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Twist1/2 activates MMP2 expression via binding to its promoter in colorectal cancer

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Abstract. – OBJECTIVE: This study aimed to characterize the effect of Twist2 on epithelial-to-mesenchymal transition (EMT) and the invasive potential of colorectal cancer (CRC) cells and to explore the mechanisms underlying the regulative effect of Twist1 and Twist2 on matrix metalloproteinase 2 (MMP2) expression in CRC.

PATIENTS AND METHODS: Data mining was performed in colorectal cancer cohort (COADREAD) in the Cancer Genome Atlas (TCGA-COADREAD). CRC LoVo and HCT116 cells were used as in vitro cell models.

RESULTS: CRC tumors with lymphatic invasion (N = 102) had a significantly higher expression of TWIST1 (p = 0.01) and TWIST2 (p = 0.02) than the lymphatic invasion negative cases (N = 228). TWIST2 overexpression enhanced EMT and the invasive potential of the CRC LoVo and HCT116 cells, while TWIST2 knockdown reversed the EMT process and weakened the invasive potential of the cells. TWIST1 and TWIST2 were co-upregulated with MMP2 and MMP9 in COADREAD cohort. TWIST1 or TWIST2 overexpression significantly elevated nuclear β-catenin accumulation, which is a known signaling pathway elevating MMP2 and MMP9 expression. More importantly, we found that both Twist1 and Twist2 could transcriptionally activate MMP2 via directly binding to its promoter. However, this mechanism was not observed in the MMP9 promoter.

CONCLUSIONS: TWIST1/2 is associated with lymphatic invasion in CRC. TWIST2 upregulation enhances EMT and the invasive potential of CRC cells. TWIST1/2 can enhance the Wnt/β-catenin signaling pathway in CRC cells. In addition, Twist1/2 can bind to the MMP2 promoter and promote its transcription.

Key Words:
Twist1, Twist2, MMP2, MMP9, Wnt/β-catenin, Colorectal cancer.

Introduction

Colorectal cancer (CRC) is one of the most common cancers across the world[1]. Although the survival of CRC has significantly improved during the past decades due to the advances in diagnosis and therapeutic strategies, about 50% of the patients still suffer local and/or distant metastasis during the disease, which is a major cause of cancer-related death[2-4]. Therefore, it is valuable to explore the molecular mechanisms of metastasis and to identify potential biomarkers that are capable of predicting patients with high-risk of metastasis. Epithelial-to-mesenchymal transition (EMT) is a reversible and dynamic process in which the cells lose epithelial characteristics and obtain mesenchymal phenotypes[5]. This process enables the cancer cells to gain stronger potential of migration and invasion and is an important mechanism of enhanced metastasis in CRC[6-8]. Transcription factors Twist1 and Twist2 are encoded by TWIST1 and TWIST2 genes respectively and belong to the basic helix-loop-helix (bHLH) transcription factor family[9]. Previous studies suggest that Twist1 and Twist2 are key regulators of EMT and drive cancer cells to display mesenchymal phenotypes in multiple types of cancer, such as gastric cancer[10], pancreatic cancer[11], and cervical cancer[12]. In patients with CRC, TWIST1 upregulation also facilitates the EMT and metastasis of the cancer cells[13,14]. However, less is known about the functional role of TWIST2 in CRC. Mechanistically, Twist1 and Twist2 can form homo- or heterodimers, which bind to the E-box DNA sequence 5'-NCANNTGN-3', thereby modulating the expression of the target genes[15]. Matrix metalloproteinases (MMPs), are a family of calcium-dependent zinc-containing endopeptidases[16]. MMP2 and MMP9 are two genes encoding MMP2 and MMP9, two gelatinases that are capable of degrading type IV collagen and promoting EMT *via* activating TGF-β, thereby enhancing CRC cell invasion and metastasis[17,18]. Several studies[19-21] found that Twist1/2 may in-

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duce MMP2 and MMP9 expression in some cancers. However, the exact mechanisms underlying the regulatory effect of Twist1/2 on MMP2 and MMP9 expression in CRC are still not fully understood. In this work, we found that Twist1/2 could increase nuclear β-catenin expression and also activate MMP2 via binding to its promoter in CRC cells. However, the direct binding was not observed in the MMP9 promoter.

Materials and Methods

Data mining in the Cancer Genome Atlas (TCGA)

Bioinformatic analysis was performed based on data in Colon and Rectal Cancer (COAD-READ) cohort in TCGA, using the UCSC Xena browser (https://xenabrowser.net/).

Promoter Scanning

The possible binding sites of Twist1 and Twist2 on the promoter region of MMP2 and MMP9 were scanned using the JASPAR Database (http://jaspar.genereg.net/).

Cell Culture and Transfection

Human colorectal cancer cell lines LoVo and HCT116 cells were obtained from Shanghai Cell Biology, Institute of the Chinese Academy of Sciences (Shanghai, China). The cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium supplemented with 100 units/mL penicillin, 100 μg/mL streptomycin, 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA) and were incubated in a cell culture incubator (Thermo Fisher Scientific, Waltham, MA, USA) at 37°C with 5% CO_2. Lentiviral TWIST1 shRNA (HSH018328-LVRH1GP), TWIST2 shRNA (HSH061355-LVRH1GP), TWIST1 and TWIST2 Lentifect™ purified lentiviral particles and the corresponding negative controls were obtained from GeneCopoeia (Rockville, MD, USA). LoVo and HCT116 cells were infected with the lentiviral particles in the presence of polybrene. This study was approved by the Ethics Committee of Huaihe Hospital, Henan University, China.

