC patients and many are responsive to 5-FU-based chemotherapy, but relapse associated with drug resistance is a frequent occurrence [1, 46, 47]. Consequently, investigating the mechanism underlying 5-FU resistance is important for improving chemotherapeutic strategies. Unlike previous works that have mainly focused on the nervous system [16, 45], we studied MEGF6 in CRC and found that it regulates resistance to 5-FU in CRC cells. However, the role of MEGF6 was not limited to the regulation of 5-FU resistance, as we also found that it influenced cell proliferation and metastasis. In addition, MEGF6 also served as a significant clinical marker to predict survival and was associated with the progression of CRC. In light of these multiple functions of MEGF6, it may serve as a novel therapeutic target in the treatment of CRC.

Taken together, the results of this study showed the clinical significance of MEGF6 in CRC. We found that suppression of MEGF6 inhibited the proliferation and suppressed the EMT via downregulating Slug, which was mediated by the TGF β /SMAD signaling pathway. Consequently, our work reveals a novel therapeutic target for the treatment of CRC.

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Disclosure Statement

No conflict of interests exists.

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