MEIS2 promotes cell migration and invasion in colorectal cancer

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Abstract. Colorectal cancer (CRC) is one of the most common types of malignancy worldwide. Distant metastasis is a key cause of CRC-associated mortality. MEIS2 has been identified to be dysregulated in several types of human cancer. However, the mechanisms underlying the regulatory role of MEIS2 in CRC metastasis remain largely unknown. For the first time, the present study demonstrated that MEIS2 serves a role as a promoter of metastasis in CRC. *In vivo* and *in vitro* experiments revealed that knockdown of MEIS2 significantly suppressed CRC migration, invasion and the epithelial-mesenchymal transition. Furthermore, microarray and bioinformatics analyses were performed to investigate the underlying mechanisms of MEIS2 in the regulation of CRC metastasis. Additionally, it was identified that a high expression of MEIS2 was significantly associated with a shorter overall survival time for patients with CRC. The present study demonstrated that MEIS2 may serve as a novel biomarker for CRC.

Introduction

Colorectal cancer (CRC) is one of the most commonly diagnosed types of malignancy, with ~134,490 new cases diagnosed worldwide (1,2). In 2016, ~49,190 CRC-associated mortalities were reported worldwide (2). Distant metastasis of cancer is a predominant cause of CRC-associated mortality (3). The 5-year survival rate of metastatic CRC is as low as ~10%. In the past few decades, several regulators of CRC metastasis have been identified, including HNRNPLL and PGE2 (4,5). HNRNPLL has been revealed to modulate alternative splicing of CD44 during the epithelial-mesenchymal transition (EMT), which leads to suppression of CRC metastasis (4). PGE2 induced an expansion of CRC stem cells to promote liver metastases in mice by activating NF-κB (5). However, to the best of our knowledge, the mechanisms underlying CRC metastasis remain unclear.

MEIS proteins, including MEIS1, MEIS2 and MEIS3, serve crucial roles in regulating the neural crest and limb development (6,7). MEIS proteins interacts with HOX or PBX proteins to form a homeoprotein-DNA complex. MEIS2, a member of the MEIS protein family, has been implicated in the pathogenesis of human cancer (8,9). MEIS2 has been revealed to be overexpressed in neuroblastoma cells and to promote neuroblastoma cell proliferation and tumorigenicity (10). In addition, MEIS2 was upregulated and required for AML1-ETO-positive AML growth (11). Conversely, a high expression of MEIS2 has been associated with an improved prognosis for patients with ovarian cancer (12). A recent study demonstrated that the protein expression level of MEIS2 was associated with a lack of biochemical recurrence and progression to clinically metastatic disease in prostate cancer (9). However, the functional roles of MEIS2 in CRC, particularly in CRC metastasis, remain unclear.

The present study aimed to investigate the role of MEIS2 in the regulation of CRC metastasis using *in vivo* and *in vitro* experiments. Furthermore, public datasets were analyzed to evaluate the prognostic value of MEIS2 in CRC. The present study provided novel information that supports the potential clinical use of MEIS2 as a prognostic marker for CRC.

Materials and methods

Cell culture. HCT116 cells were obtained from The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China) and cultured in RPMI-1640 medium (HyClone; GE Healthcare, Chicago, IL, USA)

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containing 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The HCT116 cells were cultured at 37˚C in a humidified incubator with 5% CO_2.

Lentiviral constructs and transfections. The MEIS2 shRNA sequences were obtained from Shanghai GeneChem Co., Ltd. (Shanghai, China). Recombinant lentiviral vectors carrying MEIS2 shRNA were constructed according to the manufacturer's protocol. The MEIS2 shRNA sequences were designed using an online system (http://rnaidesigner.thermofisher.com/rnaiexpress/) and purchased from Shanghai GeneChem Co., Ltd. The sequence of MEIS2 shRNA-1 was 5'-CCGGCCCATGAT TGACCAGTCAAATTTCAAGAGAATTTGACTGGTCAA TCATGGGTTTTTG-3' and the sequence of MEIS2 shRNA-2 was CCGGCCCATGATTGACCAGTCAAATTTCAAGAG AATTTGACTGGTCAATCATGGGTTTTTG. Recombinant lentiviral vectors carrying MEIS2 shRNAs were constructed with standard molecular techniques. 293T cells were transfected with the recombinant vectors to generate lentiviruses. Concentrated lentiviruses were transfected in HCT116 cells at a multiplicity of infection (MOI) of 40 in RPMI-1640 medium (HyClone; GE Healthcare) without FBS. The expression of MEIS2 in HCT116 knockdown cells was validated by quantitative real-time polymerase chain reaction (qRT-PCR).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from cells using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Subsequently, cDNA was synthesized using the RevertAid First Strand cDNA Synthesis kit (Promega Corp., Madison, WI, USA), according to the manufacturer's protocol. qPCR was performed using the iQ™ SYBR-Green SuperMix (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The following primers were used for qPCR: CEBPA forward, 5'-CCAGAAAGCTAG GTCGTGGGT-3' and reverse, 5'-TGGACTGATCGTGCTTCG TGT-3'; JUN forward, 5'-ATGGTCAGGTTATACTCCTCC TC-3' and reverse, 5'-CACATGCCACTTGATACAATCC-3'; TGFBR2 forward, 5'-TGGCTGTATGGAGAAAGA-3' and reverse, 5'-GTCAGGATTGCTGGTGTT-3'; GAPDH forward, 5'-TGACTTCAACAGCGACACCCA-3' and reverse, 5'-CAC CCTGTTGCTGTAGCCAAA-3'; MDM2 forward, 5'-GAA TCATCGGACTCAGGTACATC-3' and reverse, 5'-TCTGTC TCACTAATTGCTCTCCT-3'; CDKN1A forward, 5'-CTGTCT TGTACCCTTGTGCCT-3' and reverse, 5'-GGTAGAAATCTG TCATGCTGGT-3'; and TGFBR2 forward, 5'-CGATAACTT CTGCCACCGAT-3' and reverse, 5'-AGGGTTATGGTCAGC GAGAT-3'. The $2^{-\Delta\Delta Cq}$ method was used to calculate the relative expression levels of the target genes (13).

Wound healing assay. HCT116 cells were seeded into a 6-well plate. Once the confluency of the cells reached ~80%, a scratch was created in a monolayer of the cells using a sterile micropipette tip. The detached cells were then washed with phosphate-buffered saline (PBS). The extent of wound healing was observed at 0, 24 and 48 h. Images were captured from 5 random fields using an inverted microscope. Triplicate wells for each condition were examined.

Transwell assay. Transwell assays were performed using 8-μm pore size Transwell® plates (Corning Inc., Corning, NY, USA). The invasion assay was performed using Matrigel® (BD Biosciences, San, Jose, CA, USA), which was used to pre-coat Transwell® plates. A total of 50,000 HCT116 cells, cultured in RPMI-1640 with 2% FBS, were seeded into the upper well. In addition, RPMI-1640 with 10% FBS was added to the bottom well. Following incubation for 72 h the number of invading cells was counted.