Quantitative Real Time-PCR (qPCR)

Total RNA from the cell samples was isolated using the TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) and then was reversely transcribed into cDNA using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). Then, qPCR was performed to detect the gene expression using TaqMan Master Mix (Applied Biosystems, Foster City, CA, USA) and the StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Primers for TWIST1 were: F: 5'-GGACAAGCTGAGCAAGATTCA-3' and R: 5'-CGGAGAAGGCGTAGCTGAG-3'; and for TWIST2 were: F: 5'-ACGAGCGTCTCAGCTACGCC-3' and R: 5'-AGGTGGGTCCTGGCTTGCGG-3'. The relative mRNA expression was normalized to GAPDH RNA levels. The relative mRNA expression levels between the groups were calculated using the $2^{-\Delta\Delta CT}$ method.

Western Blot Assay

Total cellular protein, cytoplasmic protein and nuclear protein were extracted using a Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime, China). Then, a total of 30 μg protein was separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane. The membranes were incubated with primary antibodies against Twist1, Twist2, E-cadherin, N-cadherin, β-catenin, MMP2, MMP9, β-actin or histone3. All primary antibodies were purchased from Abcam (Cambridge, MA, USA). After incubation and washing, the membranes were further incubated with HRP conjugated secondary antibodies. The blot signals were visualized using the ECL Western blotting substrate (Promega, Madison, WI, USA).

Transwell Assay of Cell Invasion

Cell invasion assay was performed by using a Matrigel invasion chamber (BD Biosciences, Franklin Lakes, NJ, USA) in a 24-well cell culture plates following the manufacturer's instruction. Briefly, LoVo or HCT116 cells with TWIST2 knockdown or overexpression (5×10^4 cells/mL) were loaded into chamber inserts containing an 8 μm pore size membrane with a thin layer Matrigel matrix, with 500 μl of serum-free RPMI-1640 medium. The bottom of the well was filled with 700 μl of Roswell Park Memorial Institute-1640 medium 20% FBS as a chemo-attractant. 48 h later, cells migrating to the lower surface of the membrane were fixed with 100% methanol and the non-invading cells on the upper surface of the membrane were removed. The invading cells were stained with 0.1% crystal violet. The number of invading cells was then determined for 5 independent fields under a microscope.

TOP/FOP Luciferase Assay

To determine the effect of the TWIST1/2 expression on the Wnt/β-catenin signaling activity, TOP/FOP assay was performed according to the manufacturer's protocol (Millipore, Billerica, MA, USA). Briefly, 1×10^4 LoVo cells with TWIST1 or TWIST2 overexpression or knockdown were transiently transfected with either 2 μg of pTOPflash (TCF Reporter Plasmid) or pFOPflash (mutant, inactive TCF binding site) plasmids (Millipore, Billerica, MA, USA) and 0.5 μg of pSV40-Renilla plasmid as an internal control (Promega, Madison, WI, USA) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) for 48 h. Both Firefly and Renilla Luciferase activities were measured with a Glomax luminometer (Promega, Madison, WI, USA) using Dual-Glo Luciferase Assay System (Promega, Madison, WI, USA) according to manufacturer's instructions.

Immunoprecipitation (IP)

LoVo cells were harvested and lysed using iced cold IP lysis buffer. Then, the cell lysate was incubated on ice for 5 min with periodic mixing. The cell debris was removed by centrifugation at 13,000 g for 10 min at 4°C. Total protein lysate (500 μg) was immunoprecipitated with the agarose-immobilized antibody (5 μg of anti-Twist1, anti-Twist2 or isotype control antibodies) and incubated overnight at 4°C. The immune complexes were eluted from the agarose beads and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by immunoblot analysis.

Chromatin Immunoprecipitation Assay (ChIP)

ChIP assay was conducted by using the Upstate-ChIP Assay Kit (Upstate Biotechnology, Lake Placid, NY, USA) according to the manufacturer's instructions. Equal aliquots of chromatin supernatants from LoVo cells were subjected to immunoprecipitation with 1 μg of normal goat IgG, anti-Twist1 or anti-Twist2 overnight at 4°C with rotation. After reverse cross-link of protein/DNA complexes to free DNA, the ChIP-enriched DNA was analyzed by qPCR using the ABI 7900HT sequence detection system and SYBR green master mix. Primers used for MMP2 promoter were forward, 5'- CGCAGGAAAGGATTCAAGAG-3' and reverse, 5'-CTGGTCATCCTCACTGCTCA-3'. Hypoxanthine phosphoribosyltransferase 1 gene (HPRT1) was used as internal control.

