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MORC2 promotes development of an aggressive colorectal cancer phenotype through inhibition of NDRG1

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MORC2 (microrchidia family CW-type zinc finger 2) is a newly identified chromatin remodeling protein that functions in diverse biological processes including gene transcription. NDRG1 is a metastasis suppressor and a prognostic biomarker for colorectal cancer (CRC). However, the relationship between MORC2 and *NDRG1* transcriptional regulation and the roles of MORC2 in CRC remain elusive. Here, we showed that MORC2 downregulated *NDRG1* mRNA, protein levels, and promoter activity in CRC cells. We also found that MORC2 bound to the −446 to −213 bp region of the *NDRG1* promoter. Mechanistically, histone deacetylase sirtuin 1 (SIRT1) was involved in *NDRG1* transcriptional regulation. MORC2 was able to interact with SIRT1 and inhibit *NDRG1* promoter activity cumulatively with SIRT1. MORC2 overexpression led to a decrease of H3Ac and H4Ac of the *NDRG1* promoter. Importantly, we showed that NDRG1 was essential in MORC2-mediated promotion of CRC cell migration and invasion in vitro, as well as lung metastasis of CRC cells in vivo. Moreover, MORC2 expression correlated negatively with NDRG1 expression in CRC patients. High expression of MORC2 was significantly associated with lymph node metastasis ($P = 0.019$) and poor pTNM stage ($P = 0.02$) and the expression of MORC2 correlated with poor prognosis in colon cancer patients. Our findings thus contribute to the knowledge of the regulatory mechanism of MORC2 in downregulating *NDRG1*, and suggest MORC2 as a potential therapeutic target for CRC.

KEYWORDS
colorectal cancer, MORC2, NDRG1, SIRT1, transcriptional regulation

1 | INTRODUCTION

Colorectal cancer (CRC), also called colon cancer or large bowel cancer, is the most common type of gastrointestinal cancer and one of the major contributors to cancer-related death worldwide.[1,2] Metastasis is considered to be the leading cause of mortality in CRC patients. Recently, genetic and epigenetic alterations in CRC have been extensively studied.[3,4] However, the underlying molecular mechanisms

and primary biomarkers for metastasis are still not well determined, which is urgently required for the development of effective therapeutic interventions and methods of managing this disease.

MORC2, also known as ZCWCC1, ZCW3, or KIAA0852, is a member of the MORC family of proteins. Our previous work showed that, in gastric cancer cells, MORC2 suppressed carbonic anhydrase IX (*CAIX*), p21[Waf1/Cip1], and *ArgBP2* (Arg-binding protein 2) gene expression through histone deacetylase 4 (HDAC4),[5] HDAC1,[6] and

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EZH2,[7,8] respectively. It has been reported that MORC2 facilitated chromatin remodeling following the DNA damage response[9] and promoted lipogenesis.[10] We also showed that phosphorylation of MORC2 on serine 677 by PAK1 promoted gastric tumorigenesis.[11] It is reported that MORC2 promoted breast cancer invasion and metastasis through a PRD domain-mediated interaction with catenin delta 1.[12] Recently, it has been shown that MORC2-mutant M276I promotes metastasis of triple-negative breast cancer by regulating CD44 splicing.[13] Moreover, MORC2 promotes cancer stemness and tumorigenesis by facilitating DNA methylation-dependent silencing of Hippo signaling in hepatocellular carcinoma.[14] Additionally, *MORC2* was found to be one of the mutation hotspot oncogenes in CRCs with microsatellite instability.[15] However, the potential oncogenic roles and molecular mechanisms of MORC2 in CRC remain elusive.

N-myc downstream regulated gene 1 (*NDRG1*) is a well-characterized metastasis suppressor that has shown the potential to be developed as a target for antimetastatic therapy.[16] *NDRG1* mediates its activity through various signaling pathways and molecular motors.[17] It has been reported that NDRG1 was downregulated in CRC tissues and it was a prognostic biomarker for human colorectal cancer.[18] Moreover, NDRG1 inhibited epithelial-mesenchymal transition, migration, and invasion of CRC cells through interaction and promotion of caveolin-1 ubiquitylation.[19]

In this study, we found that MORC2 bound to *NDRG1* promoter and inhibited NDRG1 expression in CRC cells. We also show that MORC2 interacted with sirtuin 1 (SIRT1) and inhibited *NDRG1* promoter activity independently and cumulatively with SIRT1. We reveal that NDRG1 was required in MORC2-mediated promotion of CRC cell migration and invasion in vitro, as well as lung metastasis of CRC cells in vivo. Furthermore, we show the negative correlation between MORC2 and NDRG1 in CRC samples. We found that high expression of MORC2 was significantly associated with lymph node metastasis and poor pTNM stage. Decreased expression of NDRG1 was significantly related to lymph node metastasis in CRC samples. Our results might thus contribute to understanding the mechanisms of *NDRG1* transcriptional regulation and suggest MORC2 as a potential therapeutic target for CRC.

2 | MATERIALS AND METHODS

2.1 | Cell culture

HT-29, SW-480, and SW-620 cells were cultured in RPMI-1640 medium, and HEK-293 cells were cultured in DMEM, supplemented with 10% FBS, 100 μg/mL streptomycin, 100 U/mL penicillin, and 1% glutamine at 37°C in 5% CO_2 and 95% air.

2.2 | Plasmids, transient transfection, and luciferase assay

For the construction of *NDRG1* promoter-driven luciferase reporter plasmid, a series of fragments were amplified by PCR from human genomic DNA. These PCR products were digested with *Bgl*II/*Hind*III and inserted into the firefly luciferase reporter vector pGL3-basic (Promega, Madison, WI, USA). The sense primers were 5′-GGAAGA TCTACGGTGCTAAGGTTGGAAAGGG-3′ (−759 to +69 bp), 5′-GGAA GATCTCCGAGCTGGTGAGACCTACA-3′(−446 to +69 bp), and 5′-G GAAGATCTACTGCAGAGCCGACCCACAA-3′(−213 to +69 bp). The antisense primer was 5′-CCCAAGCTTGGAGCCAGGCGAGGTTTG TTTA-3′. We constructed 3× Flag-CMV-MORC2 in our laboratory.[6] Transient transfection was carried out using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Luciferase assay has been described previously in detail.[5] Cells were transfected with indicated reporter and *Renilla*-encoding plasmids. All measurements were repeated at least 3 times and luciferase values were normalized to internal *Renilla* control.