In vivo tumor metastasis assays. A total of $4x10^6$ MEIS2-knockdown or negative control HCT116 cells were transplanted into ten 5-week-old female nude mice (from the Shanghai Slake Laboratory Animal Co., Ltd., Shanghai, China) weighing 15-20 g through the lateral tail vein. The mice were housed at a temperature of 20-26˚C, a relative humidity of 40-70% and light/dark cycle of 12/12 h. Luciferase-expressing HCT116 cells were constructed. MEIS2-knockdown and control luciferase-expressing HCT116 cells were then injected into the mice and monitored using an IVIS system (IVIS; PerkinElmer, Inc., Waltham, MA, USA). The animal was sacrificed when the tumor reached 1 cm in diameter. The mice were sacrificed 7 weeks after injection with CO_2 (with the flow rate of CO_2 euthanasia displacing ≤30% of the chamber volume/min). To detect the effect of MEIS2 on tumor metastasis, the lungs were collected and hematoxylin and eosin (H&E) staining was performed. All *in vivo* study protocols were approved by the Shanghai Medical Experimental Animal Care Commission (Approval ID: ShCI-14-008).

Western blot analysis. The CRC cells were rinsed with PBS, and lysates were prepared using RIPA buffer (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). Total protein concentrations were determined using a BCA protein concentration assay kit (Beyotime Institute of Biotechnology, Haimen, China). A quantity of 50 μg protein sample was loaded to each lane before electrophoresis began. Proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes using a Bio-Rad System (Bio-Rad Laboratories, Inc.). Blotted membranes were firstly moved to blocking buffer containing 5% non-fat dry milk (diluted in TBST) at room temperature for 1 h. Western blot analysis was performed with E-cadherin (diluted 1:1,000; cat. no. 14472; Cell Signaling Technologie), Snail (diluted 1:1,000; cat. no. 3895; Cell Signaling Technologies), MEIS2 (diluted 1:1,000; cat. no. ab73164; Abcam, Cambridge, MA, USA), Twist (diluted 1:1,000; cat. no. ab50581; Abcam), JUN (diluted 1:1,000; cat. no. ab32137; Abcam), CEBPA (diluted 1:1,000; cat. no. 8178; Cell Signaling Technologies), MDM2 (diluted 1:1,000; cat. no. ab38618; Abcam), TGFBR2 (diluted 1:1,000; cat. no. ab61213; Abcam) and CDKN1A (diluted 1:1,000; cat. no. 2947; Cell Signaling Technologies), and mouse anti-GAPDH (diluted 1:1,000; cat. no. c-25778; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) at 4˚C overnight and then incubated with 2 mg/ml HRP-conjugated anti-rabbit IgG (diluted 1:5,000; cat. no. A9169; Sigma-Aldrich; Merck KGaA) for 1 h at room temperature. After washing, ECL Western Blotting reagent (Millipore; Merck KGaA) was applied for the detection. The Quantity One software package (Bio-Rad Laboratories, Inc.) was used for quantitation of the signal intensities.

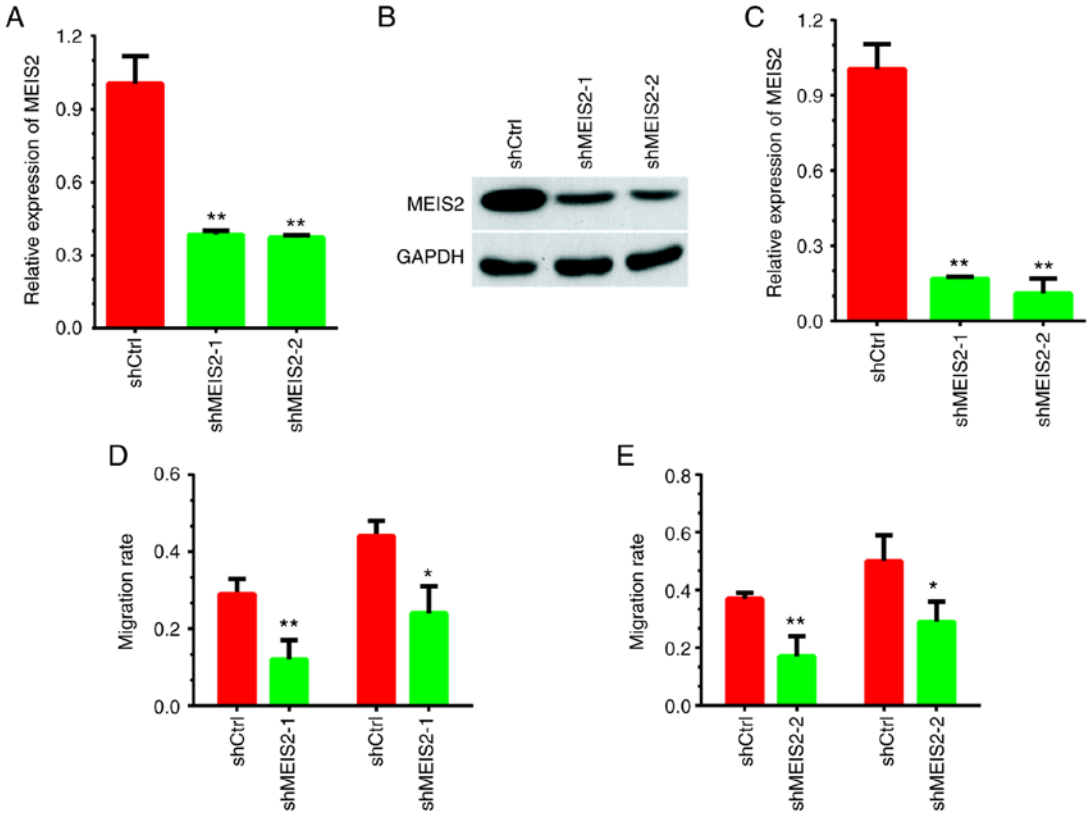

Figure 1. Knockdown of MEIS2 suppresses CRC migration. (A) RT-qPCR and (B) western blot analysis revealed that knockdown of MEIS2 suppressed the endogenous expression of MEIS2. (C) Relative values of each band normalized to GAPDH. A wound healing assay revealed that MEIS2-knockdown with (D) shRNA-1 and (E) shRNA-2 significantly inhibited migration of CRC cells. Data are presented as the mean ± standard deviation (n=3). *P<0.05, **P<0.01. CRC, colorectal cancer.