Dual Luciferase Assay

The MMP2 and MMP9 promoter plasmids (HPRM45561 and HPRM45843 respectively) were obtained from GeneCopoeia (Rockville, MD, USA). The length of the MMP2 promoter is 1591 bps, including 1405 bps upstream and 185 bps downstream of the transcriptional start site (TSS) site. Three predicted high score E-Box sites located on -1050 to -1043, -382 to -375 and -257 to -250 upstream the TSS site. Six truncated promoter sequences, including -1405 to +185, -1000 to +185, -500 to +185, -300 to +185, -200 to +185 and -100 to +185 were PCR amplified from the MMP2 promoter plasmid. The MMP2 promoter fragments and the integrate MMP9 promoter sequence were cloned into the XhoI-Hind III site of the pGL3-basic Luciferase reporter vector respectively. HEK-293 cells were plated into 12-well plates (1×10^5 cells/well) and were co-transfected with 1.5 μg reconstructed Luciferase plasmids or the empty control and 0.05 μg phRL-TK by using SuperFect (Qiagen, Hilden, Germany). 48 h after transfection, cells were lysed and the Luciferase activity was assessed using the Dual-Luciferase reporter assay system (Promega, Madison, WI, USA) with a luminometer (Promega, Madison, WI, USA). The Luciferase activity was normalized to the activity of renilla Luciferase.

Statistical Analysis

Statistical analyses were performed using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Data were presented in the form of means ± standard deviation (SD). Data were analyzed for statistical significance by two-tailed Student's *t*-test or ANOVA with Student-Newman-Keuls test as a post-hoc test. $p < 0.05$ was considered statistically significant.

Results

TWIST1/2 Upregulation is Associated With Lymphatic Invasion in CRC

Twist1 and Twist2 are two important transcription factors in the metastasis of multiple types of cancer. By data mining in TCGA-COADREAD, we assessed the association between the expression of TWIST1 and TWIST2 and lymphatic invasion of CRC. Based on data of 330 patients with primary CRC and known lymph nodal status, we

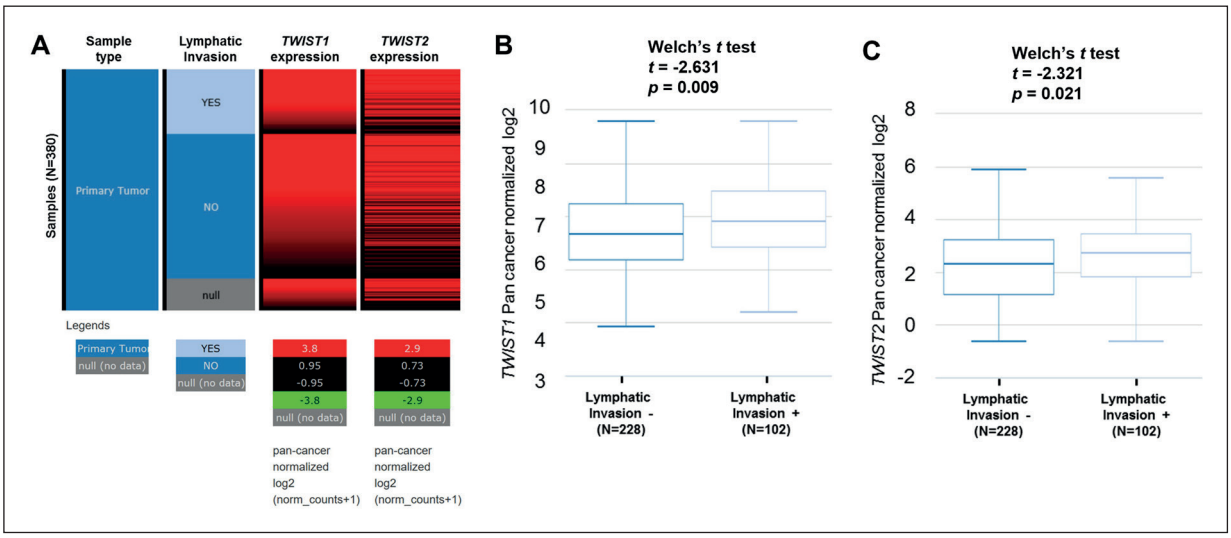

Figure 1. TWIST1/2 upregulation is associated with lymphatic invasion in CRC. ***A-C,*** Heat map ***A,*** and bar chart ***B-C,*** of the association between TWIST1 expression ***B,*** or TWIST2 expression ***C,*** and the status of lymphatic invasion in CRC. Analysis was performed using the UCSC Xena browser.

observed that the tumors with lymphatic invasion (N = 102) had a significantly higher expression of TWIST1 (p = 0.009) (Figure 1A-B) and also a substantially higher expression of TWIST2 (p = 0.021) (Figure 1A, C).

TWIST2 Upregulation Enhances EMT of CRC Cells

Twist1 has a well-characterized effect on enhancing EMT of some cancer cells, including CRC cells[10-12,22]. However, the impact of TWIST2 on EMT of CRC cells is still not well characterized. To investigate the impact of TWIST2 on EMT of CRC cells, LoVo and HCT116 cells were firstly transfected with lentiviral TWIST2 shRNA or TWIST2 expression particles respectively (Figure 2A-D). Western blot data showed that TWIST2 inhibition significantly increased E-cadherin expression and reduced N-cadherin in both LoVo and HCT116 cells (Figure 2B and D). On the contrary, TWIST2 overexpression markedly decreased E-cadherin expression, but increased N-cadherin in the cells (Figure 2B and D). EMT is an important mechanism of enhanced cancer cell invasion. By performing transwell assay, we confirmed that TWIST2 knockdown weakened the invasive capability of both LoVo and HCT116 cells (Figure 2 E-F). In comparison, its overexpression significantly enhanced their capability of invasion (Figure 2 E-F).