2.3 | Lentiviral vector production and generation of stable cell lines

Flag-vector lentivirus, Flag-MORC2 lentivirus, nonsilencing (NC)-shRNA lentivirus, MORC2-shRNA lentivirus, SIRT1-shRNA lentivirus, and NDRG1-shRNA lentivirus were purchased from GeneChem (Shanghai, China). HT-29, SW-620, and SW-480 cells were transfected with various plasmids using lentivirus according to the manufacturer's instructions. Stable clonal cell lines were selected with 2 μg/mL puromycin.

2.4 | Immunoprecipitation and western blot analyses

Immunoprecipitation and western blot analyses have been described previously in detail.[5] The samples were incubated with anti-MORC2 (Bethyl Laboratories, Montgomery, TX, USA), anti-NDRG1 (Cell Signaling Technology, Danvers, MA, USA) and anti-SIRT1 (Cell Signaling Technology) antibodies.

2.5 | RNA isolation, reverse transcription, and real-time PCR

RNA was extracted with TRIzol (Invitrogen). cDNA was synthesized by reverse transcription using an RT reaction kit (Takara, Dalian, China), according to the manufacturer's instructions. Real-time PCR was carried out according to the protocol used in our previous study.[5] The primers for *NDRG1* were: 5′-TGGACCCAACAAAGACCACT-3′ (sense) and 5′-CCATCCAGAGAAGTGACGCT-3′ (antisense); and for β-*actin* were: 5′-TCGTGCGTGACATTAAGGAG-3′ (sense) and 5′-ATGCCAGGGTACATGGTGGT-3′ (antisense). Gene expression levels were calculated relative to the housekeeping gene β-*actin* by using Stratagene Mx 3000P software (Agilent Technologies Inc., CA, USA).

2.6 | Tissue samples and immunohistochemical staining

Nontumor colon tissues (5 cm away from the cancer edge) from 36 patients and human CRC tissues from 119 patients undergoing

radical colon resection were obtained at the First Hospital of China Medical University (Shenyang, China). Fresh samples were snap frozen in liquid nitrogen immediately after resection and stored at −80°C. All samples were obtained with patients' informed consent. The samples were histologically confirmed by staining with H&E. The histological grade of cancer was assessed according to criteria set by the WHO. The TNM classification was undertaken according to the standard criteria of the 7th TNM staging system. Immunohistochemistry has been described previously,[20] and immunohistochemical results were judged by HSCORE (histological score).[21]

2.7 | Transwell migration and invasion assays and wound healing assays

Transwell migration and invasion assays have been described previously.[22] The number of migrated cells was counted in 5 representative microscopic fields that were photographed. Three independent experiments were carried out. The invasion assay was undertaken using BD BioCoat Matrigel invasion chambers (8 mm pore size; BD Biosciences, San Jose, CA, USA). The same procedures described above were used, except that the filters were precoated with 100 mL Matrigel at a 1:4 dilution in 1640 medium to form a genuine reconstituted basement membrane. For wound healing assays, cells were seeded and grown to confluency. Then the cells were gently scratched with tips to create a mechanical wound. Images were taken at 0, 24, and 48 hours using a microscope.

2.8 | Nude mice xenografts

Five- to 6-week-old female athymic nude BALB/c mice were purchased from Vital River Laboratory Animal Technology (Beijing, China). To induce tumor formation, 5×10^6 tumor cells were injected into the tail vein. Eight weeks after injection, liver and lung samples were collected and subjected to histological examination. Visible lung metastases were measured and counted using a microscope. All experimental procedures involving animals were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication no. 80-23, revised 1996) and were undertaken according to the institutional ethical guidelines for animal experiments.

2.9 | Chromatin immunoprecipitation

Cells were cross-linked with 1% formaldehyde at 37°C for 10 minutes, resuspended in lysis buffer (50 mmol/L Tris-HCl [pH = 8.1], 10 mmol/L EDTA, 1% SDS, and protease inhibitor cocktail). DNA was sheared by sonication to lengths between 200 and 1000 bp. Protein-DNA complexes were precipitated by anti-MORC2 antibody (Bethyl Laboratories) and control IgG, respectively, followed by the elution of the complex from the Ab. Real-time PCR was carried out with primers specific for the *NDRG1* promoter region: 5′-CCGAGCT GGTGAGACCTACA-3′(sense) and 5′-TTGTGGGTCGGCTCTGCAGT-3′(antisense).

2.10 | Cell viability and cell cycle analysis

Cells were seeded in 96-well plates (2000 cells/well) in triplicate, and cell viability was evaluated by CCK-8 (Dojindo Laboratories, Kumamoto, Japan). For cell cycle analyses, cells were harvested and fixed in 75% ethanol overnight. After washing with PBS, cells were stained with propidium iodide and analyzed on a BD FACSVerse flow cytometer (BD Biosciences).

2.11 | Statistical analysis

The statistical analysis was carried out using SPSS (17.0) software (SPSS, Chicago, IL, USA). Data of MORC2 and NDRG1 expression in CRC were analyzed by Spearman's rank correlation coefficient test. Mann-Whitney U test was used to analyze the association between MORC2 or NDRG1 expression and clinical features. Survival curve was estimated by the Kaplan-Meier method. The statistical significance of difference was analyzed by ANOVA. Statistical significance was defined as $P < 0.05$. All experiments were repeated 3 times, and data were expressed as the mean ± SD from a representative experiment.

3 | RESULTS

3.1 | MORC2 downregulates NDRG1 in colorectal cancer cells

To search MORC2 target genes, we undertook DNA microarray hybridization (Affymetrix, Santa Clara, CA, USA) using stable pcDNA3.1 or MORC2-overexpressed gastric cancer SGC-7901 cells[5] and found various MORC2 target genes, most of which were downregulated. The genes that were downregulated or upregulated by MORC2 are listed in Tables S1 and S2. *NDRG1* was one of the genes that was downregulated by MORC2 in gastric cancer SGC-7901 cells. Importantly, emerging evidence suggests that NDRG1 can serve as a biomarker for colorectal cancer and has tumor suppressive properties in colorectal cancer.[23,24] These findings prompted us to investigate whether MORC2 regulates NDRG1 in CRC cells. Quantitative (q)RT-PCR and western blot analyses were carried out in stable empty vector or MORC2-overexpressed HT-29 and SW-620 cells. The results showed that MORC2 overexpression downregulated the mRNA and protein level of NDRG1 (Figure 1A,B). In contrast, stable knockdown of MORC2 increased the expression of NDRG1 mRNA and protein (Figure 1C-F).