Microarray and expression datasets. Total RNA was extracted using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and was quantified by the NanoDrop ND-2000 (Thermo Fisher Scientific, Inc.). Global expression of mRNAs in 3 MCM10 shCtrl samples and 3 shMCM10 were examined using the GeneChip PrimeView Human Gene Expression Array (Thermo Fisher Scientific, Inc.). The raw data of the mRNA expression profiles were downloaded and analyzed by R language software. Background correction, quartile data normalization, and probe summarization were applied for the original data. The limma method in Bioconductor (http://www.bioconductor.org/) was used to identify genes which were differentially expressed between two groups; the significance of DEGs was calculated by t-test and was represented by a P-value. The threshold set for upregulated and downregulated genes was a fold-change ≥1.5.

Microarray data. The gene expression profiles of MEIS2 in CRC by analyzing a series of public datasets, including The Cancer Genome Atlas (TCGA), GSE17536 (14), GSE17537 (14) and GSE41258 (15) datasets. TCGA dataset included 10 normal colon samples and 367 CRC samples. GSE17536 dataset included 177 patients with CRC from the Moffitt Cancer Center (Tampa, FL, USA) were used as the independent dataset. GSE17537 dataset included 55 CRC patients from Vanderbilt Medical Center (Nashville, TN, USA). GSE41258 dataset included 54 normal colon samples, 13 normal liver samples, 7 normal lung samples and 186 primary CRC samples. Moreover, we analyzed the protein levels of MEIS2 in CRC by analyzing Human Protein Atlas database (https://www.proteinatlas.org/).

Functional enrichment analysis. The Database for Annotation, Visualization, and Integrated Discovery (DAVID) online tool (version 6.8; david.ncifcrf.gov) was applied to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. Adjusted P<0.05 was considered to indicate statistical significance.

Statistical analysis. SPSS 13.0 software (SPSS, Inc., Chicago, IL, USA) was used to perform statistical analysis. Each experiment was performed 3 times. Student's t-test was used to calculate the statistical significance between 2 groups. For >2 groups, one-way analysis of variance followed by Newman-Keuls post hoc test was used. P<0.05 was considered to indicate a statistically significant difference.

Results

Knockdown of MEIS2 suppresses cell migration in CRC. The present study performed loss-of-function assays by silencing MEIS2 expression to investigate its role in CRC. As presented in Fig. 1A-C, shRNA-mediated silencing of MEIS2 led to a significant decrease in both mRNA and protein expression levels of MEIS2 in HCT116 cells. Subsequently, the effects of MEIS2 on cell migration were examined by

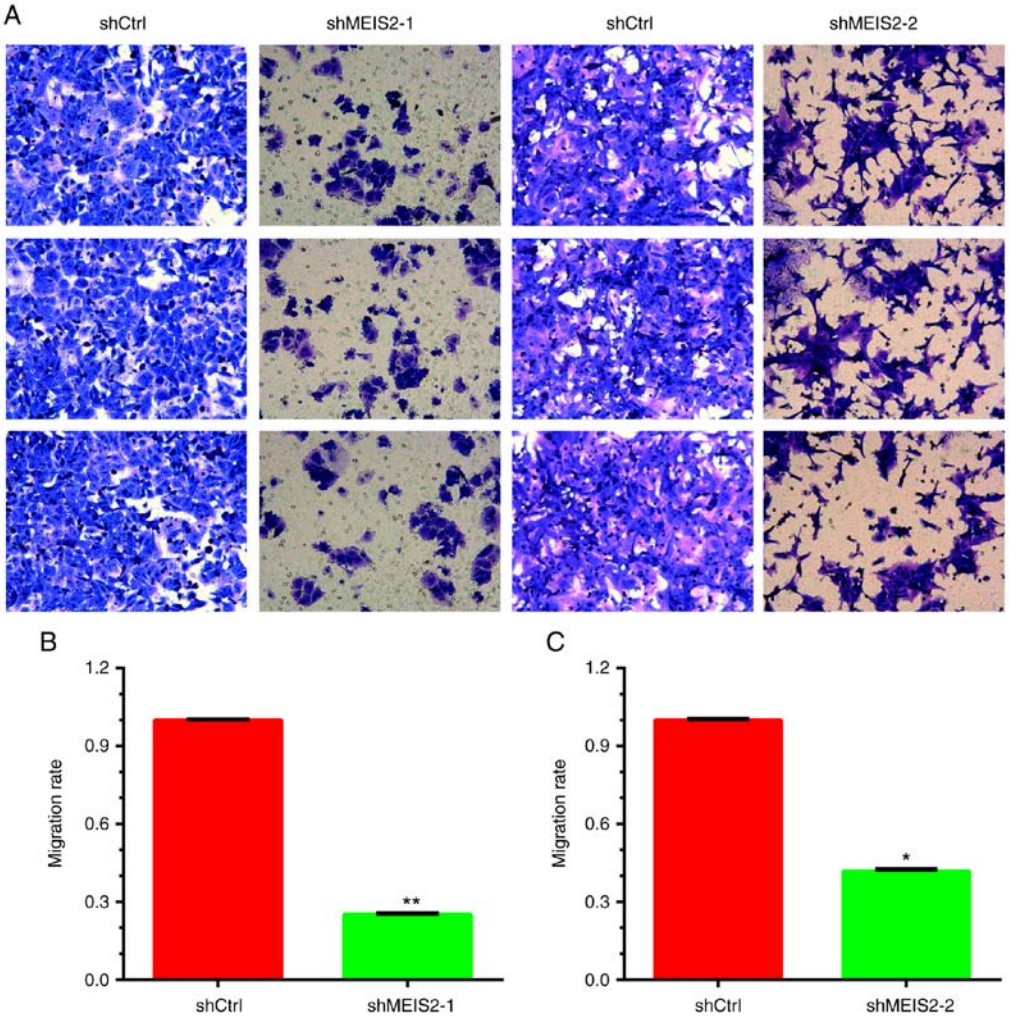

Figure 2. Knockdown of MEIS2 suppresses CRC migration. (A) A Transwell assay revealed that MEIS2-knockdown significantly inhibited cell migration in CRC cells. Quantitative analysis demonstrated that MEIS2-knockdown with (B) shRNA-1 and (C) shRNA-2 significantly inhibited migration of CRC cells. Data are presented as the mean ± standard deviation (n=3). *P<0.05, **P<0.01. CRC, colorectal cancer.

wound healing assay. Compared with the negative control group, MEIS2-knockdown significantly suppressed the number of HCT116 cells migrating toward the wound area (Fig. 1D-E and S1).

Furthermore, a Transwell assay was performed to investigate the role of MEIS2 in the regulation of migration. It was revealed that the number of migrating HCT116 cells decreased by ~70 and 60% in the shRNA-1 (Fig. 2A and B) and shRNA-2 (Fig. 2A and C) knockdown groups, respectively, compared with the negative control group.