MMP2 is Associated With Lymphatic Invasion in CRC

Interestingly, by comparing the expression of MMP2 and MMP9 in CRC cases with or without lymphatic invasion, we observed that MMP2 expression was significantly higher in the lymphatic invasion positive group than in invasion negative control (p = 0.001, Figure 3A-B). However, no significant difference was observed in MMP9 expression between the two groups (p=0.125, Figure 3A, C). These results suggest that MMP2 upregulation might play an important role in the lymphatic invasion of CRC. Therefore, we decided to investigate the underlying mechanisms of its dysregulation.

TWIST1/2 Upregulation is Associated With Increased MMP2 and MMP9 Expression

In patients with primary CRC in TCGA-COADREAD cohort (N = 380), both TWIST1 and TWIST2 had a strong positive correlation with MMP2 (Pearson's r = 0.85 and 0.84 respectively) (Figure 4A-B, Figure 5A-B). In comparison, although TWIST1 and TWIST2 were also positively correlated with MMP9 expression, the correlation was less strong (Pearson's r = 0.60 and 0.62 respectively) (Figure 4A and C, Figure 5A and C).

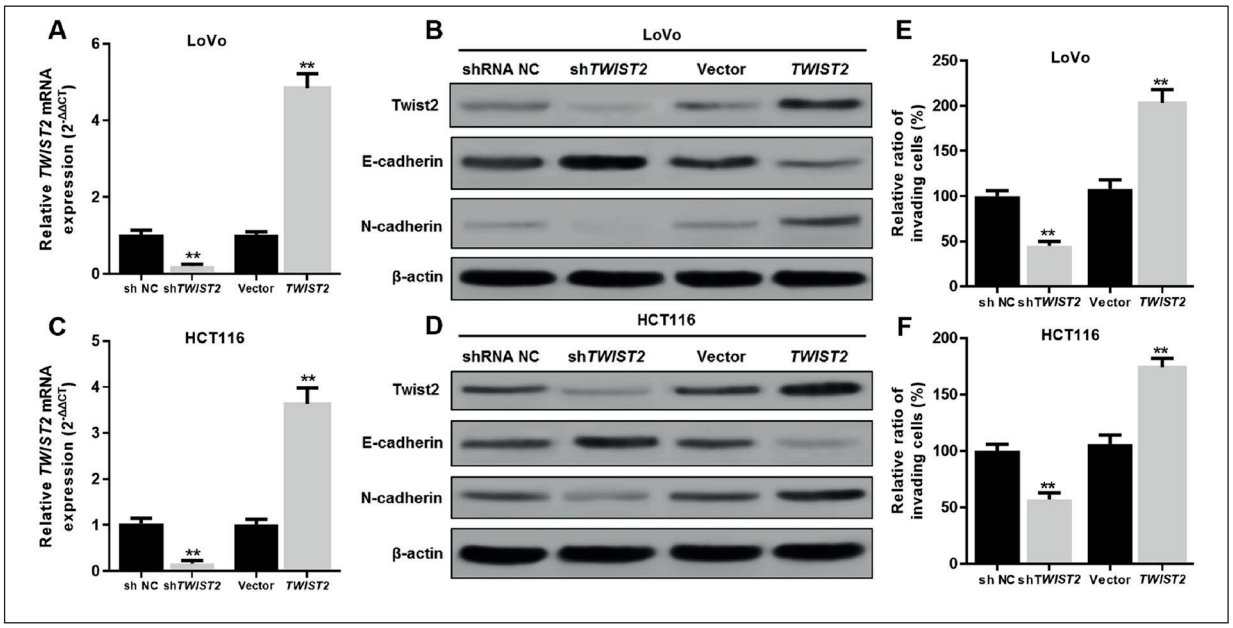

Figure 2. TWIST2 upregulation enhances EMT in CRC cells. ***A*** and ***C*,** qPCR analysis of TWIST2 mRNA expression in LoVo A, and HCT116 cells. ***C*,** 24 h after transfection of lentiviral TWIST2 shRNA or TWIST2 expression particles. ***B*** and ***D*,** Immunoblotting analysis of Twist2, E-cadherin and N-cadherin expression in LoVo ***B*,** and HCT116. ***D*,** 48 h after transfection of lentiviral TWIST2 shRNA or TWIST2 expression particles. ***E-F*,** Quantitation of relative ratio of invading LoVo ***E*,** or HCT116. ***F*,** cells with TWIST2 knockdown or overexpression in transwell assay. $^{**}p < 0.01$.

Twist1 and Twist2 Can Enhance the Wnt/β-Catenin Signaling and Increase MMP2 Expression via Directly Binding to its Promoter

Previous studies[23,24] reported that the Wnt/β-catenin signaling could induce MMP2 and MMP9 expression. In this work, we hypothesized that Twist1 and Twist2 could also modulate the Wnt/β-catenin signaling pathway in CRC cells. To verify this hypothesis, LoVo cells were infected with lentiviral TWIST1 or TWIST2 expression particles (Figure 6 A-B) or lentiviral