3.2 | MORC2 binds to *NDRG1* promoter and inhibits *NDRG1* promoter activity

In order to elucidate whether the decrease in *NDRG1* mRNA is dependent on MORC2 as a regulator of transcription, we examined the regulation of the *NDRG1* promoter. As a reporter, we used the −759 to +69 fragment of the *NDRG1* promoter fused to the luciferase reporter gene. This region has been shown to be sufficient for the transcription induction of the *NDRG1* gene in neuroblastoma cells.[25] The luciferase assay results showed that MORC2 downregulated *NDRG1* promoter activity in a dose-dependent manner
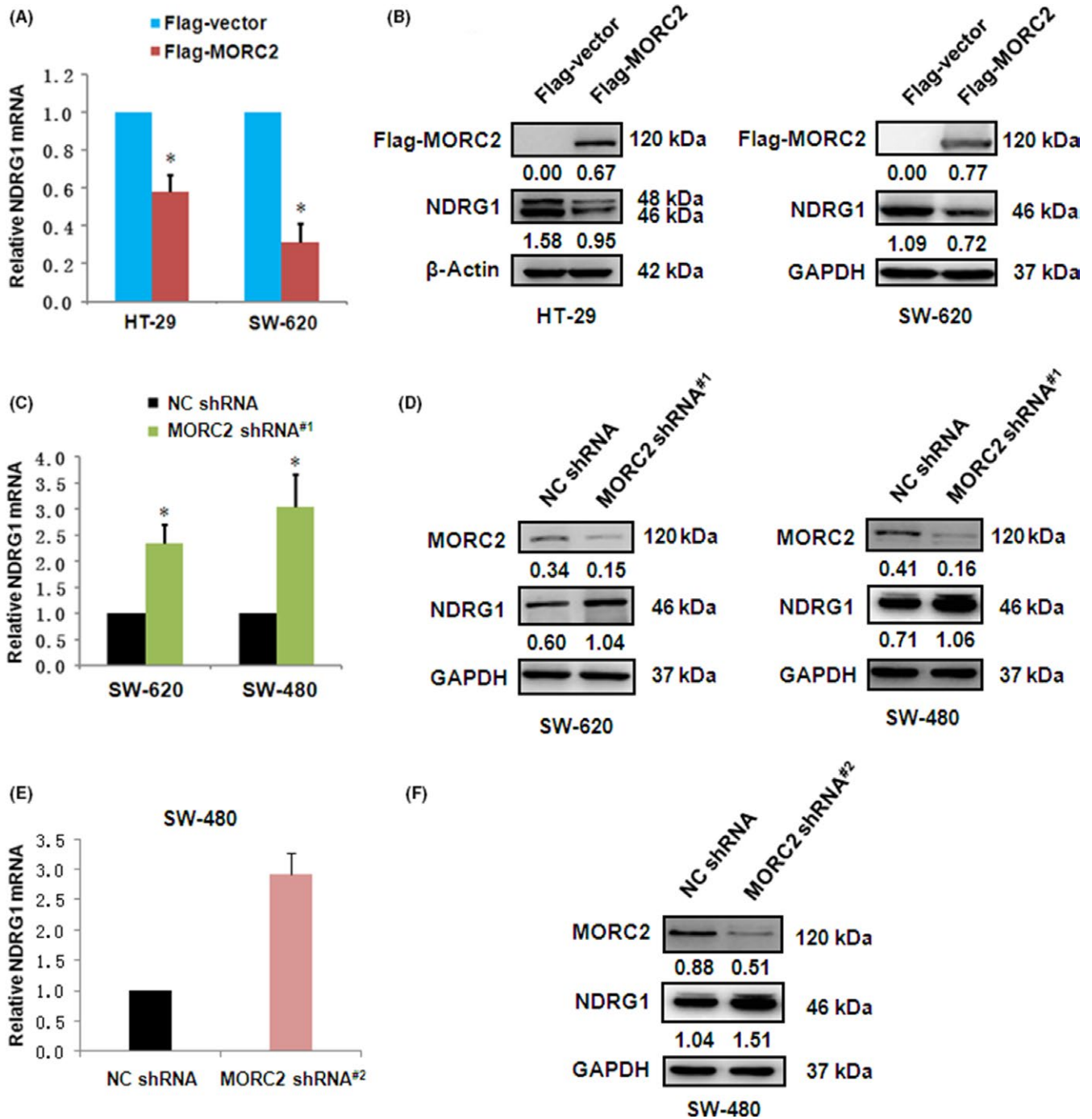
FIGURE 1 MORC2 downregulates NDRG1 in colorectal cancer cells. A,B, MORC2 overexpression downregulated *NDRG1* mRNA and protein expression. Flag-MORC2 or vector control was stably transfected into HT-29 cells (left panel) and SW-620 cells (right panel). A, mRNA level was examined by quantitative RT-PCR analysis. *P < 0.05. B, Protein level was analyzed by western blot. C-F Specific knockdown of MORC2 upregulated *NDRG1* mRNA and protein levels. C,E, mRNA level was estimated by quantitative RT-PCR. D,F, Protein level was analyzed by western blot. NC, negative control

(Figure 2A,B). To determine which region is required for the repression function of MORC2 on *NDRG1* transcription, a series of 5′ promoter deletion mutants of the *NDRG1* promoter proximal to the transcriptional initiation site were transfected into HEK-293 cells with or without MORC2. Significant decrease of *NDRG1* promoter activity was observed in pGL3-NDRG1(−759/+69) and pGL3-NDRG1(−446/+69), but not in pGL3-NDRG1(−213/+69)