Knockdown of MEIS2 inhibits cell invasion in CRC. The invasive ability of cells transfected with MEIS2 shRNAs was assessed by Matrigel cell invasion assay. The results indicated that knockdown of MEIS2 significantly suppressed the invasion of CRC cells (P<0.05). The number of invading HCT116 cells decreased by ~80 and 45% in the shRNA-1 (Fig. 3A and B) and shRNA-2 (Fig. 3A and C) knockdown groups, respectively, compared with the negative control group.

Knockdown of MEIS2 inhibits EMT in CRC. To investigate whether MEIS2 promotes metastasis by regulating EMT, the protein levels of E-cadherin, Twist and Snail were detected following MEIS2 knockdown in CRC cells. Western blot analysis revealed that the expression level of E-cadherin increased, while Twist and Snail levels decreased in MEIS2-knockdown HCT116 cells compared with the control groups (Fig. 4A and B).

Knockdown of MEIS2 inhibits CRC metastasis in vivo. Since it was demonstrated that MEIS2 knockdown inhibited cell motility *in vitro*, the effect of MEIS2 on CRC metastasis was further validated *in vivo*. The results demonstrated that MEIS2-knockdown significantly inhibited CRC metastasis *in vivo*. The luciferase signaling in the MEIS2-knockdown group was significantly decreased compared with the control groups (Fig. 5A and C).

Furthermore, histological analysis was performed to confirm that MEIS2-knockdown inhibits the formation of lung metastasis. It was revealed that the number of lung metastasis nodules was markedly lower in the MEIS2-knockdown group compared with the control group (Fig. 5B).

Microarray analysis reveals targets of MEIS2 in CRC. Previous studies have indicated that MEIS2 is involved in the regulation of gene transcription by interacting with

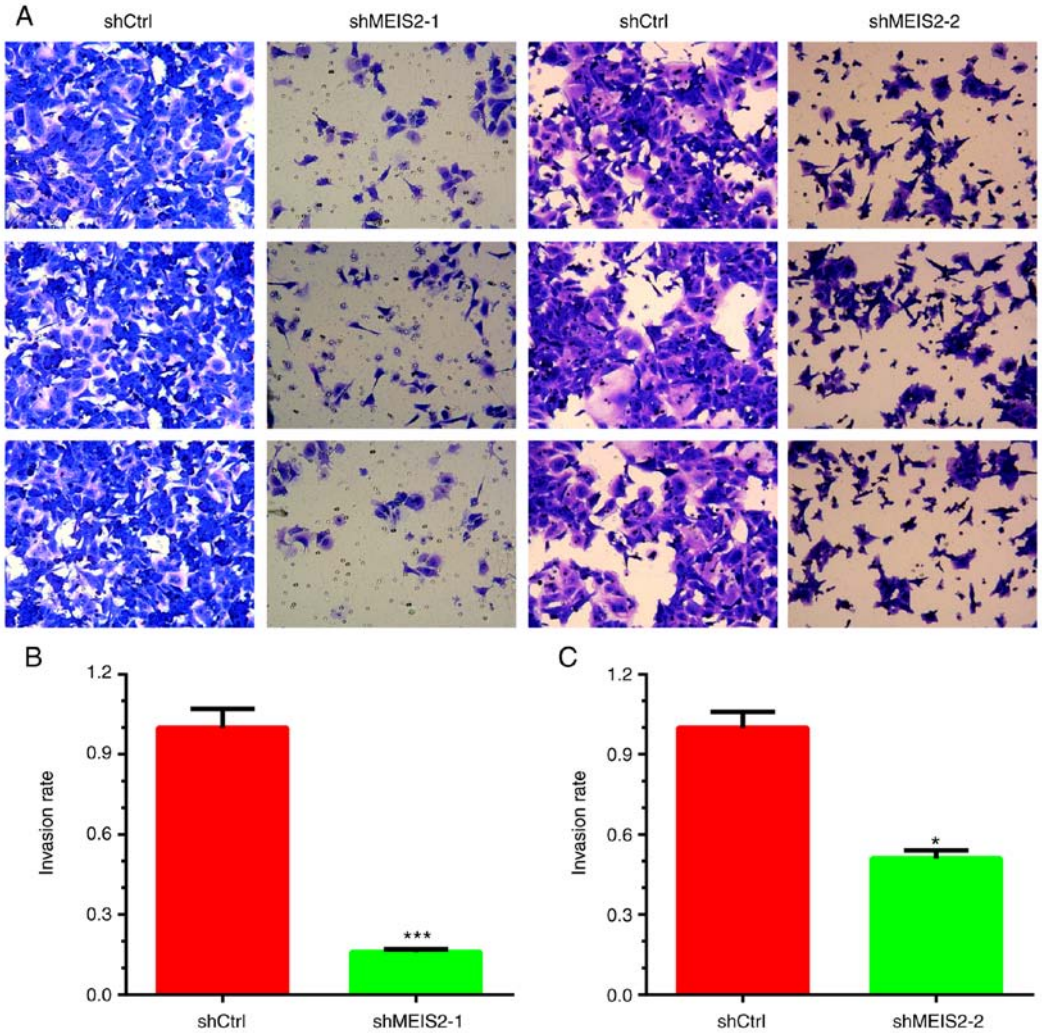

Figure 3. Knockdown of MEIS2 suppresses CRC invasion. (A) A Transwell Matrigel assay revealed that MEIS2-knockdown significantly inhibited invasion of CRC cells. Quantitative analysis demonstrated that MEIS2-knockdown with (B) shRNA-1 and (C) shRNA-2 significantly inhibited invasion of CRC cells. Data are presented as the mean ± standard deviation (n=3). *P<0.05, ***P<0.001. CRC, colorectal cancer.

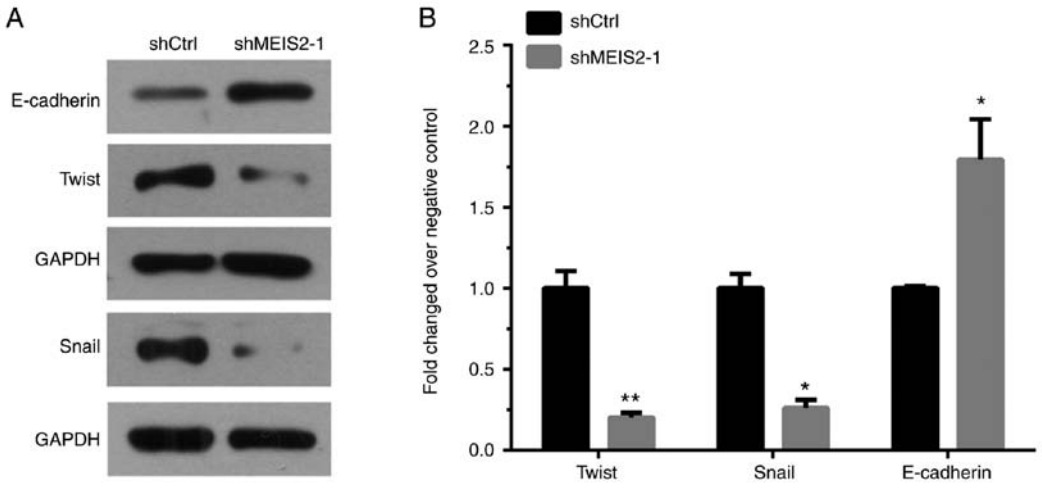

Figure 4. Knockdown of MEIS2 suppresses EMT. (A) MEIS2-knockdown markedly decreased the protein levels of Twist and Snail, and increased the protein level of E-cadherin. (B) Relative values of each band normalized to GAPDH. EMT, epithelial-mesenchymal transition. *P<0.05, **P<0.01.