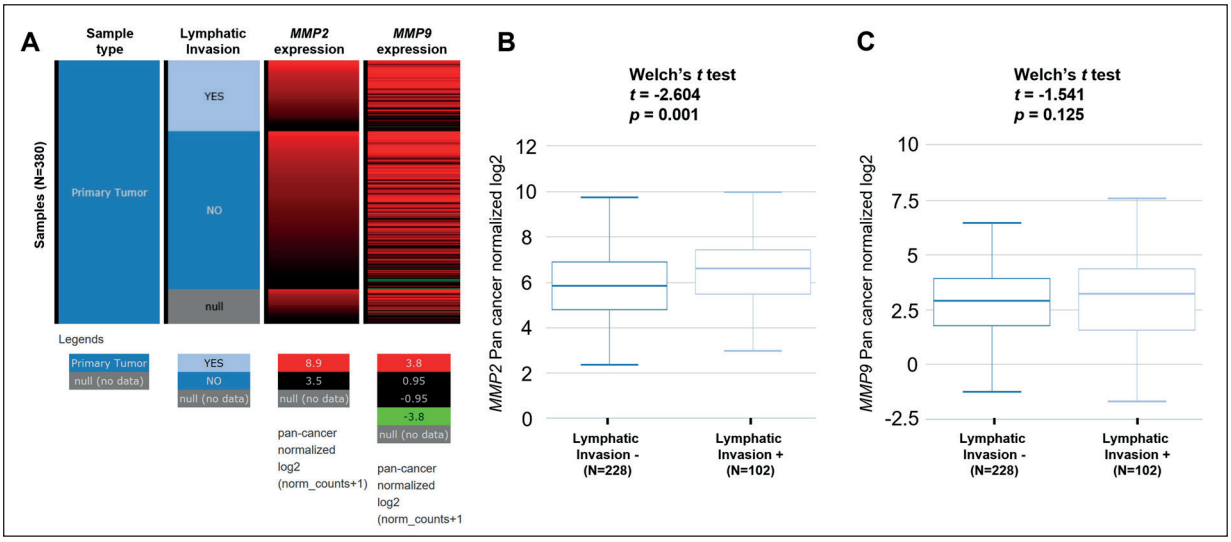

Figure 3. MMP2 is associated with lymphatic invasion in CRC.***A-C*,** Heat map ***A*,** and bar chart ***B-C*,** of the association between MMP2 expression ***B*,** or MMP9 expression ***C*,** and the status of lymphatic invasion in CRC. Analysis was performed using the UCSC Xena browser.

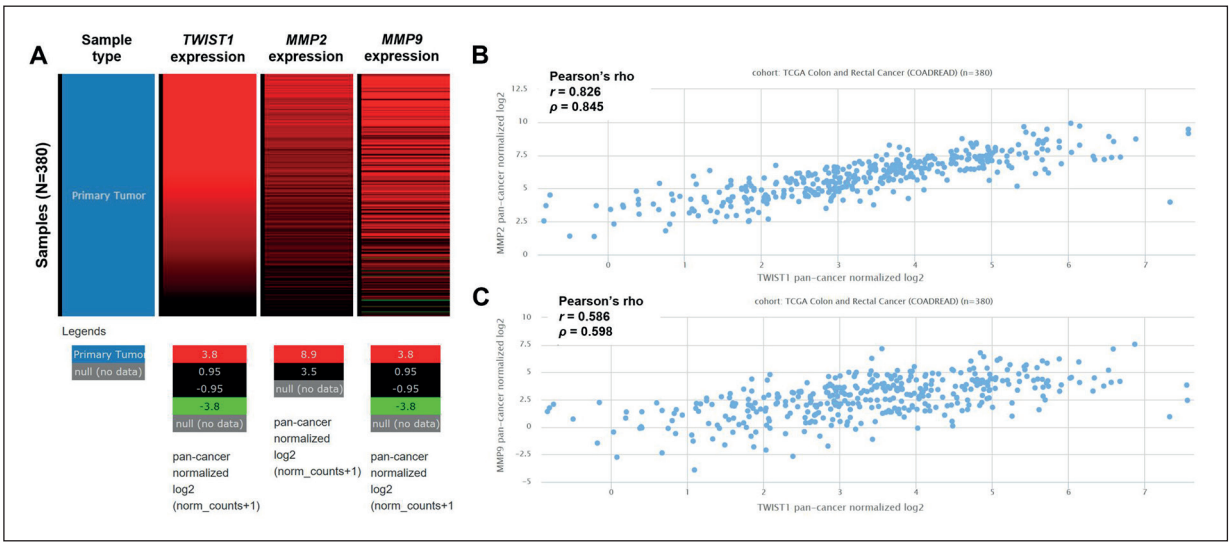

Figure 4. TWIST1 is highly correlated with MMP2 expression, but is moderately correlated with MMP9 expression. ***A-C,*** Heat map of TWIST1, MMP2 and MMP9 ***A,*** and regression analysis of the expression between TWIST1 and MMP2 ***B,*** and between TWIST1 and MMP9 ***C,*** in COADREAD cohort in TCGA. Analysis was performed using the UCSC Xena browser.