(Figure 2C), indicating that the −446 to −213 bp region played an important role in the suppression function of MORC2 on *NDRG1* transcription. In order to verify the binding of MORC2 to the *NDRG1* promoter, ChIP assays were carried out. The results showed that MORC2 bound to the −446 to −213 bp region of *NDRG1* promoter, but not to the −759 to −446 bp or −213 to +69 bp region in Flag-MORC2 transfected cells (Figure 2D). Our
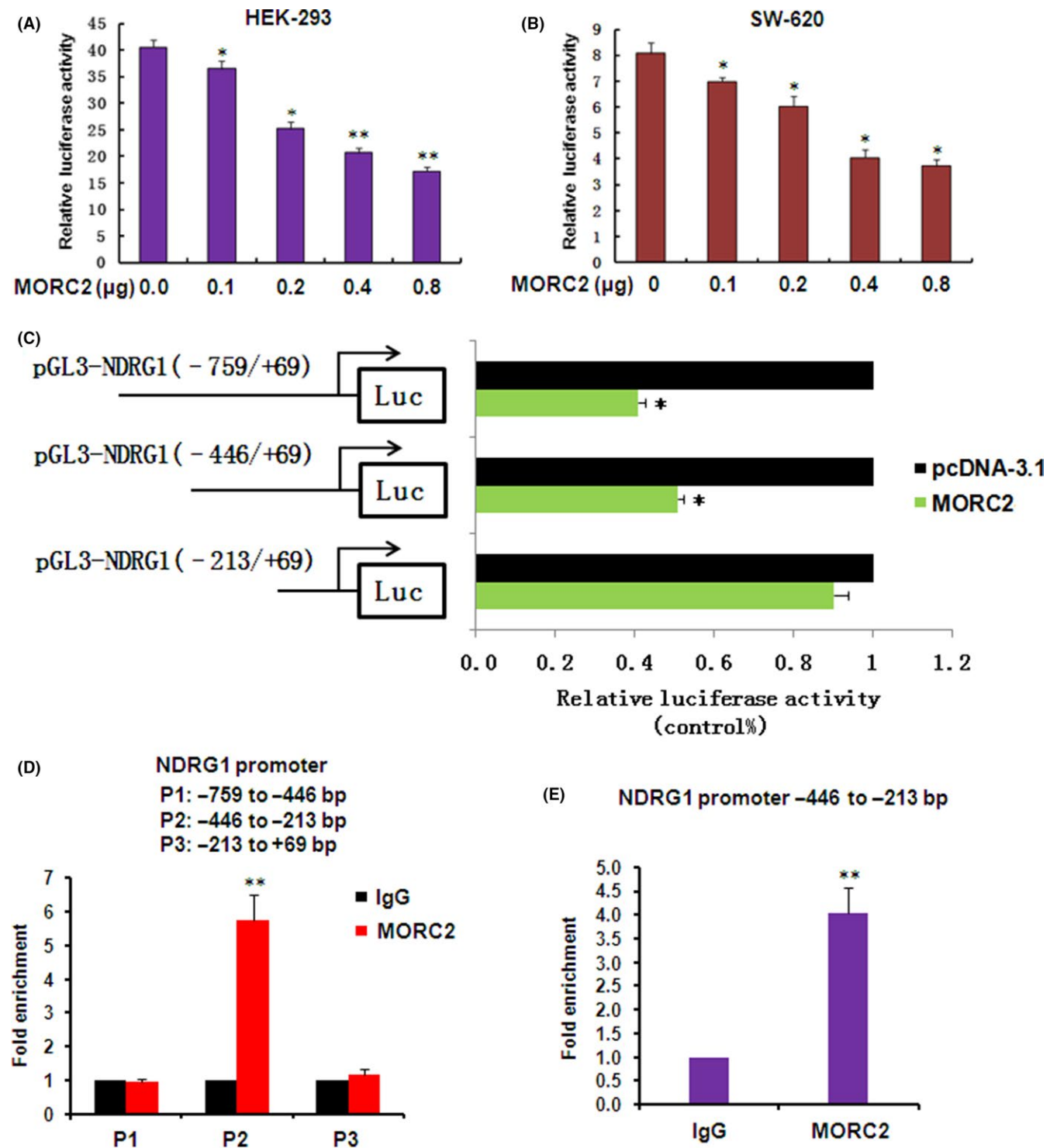
FIGURE 2 MORC2 binds to *NDRG1* promoter and inhibits its activity. A,B, HEK-293 and SW-620 colon cancer cells were transiently transfected with pGL3-NDRG1-luc reporter plasmid, pRL-TK vector along with Flag-MORC2 as indicated. Luciferase activities were determined and normalized to pRL-TK (*Renilla*) activity 24 hours after transfection. *$P < 0.05$, **$P < 0.01$. C, Left panel, schematic representation of a series of 5′-deleted *NDRG1* promoter/luciferase constructs. Bent arrow indicates transcription initiation site. Right panel, HEK-293 cells were transiently transfected with various *NDRG1* promoter deletion vectors indicated in the left panel with or without Flag-MORC2 expression vector as indicated. Results are expressed as a percentage of the MORC2-untransfected control that is taken as 100%. *$P < 0.05$ compared with control. D, ChIP assays were carried out using MORC2 Abs, and appropriate negative control Abs (IgG), in MORC2 overexpressed (Flag-MORC2) SW-480 cells, followed by quantitative PCR with primers amplifying the *NDRG1* promoter region (−759 to −446 bp, −446 to −213 bp, and −213 to +69 bp). Data are plotted as fold-enrichment of specific Ab binding over IgG control. E, ChIP assays were carried out using IgG and MORC2 Abs in SW-480 cells, followed by quantitative PCR with primers amplifying the *NDRG1* promoter region (−446 to −213 bp)

ChIP-qPCR results verified that endogenous MORC2 also bound to the −446 to −213 bp region of *NDRG1* promoter (Figure 2E).

3.3 | MORC2 inhibits NDRG1 expression by decreasing histone acetylation level of *NDRG1* promoter

Our previous work showed that MORC2 downregulated *CAIX* transcription by recruiting HDAC4.[5] Sirtuin 1, a member of the class III HDACs, has been implicated in the carcinogenesis and progression of various cancers,[26] so we wondered whether SIRT1 participates in MORC2-mediated