HOX and PBX proteins to form protein-DNA complex. However, the targets of MEIS2 in certain human cancer types, including CRC, remain unknown. The present study performed microarray analysis, which identified

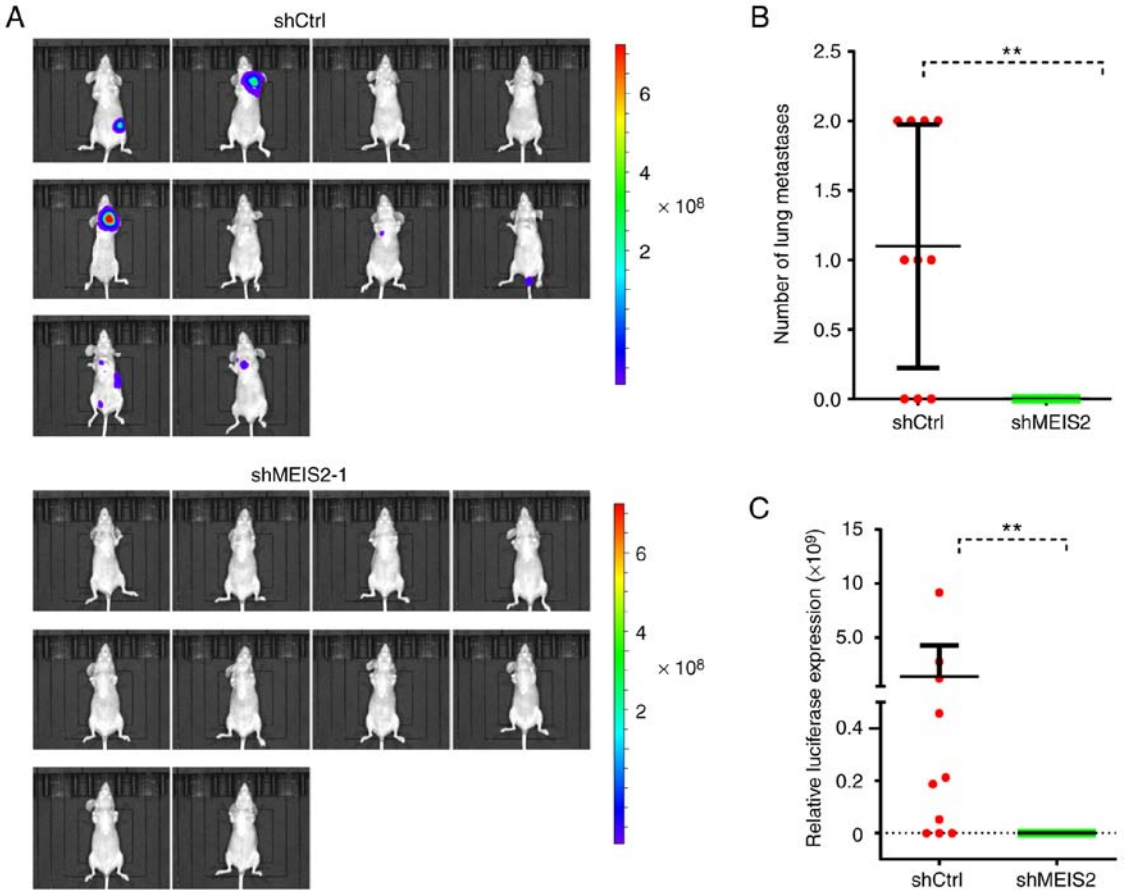

Figure 5. Knockdown of MEIS2 suppresses CRC invasion *in vivo*. (A) MEIS2-knockdown significantly inhibited CRC invasion *in vivo*. (B) The number of lung metastasis nodules was significantly decreased in the MEIS2-knockdown group compared with the control group. (C) The luciferase activity was significantly lower in the MEIS2-knockdown group compared with the control groups. Data are presented as the mean ± standard deviation (n=3). **P<0.01. CRC, colorectal cancer.

338 upregulated and 317 downregulated genes following MEIS2-knockdown (Fig. 6A). The top 10 upregulated genes are presented in Table I and the top 10 downregulated genes are revealed in Table II. Bioinformatics analyses, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes pathway analysis, were subsequently performed.

As anticipated, it was revealed that the downregulated genes following MEIS2-knockdown were associated with negative regulation of apoptotic process, chondroitin sulfate biosynthetic process, regulated exocytosis, ER-associated misfolded protein catabolic process, protein kinase B signaling, Golgi organization, Rap1 signaling pathway and glycosaminoglycan biosynthesis. Furthermore, upregulated genes following MEIS2-knockdown were identified to be involved in regulating cilium assembly, response to stress, protein transport, cellular response to starvation, negative regulation of TOR signaling, mitotic spindle organization, p53 signaling pathway, FoxO signaling pathway, carbohydrate digestion and absorption, endocytosis, and PI3K-Akt signaling pathway (Fig. 6B-E).

To further validate the microarray analysis results, the expression levels of several key pathway regulators were detected using RT-qPCR following MEIS2-knockdown in CRC cells. As presented in Fig. 7A, it was revealed that CEBPA was downregulated, and JUN, TGFBR2, MDM2 and CDKN1A were upregulated following MEIS2-knockdown in HCT116 cells. Western blot analysis also revealed similar results (Fig. 7B and C).

Higher MEIS2 expression is associated with shorter overall survival time in CRC. Next, the protein levels of MEIS2 in CRC tissue and normal colorectal tissue were analyzed using the Human Protein Atlas database. As revealed in Fig. S2, it was demonstrated that the MEIS2 protein levels in CRC samples were high. In addition, the MEIS2 protein levels in normal colon and rectum samples were also high. Furthermore, the present study analyzed whether the dysregulation of MEIS2 was correlated with overall survival time in CRC by analyzing a series of public datasets, including The Cancer Genome Atlas (TCGA), GSE17536 (14), GSE17537 (14) and GSE41258 (15) datasets. Analysis of TCGA data revealed that a high expression level of MEIS2 was correlated with a shorter overall survival time for patients with CRC (Fig. 8A). Subsequently, the Gene Expression Omnibus datasets, including GSE17536, GSE17537 and GSE41258, were further analyzed to validate the aforementioned analysis. A similar result was observed, where the overall survival time was shorter in the MEIS2-high group compared with the MEIS2-low group (Fig. 8B-D). These results indicated that the dysregulation of MEIS2 could serve as a novel biomarker for CRC.