TWIST1 or TWIST2 shRNA (Figure 6 C-D). TWIST1 or TWIST2 overexpression significantly elevated nuclear β-catenin expression, while TWIST1 or TWIST2 knockdown significantly reduced nuclear β-catenin expression (Figure 6A-D). To further verify the role of TWST1/TWIST2 in the Wnt/β-catenin signaling pathway, LoVo cells with or without TWST1/TWIST2 overexpression or knockdown were subjected to TOP/FOP Luciferase assay. The results showed that TWST1 or TWIST2 overexpression significantly increased the TOP/FOP Luciferase reporter activity, while TWST1 or TWIST2 knockdown reduced the activity (Figure 6E). By performing qPCR and immunoblotting assay, we confirmed that enforced TWIST1 or TWIST2 expression significantly increased MMP2 and MMP9 expression at both mRNA and protein level (Figure 6F-I). However, since TWIST1 and TWIST2 were strongly correlated with MMP2 but were

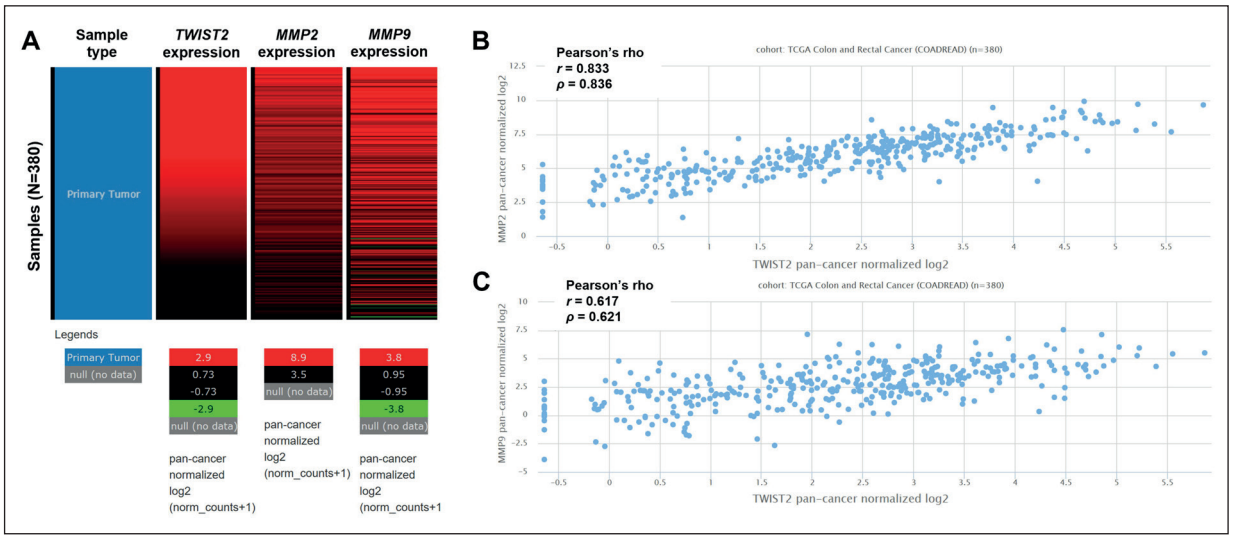

Figure 5. TWIST2 is highly correlated with MMP2 expression, but is moderately correlated with MMP9 expression. ***A-C,*** Heat map of TWIST2, MMP2 and MMP9 ***A,*** and regression analysis of the expression between TWIST2 and MMP2 ***B,*** and between TWIST2 and MMP9 ***C,*** in COADREAD cohort in TCGA. Analysis was performed using the UCSC Xena browser.