NDRG1 transcriptional regulation. Then we used sirtinol, a specific inhibitor of SIRT1, to treat stable empty vector or MORC2 overexpressed SW-620 cells. Obviously, the inhibition of NDRG1 by MORC2 was inverted after treatment with sirtinol (Figure 3A,B). To further detect whether SIRT1 plays a role in downregulation of NDRG1, qRT-PCR and western blot were carried out. As can been seen in Figure 3C,D, depletion of the endogenous SIRT1 by specific shRNA resulted in an increase of NDRG1 mRNA and protein level. Moreover, SIRT1 inhibited *NDRG1* promoter activity in a dose-dependent manner (Figure 3E). To test whether MORC2 could physically interact with SIRT1, we carried out immunoprecipitation followed by western blot analysis. We transfected HCT-116 cells with Flag-tagged MORC2 or Flag
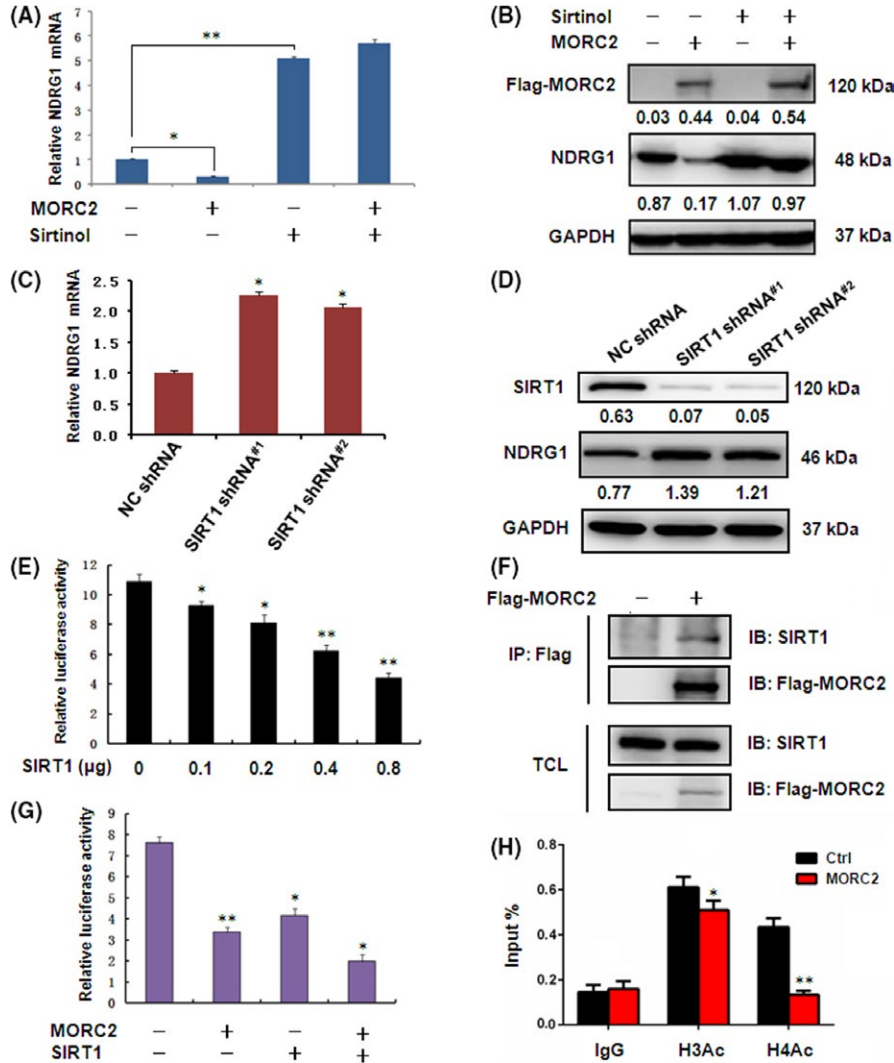

FIGURE 3 MORC2 inhibits NDRG1 expression by decreasing the histone acetylation level of the *NDRG1* promoter region. A,B, SW-620 cells stably transfected with Flag-MORC2 or vector control were treated with 50 μmol/L sirtinol for 24 hours. A, *NDRG1* mRNA level was measured by quantitative (q)RT-PCR. B, Protein levels of MORC2 and NDRG1 were measured by western blot. C,D, Endogenous sirtuin 1 (SIRT1) in SW-620 cells was knocked down by shRNA targeting SIRT1 and lentivirus infection. C, *NDRG1* mRNA level was measured by qRT-PCR. *$P < 0.05$. D, Protein levels of SIRT1 and NDRG1 were measured by western blot. E, SW-620 cells were transfected with pGL3-NDRG1-luc reporter construct, pRL-TK plasmid, and SIRT1 expression vector as indicated. Luciferase activities were determined and normalized to *Renilla* activity 24 hours after transfection. *$P < 0.05$, **$P < 0.01$. F, For the immunoprecipitation (IP), cell lysates were immunoprecipitated by anti-Flag Ab, and precipitates were immunoblotted (IB) with anti-SIRT1 and anti-Flag Abs. G, MORC2 and SIRT1 were transiently transfected into SW-620 cells as indicated, and the promoter activity was estimated by luciferase assays. *$P < 0.05$, **$P < 0.01$. H, ChIP-qPCR was carried out using H3Ac and H4Ac Abs, and negative control Abs (IgG) in control and MORC2 overexpressed SW-480 cells, followed by qPCR with primers amplifying the *NDRG1* promoter region (−446 to −213 bp). *$P < 0.05$, **$P < 0.01$

empty vector. The results indicated that Flag-tagged MORC2 could interact with endogenous SIRT1 in vivo (Figure 3F). To investigate how the *NDRG1* promoter activity was affected by MORC2 and SIRT1, we undertook luciferase assays. The results showed that MORC2 was able to inhibit *NDRG1* promoter activity independently and cumulatively with SIRT1 (Figure 3G). To detect the influence of MORC2 on the acetylation level of histone H3 and H4 in the *NDRG1* promoter, ChIP-qPCR was carried out. The results showed that MORC2 overexpression led to a significant decrease of H4Ac and a slight decrease of H3Ac at the −446 to −213 bp region in NDRG1 promoter (Figure 3H). These data suggest that MORC2 and SIRT1 inhibit *NDRG1* transcription cumulatively and MORC2 inhibits NDRG1 expression by decreasing the histone acetylation level of the NDRG1 promoter.

3.4 | NDRG1 is essential in MORC2-mediated promotion of CRC cell migration and invasion

To study the role of MORC2 in CRC cell migration and invasion, we used HT-29 and SW-480 cells that stably express MORC2 and analyzed their migratory capacities in vitro. Compared with the control, MORC2 overexpression enhanced the migration of cells, as shown by Transwell assays (Figure 4A) and wound healing assays (Figures 4B,S1). We carried out CCK-8 cell growth assays and flow cytometric analyses to evaluate whether MORC2 affected cell proliferation and the cell cycle. As shown in Figure S2A, MORC2 overexpression or MORC2 and

SIRT1 overexpression did not affect cell proliferation in SW-480 cells (Figure S2A). The flow cytometric analysis showed that MORC2 significantly inhibited cell cycle progression in HCT-116 cells (Figure S2B) and slightly inhibited cell cycle progression in SW-480 cells (Figure S2C). As NDRG1 is a potent suppressor of metastasis in colon cancer,[27] and MORC2 inhibit NDRG1 expression in CRC cells, we wondered whether NDRG1 is required for MORC2-mediated promotion of CRC cell migration and invasion. We examined the effect of NDRG1 knockdown on shMORC2-induced reduction of cell migration and invasion capacities in SW-480 cells. As shown in Figure 4C,D, NDRG1 knockdown abrogated the shMORC2-induced reduction of the migration and invasion ability of SW-480 cells, indicating that NDRG1 is essential in MORC2-mediated promotion of CRC cell migration and invasion.