Table I. The top 10 upregulated genes after MEIS2 knockdown in CRC cell line.

Entrez	Gene symbol	Fold-change	logFC	Regulation	P-value	FDR
5899	RALB	2.42	1.27	Upregulation	4.87E-14	3.46E-11
10769	PLK2	2.46	1.3	Upregulation	2.03E-11	2.97E-09
23639	LRRC6	2.52	1.33	Upregulation	1.69E-11	2.63E-09
9270	ITGB1BP1	2.57	1.36	Upregulation	5.17E-10	3.39E-08
23568	ARL2BP	2.71	1.44	Upregulation	5.11E-17	7.51E-13
5824	PEX19	2.75	1.46	Upregulation	1.08E-13	6.16E-11
4193	MDM2	2.85	1.51	Upregulation	8.80E-09	3.14E-07
5887	RAD23B	2.93	1.55	Upregulation	4.03E-16	1.57E-12
51200	CPA4	3.51	1.81	Upregulation	5.73E-17	7.51E-13
5906	RAP1A	22.75	4.50	Upregulation	6.09E-27	2.39E-22

CRC, colorectal cancer; FDR, false discovery rate.

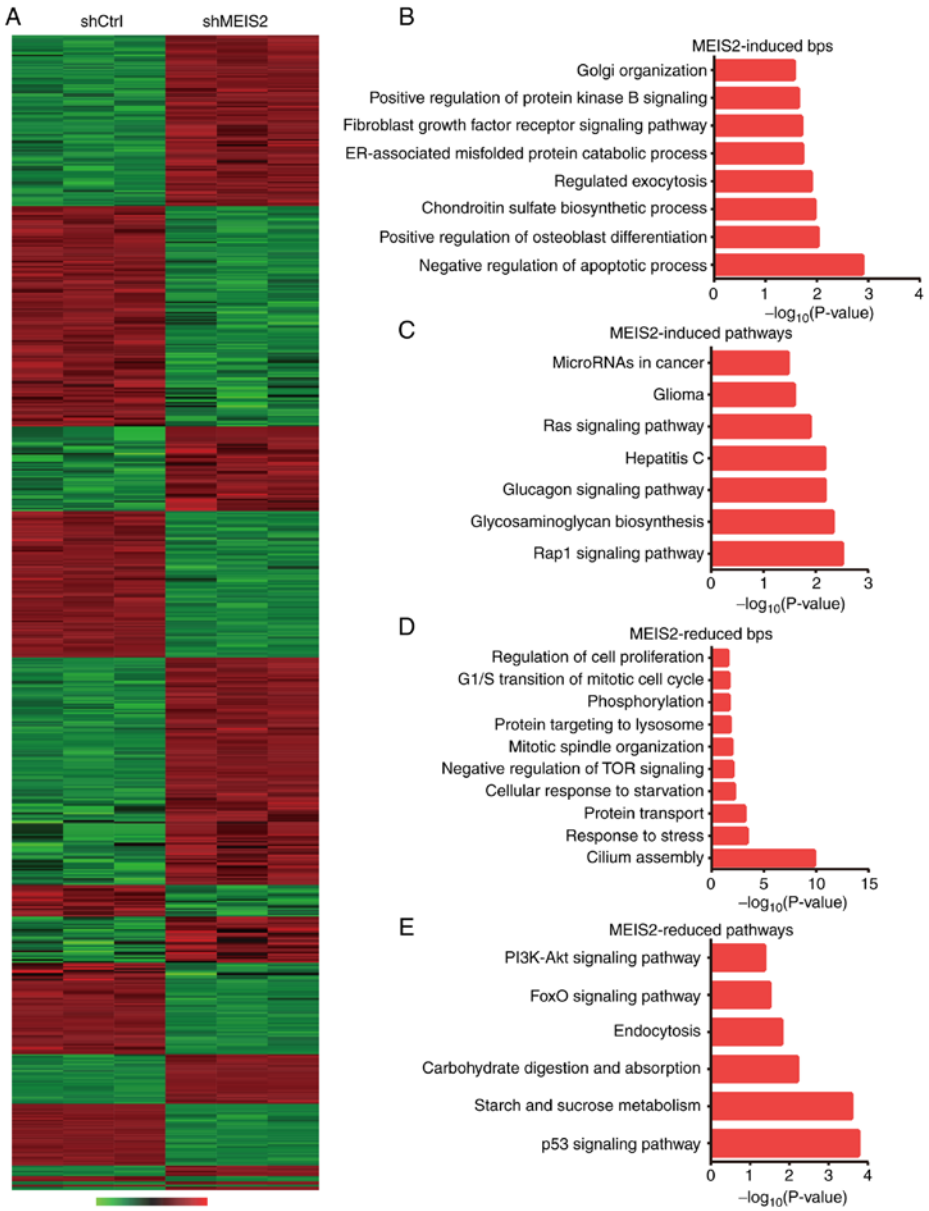

Figure 6. Identification of genes regulated by MEIS2 in CRC. (A) A heatmap revealed the differentially expressed genes following MEIS2-knockdown. (B) GO and (C) KEGG analysis revealed the potential roles of the genes downregulated following MEIS2-knockdown in HCT116 cells. (D) GO and (E) KEGG analysis revealed the potential roles of the genes upregulated following MEIS2-knockdown in HCT116 cells. CRC, colorectal cancer; bps, biological processes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table II. The top 10 downregulated genes after MEIS2 knockdown in CRC cell line.

Entrez	Gene symbol	Fold-change	logFC	Regulation	P-value	FDR
9802	DAZAP2	-2.83	-1.50	Downregulation	1.29E-16	1.01E-12
81839	VANGL1	-2.81	-1.49	Downregulation	5.55E-14	3.63E-11
7504	XK	-2.77	-1.47	Downregulation	9.71E-13	2.75E-10
8519	IFITM1	-2.74	-1.45	Downregulation	2.53E-16	1.34E-12
154807	VKORC1L1	-2.66	-1.41	Downregulation	2.01E-12	4.95E-10
5880	RAC2	-2.53	-1.34	Downregulation	5.26E-16	1.72E-12
9474	ATG5	-2.53	-1.34	Downregulation	1.45E-12	3.80E-10
79801	SHCBP1	-2.50	-1.32	Downregulation	1.03E-13	5.95E-11
5160	PDHA1	-2.42	-1.27	Downregulation	2.55E-12	5.77E-10
55591	VEZT	-2.39	-1.25	Downregulation	1.43E-09	7.27E-08

CRC, colorectal cancer; FDR, false discovery rate.