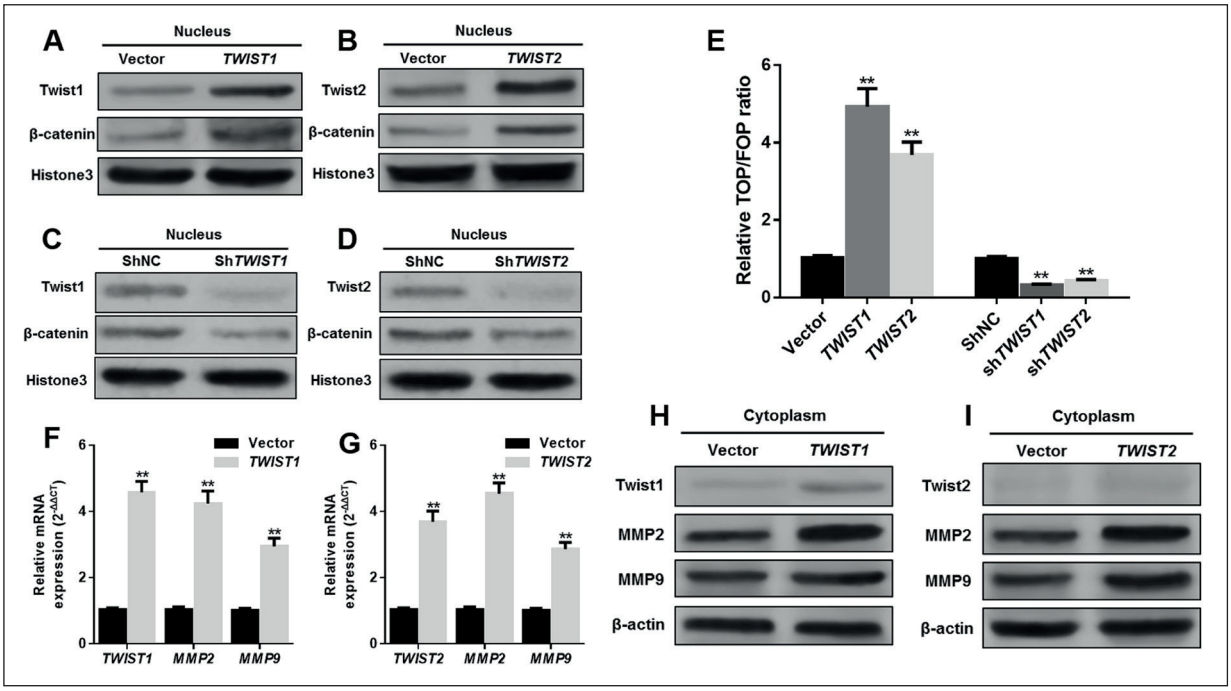

Figure 6. Twist1 and Twist2 can modulate the Wnt/β-catenin signaling pathway in LoVo cells. ***A-D,*** Western blot analysis of nuclear expression of Twist1, Twist2 and β-catenin expression in LoVo cells 48 h after infection of lentiviral TWIST1 expression particles ***A,*** lentiviral TWIST2 expression particles ***B,*** lentiviral TWIST1 shRNA ***C,*** or lentiviral TWIST2 shRNA ***D. E,*** TOP/FOP Luciferase reporter assays in LoVo cells with indicating transfection. ***F-G,*** qPCR analysis of TWIST1, TWIST2, MMP2 and MMP9 mRNA expression in LoVo cells 48 h after infection of lentiviral TWIST1 expression particles ***C,*** or lentiviral TWIST2 expression particles ***D. H-I,*** Western blot analysis of cytoplasmic expression of Twist1, Twist2, MMP2 and MMP9 expression in LoVo cells 48 h after infection of lentiviral TWIST1 expression particles ***H,*** or lentiviral TWIST2 expression particles ***I.***

moderately correlated with the MMP9 expression in TCGA-COADREAD, we decided to explore whether other mechanisms are involved in their co-upregulation. By performing bioinformatic analysis using the JASPAR database, we found three high score E-Box sites in the promoter region of MMP2 (Figure 7A), but failed to identify similar sites in the MMP9 promoter. To verify these binding sites, Luciferase reporter constructs carrying truncated MMP2 promoter sequences or integrate MMP9 promoter sequence were generated. Truncation of the predicted E-Box binding sites resulted in a significant decrease in the Luciferase activity in HEK-293 cells pre-infected with lentiviral TWIST1 or TWIST2 expression particles (Figure 7B-C). In comparison, TWIST1 or TWIST2 overexpression did not influence the Luciferase expression of the reporter with integrate MMP9 promoter sequence (Figure 7D-E). By performing IP, we observed that TWIST1 and TWIST2 were mutually co-immunoprecipitated with their respective antibodies in LoVo cells (Figure 7F). To further verify the direct binding of TWIST1 and TWIST2 to MMP2 promoter, ChIP-qPCR was performed. The results confirmed that the DNA samples from LoVo cells immunoprecipitated by TWIST1 or TWIST2 antibodies had significantly higher enrichment of MMP2 promoter fragments than that from input control (Figure 7F-G), suggesting that the TWIST1 and TWIST2 can effectively bind to the MMP2 promoter. Based on these findings, we infer that besides the Wnt/β-catenin signaling, TWIST1 and TWIST2 can also transcriptionally activate MMP2 via directly binding to its promoter (Figure 7H).

Discussion

Invasion into lymph nodal is clinically important in CRC. Our data mining in COADREAD cohort in TCGA confirmed that both TWIST1 and TWIST2 upregulation are associated with lymphatic invasion in CRC. TWIST1 upregulation has been reported in CRC and their upregulation is associated with clinicopathological features and may predict a poor prognosis[13,14].