3.5 | NDRG1 is required for MORC2-mediated promotion of CRC cell lung metastasis

To further examine the effect of MORC2 and NDRG1 on CRC cell metastasis in vivo, shNC, shMORC2, and shMORC2 + shNDRG1 SW-480 cells were injected into nude mice through the tail vein. Eight weeks later, mice were killed and necropsied to find metastases in the lung. Indeed, SW-480 cells stably silencing MORC2 developed less metastases in the lung of nude mice than the shNC group and shMORC2 + shNDRG1 group (Figure 5A,B). Lung
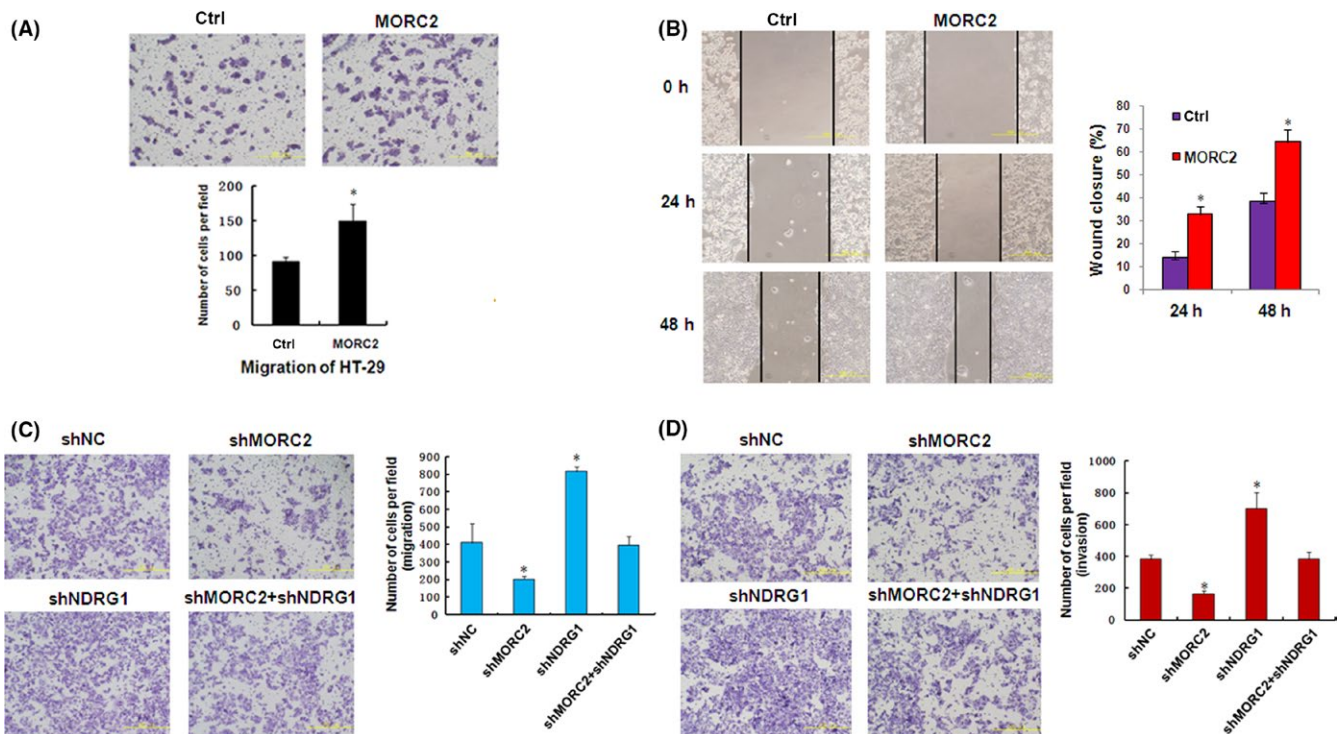

FIGURE 4 NDRG1 is essential in MORC2-mediated promotion of colorectal cancer cell migration and invasion. A, Migratory capacities of HT-29 cells was measured by Transwell assay after MORC2 overexpression. Representative images and quantitative data of 3 independent experiments are shown. *P < 0.05. B, Wound healing assays were undertaken to detect the migratory capacity of HT-29 cells after MORC2 overexpression. Representative images of wound healing assays are presented from 3 independent experiments. Histograms represent the wound closure rates at the indicated times. *P < 0.05. C,D, Migratory and invasive capacities of SW-480-shNC (negative control), SW-480-shMORC2, SW-480-shNDRG1, and SW-480-shMORC2 + shNDRG1 were measured by Transwell assay. Representative images and quantitative data of 3 independent experiments are shown. *P < 0.05. Ctrl, control

metastasis was detected in all of the 8 mice in the shNC group, 1 of 9 mice in the shMORC2 group, and 8 of 9 mice in the sh-MORC2 + shNDRG1 group (Figure 5C). Hematoxylin–eosin and immunohistochemical staining confirmed that shMORC2 group resulted in much less marked metastatic spread to the lungs of the mice compared with the shNC group and shMORC2 + shNDRG1 group (Figure 5D). These findings indicate that NDRG1 is required for MORC2-mediated promotion of CRC cell pulmonic metastasis.