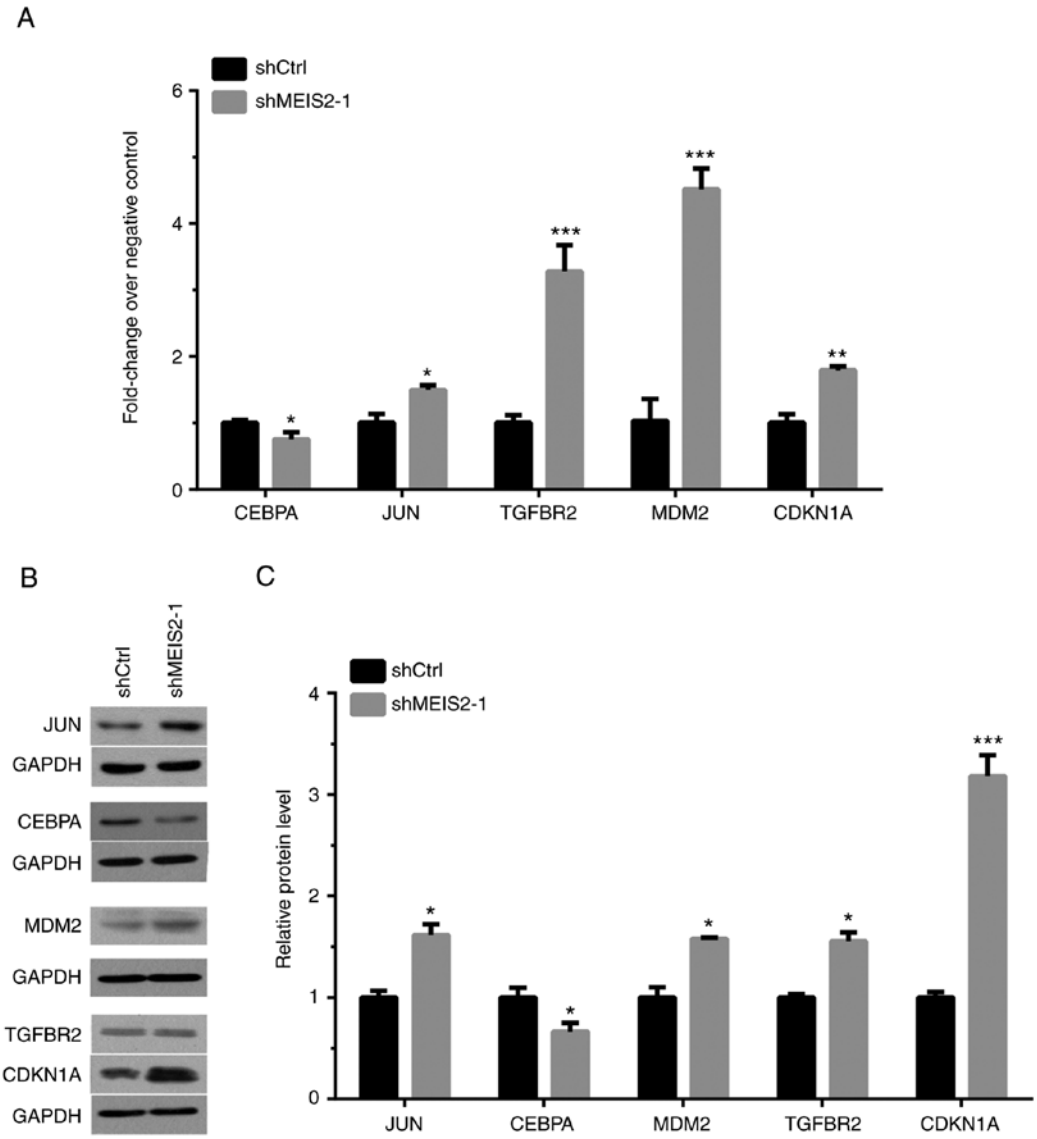

Figure 7. Knockdown of MEIS2 regulates cell proliferation-associated genes. (A) RT-qPCR analysis revealed that MEIS2-knockdown regulated the expression of CEBPA, JUN, TGFBR2, MDM2 and CDKN1A. Data are presented as the mean ± standard deviation (n=3). (B) Western blot analysis demonstrated that MEIS2-knockdown regulated the expression of CEBPA, JUN, TGFBR2, MDM2 and CDKN1A. (C) Relative values of each band normalized to GAPDH. Data are presented as mean ± SD (vertical bars). At least 3 independent experiments were performed. *P<0.05, **P<0.01, ***P<0.001.