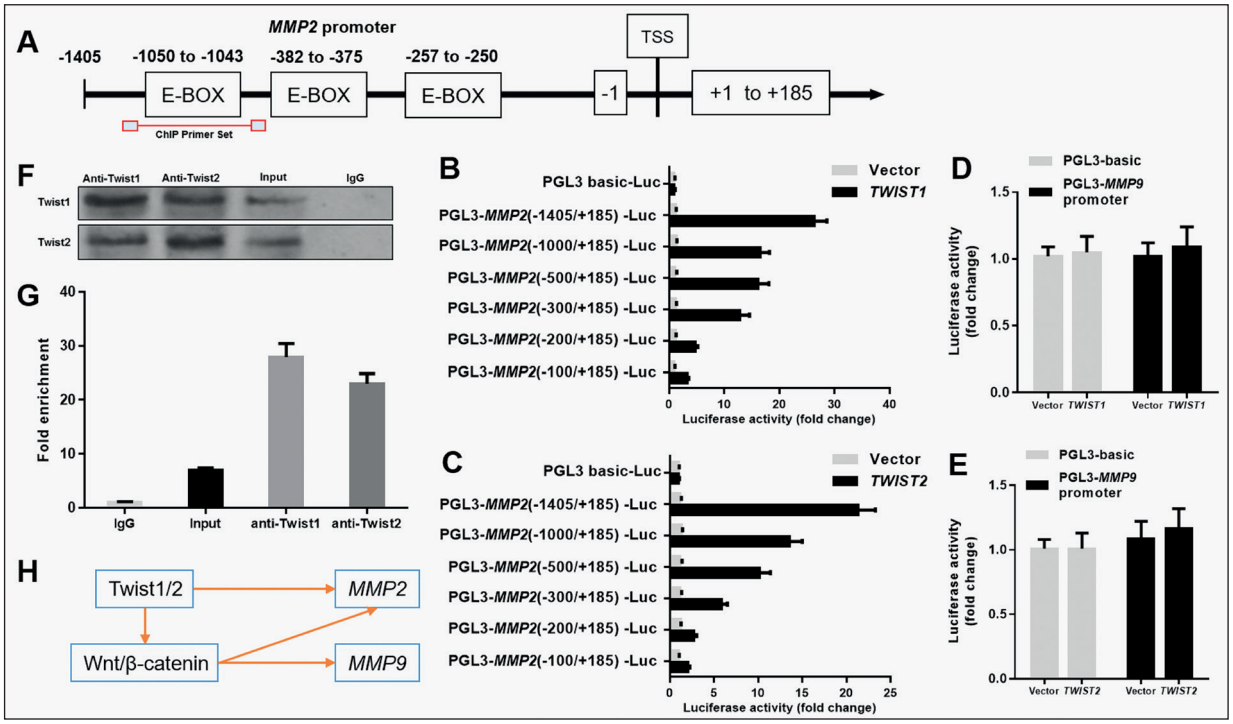

Figure 7. Twist1 and Twist2 can increase MMP2 expression via directly binding to its promoter. ***A,*** Predicted high score E-BOX sites in MMP2 promoter region. ***B-E,*** The luciferase reporter constructs carrying truncated MMP2 promoter sequences ***B-C,*** or intact MMP9 promoter sequence ***D-E,*** were introduced into HEK-293 cells pre-infected with lentiviral TWIST1 expression particles ***B*** and ***D,*** or lentiviral TWIST2 expression particles ***C*** and ***E.*** Luciferase activity was measured 48 h post-transfection. ***F,*** LoVo cells were collected and immunoprecipitation was performed using Twist1 or Twist2 antibody; Western blot of Twist1 and Twist2 is shown. ***G,*** Fold-enrichment of Twist1 or Twsit2 binding at MMP2 promoter relative to background in LoVo cells was measured by CHIP-qPCR. The primer set for ChIP-qPCR is indicated in Figure A. Upon normalization to HPRT1, results were expressed as n-fold compared to IgG. ***H,*** Schematic regulative effect of Twist1 and Twist2 on MMP2 and MMP9 expression. $^{**}p < 0.01$.

Functionally, TWIST1 can cooperate with AP-1 to upregulate integrin alpha5 expression, leading to enhanced invasion and EMT of CRC cells[25]. In addition, TWIST1 upregulation may also confer enhanced tumor cell stemness to CRC[26]. Less is known about the functional role of TWIST2 in CRC. TWIST2 is a candidate invasion driver gene in CRC[27] and its upregulation might be a valuable adverse prognostic marker, particularly for patients in stage III and IV[28]. Although TWIST2 has a well-characterized effect on promoting EMT in some cancers[29,30], its regulation on EMT in CRC cells is still not conclusive. In this work, we further studied the regulative effect of TWIST2 on EMT of CRC LoVo and HCT116 cells. Our data showed that TWIST2 overexpression enhanced EMT and invasive potential of the cells, while TWIST2 knockdown reversed the EMT process and weakened their invasive capability. As transcriptional factors, TWIST1 and TWIST2 may exert regulative effects on a series of genes. Twist1 binds to an evolutionarily conserved E-box on the proximate Snail2 promoter and induces its transcription in breast cancer cells, which act together to promote EMT and tumor metastasis[31]. Twist1 can also bind to the E-boxes of MAGEA4 promoter sequence and enhance its transcription, which is a highly expressed oncogene in a variety of malignancies and is significantly correlated with tumor cell invasiveness and aggressiveness[32]. As to TWIS2, it can regulate CD24 expression by binding directly to the E-box region in CD24 promoter in hepatocellular carcinoma[30]. However, their downstream transcriptional targets in CRC are not well identified. Some previous studies reported that TWIST1 and TWIST2 upregulation could also induce MMP2 and/or MMP9 upregulation, which contributes to TWIST1/TWIST2 induced EMT in some types of cancer, such as hepatocellular carcinoma[20], glioblastoma[19], cervical cancer[33] and ovarian cancer[34]. Mechanistically,

TWIST2 might promote β-catenin release from the E-cadherin/β-catenin complex, thereby enhancing nuclear β-catenin accumulation and the subsequent activation of MMP2 and MMP9 in cervical cancer cells[33]. TWIST1 can increase the MMP2 expression in glioblastoma *via* directly binding to the E-box in its promoter region[19]. In CRC, MMP2 and MMP9 upregulation directly lead to enhanced cell invasion and metastasis[17,18] and may also predict a poor prognosis[35,36]. By data mining in COADREAD cohort in TCGA, we observed that TWIST1 and TWIST2 were highly co-upregulated with MMP2 but were moderately correlated with MMP9 expression. This inconsistency triggered our interests to investigate the underlying mechanisms. Our data showed that TWIST1 or TWIST2 overexpression significantly elevated nuclear β-catenin expression, suggesting that the enhanced Wnt/β-catenin signaling pathway is one of the mechanisms of MMP2 and MMP9 upregulation in CRC cells. More importantly, we found that both TWIST1 and TWIST2 can transcriptionally activate MMP2 by directly binding to its promoter. However, this mechanism was not observed in the MMP9 promoter.

Conclusions

We fiound that TWIST1/2 is associated with lymphatic invasion in CRC. TWIST2 upregulation enhances EMT and the invasive potential of CRC cells. TWIST1/2 can enhance the Wnt/β-catenin signaling pathway in CRC cells. In addition, Twist1/2 can bind to the MMP2 promoter and promote its transcription.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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