3.6 | Negative correlation of MORC2 and NDRG1 expression in CRC samples

In order to detect the expression of MORC2 and NDRG1 and the correlation between MORC2 and NDRG1 in CRC samples, 36 noncancerous tissues and 119 CRC tissues were immunostained for MORC2 and NDRG1. Representative images are shown in Figure 6A. Unlike noncancerous tissue, high expression level of
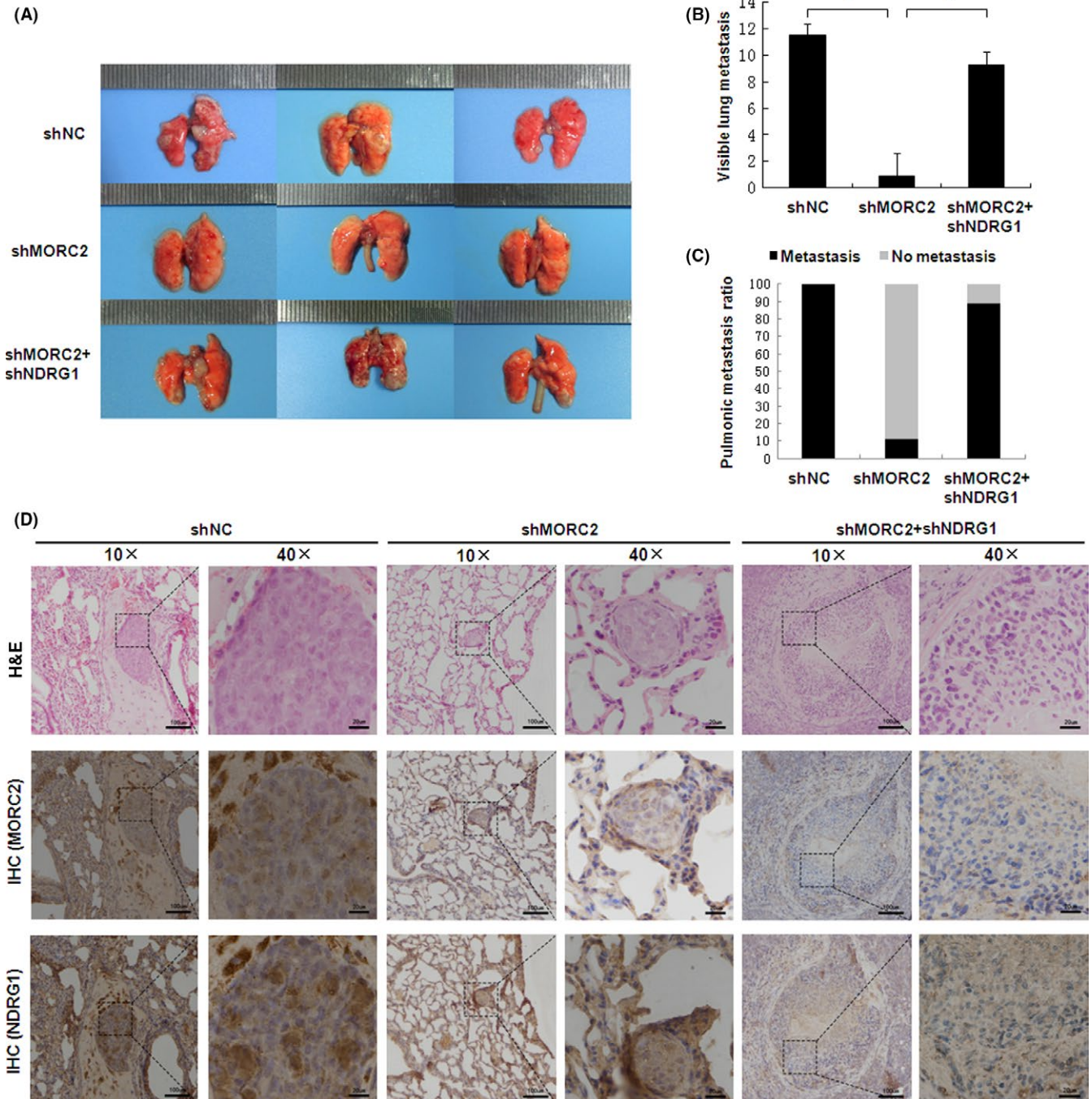

FIGURE 5 NDRG1 is required for MORC2-mediated promotion of CRC cell pulmonic metastasis. 5×10^6 SW-480-shNC, SW-480-shMORC2, and SW-480-shMORC2 + shNDRG1 cells were injected into nude mice through the tail vein. A, After 8 weeks, mice were killed and lungs were macroscopically photographed. B, Graphical representation of the number of metastatic nodules in the lungs of each mouse (mean ± SD), *$P < 0.05$. C, Bar charts show the pulmonic metastasis ratio. D, Metastatic tumors were stained by H&E and evaluated for MORC2 and NDRG1 expression by immunohistochemistry

MORC2 and low expression level of NDRG1 were observed in CRC tissues (Figure 6A). More importantly, the MORC2 expression level was negatively correlated with the NDRG1 expression level (Figure 6B, $r = -0.782$, $P < 0.0001$). To better understand the correlation between MORC2 or NDRG1 expression and progression of CRC, these samples were divided into 2 groups based on MORC2 or NDRG1 levels (histological score). The data showed that high expression of MORC2 was significantly associated with lymph node metastasis ($P = 0.019$) and poor pTNM stage ($P = 0.02$) (Table 1), but not with tumor size ($P = 0.916$). The data also showed that decreased expression of NDRG1 was significantly related to lymph node metastasis ($P = 0.003$) (Table 2). Moreover, Kaplan-Meier curves using The Cancer Genome Atlas database showed that colon cancer patients with higher MORC2 expression had a significantly shorter overall survival ($P = 0.033$, Figure 6C). Taken together, these data indicate that MORC2 is overexpressed in CRC tissues, leading to the decreased expression of NDRG1 and influencing the lymph node metastasis of CRC. The expression of MORC2 correlates with poor prognosis in colon cancer patients.