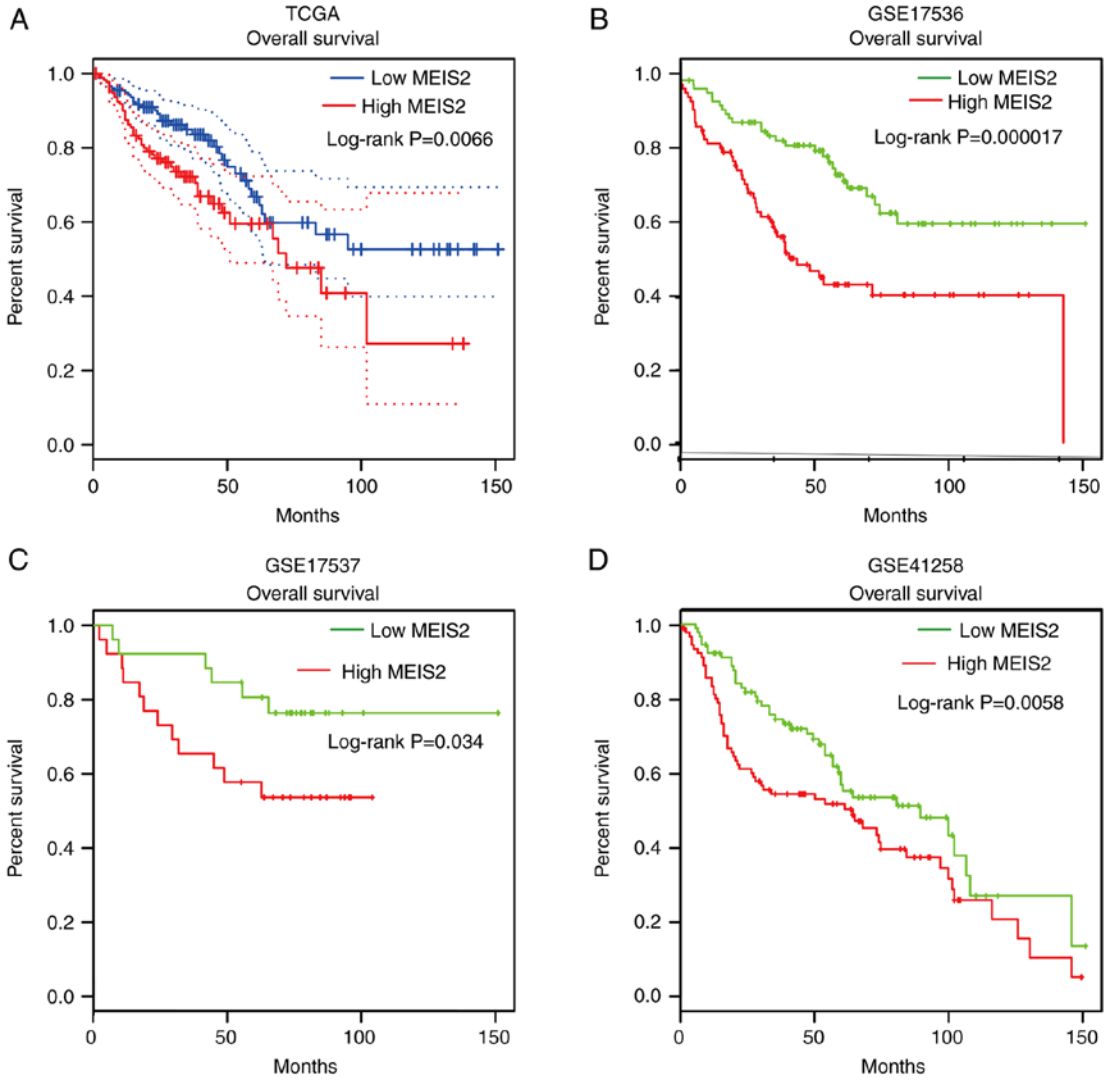

Figure 8. High MEIS2 expression levels are associated with shorter overall survival and disease-free survival times in CRC. Analysis of (A) TCGA, (B) GSE17536, (C) GSE17537 and (D) GSE41258 data revealed that a high MEIS2 expression level is associated with a shorter overall survival time for patients with CRC. The dashed/dotted lines represent the 95% confidence interval of survival curves. CRC, colorectal cancer.

Discussion

MEIS2 has been identified to be involved in the tumorigenesis of human cancer. Previous studies have demonstrated that MEIS2 served crucial roles in cancer proliferation, and was dysregulated in neuroblastoma (10), AML1-ETO-positive AML (11), and ovarian cancer (12). A recent study regarding prostate cancer revealed that MEIS2 may be associated with the progression of metastasis, since tumor expression of MEIS2 was correlated with clinically metastatic disease (8,9). However, to the best of our knowledge, the roles of MEIS2 in the regulation of CRC metastasis progression remain unknown. For the first time, the present study determined the effect of MEIS2 on CRC metastasis using *in vivo* and *in vitro* assays.

Distant metastasis of cancer is a key cause of CRC-associated mortality. In the past few decades, several genes have been reported to be regulators of CRC metastasis. For example, Qi *et al* (16) revealed that BOP1, CKS2 and NFIL3 served as new targets of the Wnt pathway and influenced CRC metastasis in mice. miR-224 has also been revealed to act as a promoter of metastasis by suppressing SMAD4 in CRC (17). However, to the best of our knowledge, the detailed mechanisms underlying CRC metastasis remain to be further investigated. The present study demonstrated that silencing of MEIS2 significantly suppressed cell metastasis. Furthermore, it was determined that MEIS2-knockdown suppressed EMT progression by inducing E-cadherin expression and reducing Twist and Snail expression. In addition, this result was validated using an *in vivo* model by transplanting HCT116 cells into nude mice through the tail vein. In summary, these analyses indicated that MEIS2 acted as a promoter of metastasis in CRC.

To investigate the detailed mechanisms of MEIS2 in CRC progression, the present study performed microarray analysis to identify MEIS2 targets. GO analysis revealed that MEIS2 was significantly associated with regulating the apoptotic process, protein kinase B signaling, the Rap1 pathway, TOR signaling, the FoxO pathway, the PI3K/Akt pathway, mitotic spindle organization and the p53 pathway. A number of studies have indicated that these pathways serve crucial roles in CRC progression. For example, PI3K-Akt signaling has been identified to be involved in regulating cell growth, cell

apoptosis and cell metastasis in CRC (18-21). Furthermore, in the present study, RT-qPCR and western blot analysis demonstrated that the expression levels of TGFBR2, CDKN1A, JUN and MDM2 increased, and the level of CEBPA decreased following knockdown of MEIS2 in HCT116 cells. Numerous studies have reported that these genes serve key roles in a number of human cancer types, including CRC. For example, TGFBR2, a key member of the TGF-β signaling pathway, has been revealed to act as a suppressor of metastasis, since downregulation of TGFBR2 promoted migration and invasion in CRC (22,23). CDKN1A, a widely studied cell cycle regulator, was involved in regulating CRC proliferation (24,25). JUN, a core member of the AP-1 complex, regulated CRC progression via transcriptional regulation of various targets, including miR-22 (26-28).

Notably, the 5-year survival rate of metastatic CRC is as low as ~10%. In the past few decades, numerous studies have aimed to identify biomarkers for CRC. Several genes have been identified to be dysregulated and associated with tumor progression in CRC. For example, serum CNPY2 isoform 2 was revealed to be upregulated in tumor samples and served as a novel biomarker for early detection of CRC (29). miR-6852 was downregulated and correlated with an improved prognosis for patients with CRC (30). However, there remains an urgent requirement to identify new biomarkers for CRC. By analyzing Human Protein Atlas database, it was revealed that the MEIS2 protein levels in CRC samples were high. In addition, MEIS2 protein levels in normal colon and rectum samples were also high. By analyzing a series of public datasets, including GSE17536, GSE17537, GSE41258 and TCGA datasets, it was revealed that a high MEIS2 expression level was associated with a poor prognosis for patients with CRC. These results revealed that MEIS2 may not regulate CRC tumorigenisis but participated in regulation CRC progression.

In summary, to the best of our knowledge, the present study demonstrated for that first time that MEIS2 acted as a promoter of metastasis in CRC. Using *in vivo* and *in vitro* experiments it was revealed that knockdown of MEIS2 significantly suppressed CRC migration, invasion and EMT. Furthermore, microarray and bioinformatics analyses were performed to investigate the underlying mechanisms of MEIS2 in the regulation of CRC metastasis. In addition, it was identified that MEIS2 was associated with a shorter overall survival time for patients with CRC. In conclusion, the present study demonstrated that MEIS2 may serve as a novel biomarker for CRC.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

RC, ST and XH conceived the experiments and drafted the manuscript. ZW, BC and HY conducted the experiments. QD, BZ, XM, WP, YT and QY analyzed and interpreted the data. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

All *in vivo* study protocols were approved by the Shanghai Medical Experimental Animal Care Commission (Approval ID: ShCI-14-008).

Patient consent for publication

Not applicable.

Competing interests

The authors state that they have no competing interests.

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