4 | DISCUSSION

In our previous study, we found that MORC2 could act as a transcriptional repressor and inhibit the transcription of a number of target genes, including *CAIX*, *p21^Waf/Cip1*, and *ArgBP2* in gastric cancer cells.[5-7] We also reported the potential role of the PAK1-MORC2
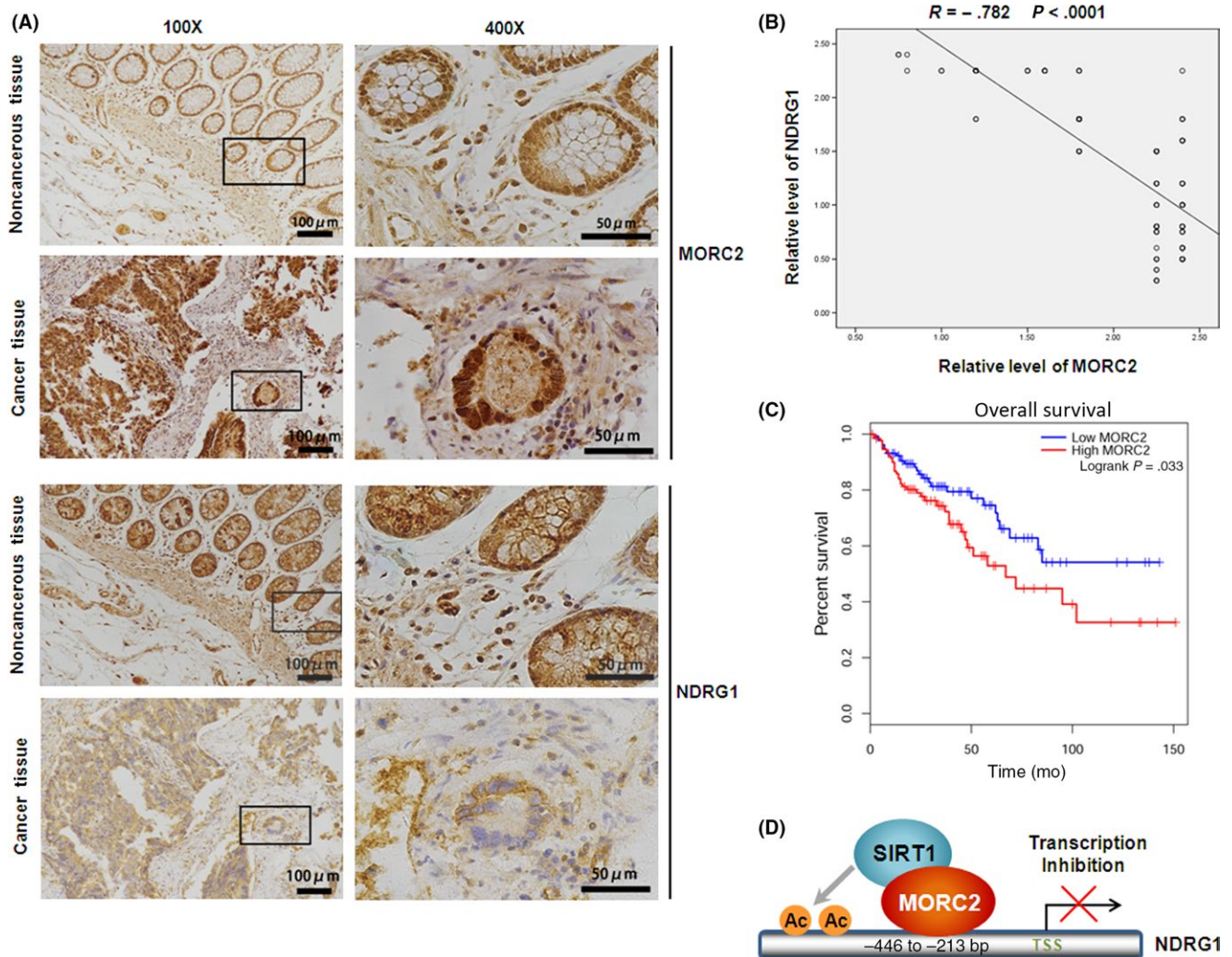

FIGURE 6 Negative correlation of MORC2 and NDRG1 expression in colorectal cancer (CRC) samples. A, Representative images of immunohistochemical staining for CRC specimens incubated with MORC2 or NDRG1. Intensity value is expressed as histological score (HSCORE). Data were analyzed by Mann-Whitney *U* test. B, Spearman's rank test was used to analyze the correlation between MORC2 relative expression and NDRG1 relative expression in 119 CRC samples. C, Kaplan-Meier overall survival curve of colon cancer patients based on MORC2 expression using The Cancer Genome Atlas database; $P = 0.033$. D, Proposed schematic model showing the roles of MORC2 and SIRT1 in regulation of the *NDRG1* gene transcription. In our model, MORC2 binds the −446 to −213 bp region of *NDRG1* promoter, and interacts with sirtuin 1 (SIRT1), leading to the a decrease of histone H3 and H4 acetylation levels of the *NDRG1* promoter and thus the transcriptional repression of the *NDRG1* gene. TSS, Transcription start site

axis in gastric tumorigenesis.[10] Other studies revealed that MORC2 promoted chromatin remodeling during the DNA-damage response and had a cytosolic role in lipogenesis and adipogenesis.[8,9] However, little is known about its functions in colorectal cancer. Here, we show that MORC2 is upregulated in CRC tissues (Figure 6A), which is consistent with the findings of another group.[28] We provide the first evidence that MORC2 promotes CRC cell migration and invasion in vitro and metastasis in vivo (Figures 4,5). Our data also show that MORC2 inhibits cell cycle progression in HCT-116 cells (Figure S2B), indicating that increased migration and invasion by MORC2 are not due to increased proliferation.

NDRG1 is a member of the NDRG family. Previous studies have revealed that NDRG1 shows tissue-specific expression patterns in different human cancers. NDRG1 is downregulated in colorectal cancer,[29] prostate cancer,[30] and glioma,[31] whereas it is upregulated in breast cancer,[32] liver cancer,[33] and lung cancer.[34] Our study shows the low expression of NDRG1 in colorectal cancer samples compared with noncancerous tissues (Figure 6A), which is consistent with a previous study. NDRG1 plays pleiotropic roles in tumor metastasis depending on cell context. It inhibits tumor progression

and metastasis in colon, prostate, and breast cancers, indicating that NDRG1 is an effective metastasis suppressor in these cancers.[35] However, NDRG1 promotes portal vein invasion and intrahepatic metastasis in hepatocellular carcinoma.[36] In this study, we show that NDRG1 prevents the migration and invasion of CRC cells (Figure 4C,D). Importantly, NDRG1 is essential in MORC2-mediated enhancement of CRC cell migration and invasion (Figure 4C,D).

NDRG1 is regulated by a variety of factors and stress related to cancer progression, including metal ions, cytokines, oncogenes, tumor suppressors, hormones, and a hypoxic microenvironment.[37] As the name implies, N-myc transcriptionally repress NDRG1 expression in human neuroblastomas cells.[38] The von Hippel-Lindau protein has been reported to inhibit NDRG1 expression in renal cancer cells.[39] However, NDRG1 expression is able to be promoted by hypoxia-inducible factor-1α, p53, PTEN, and eIF3a.[24] Here, our data show that MORC2 downregulated NDRG1 promoter activity in a dose-dependent manner (Figure 2A,B). Through a series of deletions of the NDRG1 promoter luciferase constructs, we found that the −446 to −213 bp region was important for the promoter activities of NDRG1 (Figure 2C). Moreover, ChIP assay indicated the enrichment

TABLE 1 Expression of MORC2 during colon cancer progression

Feature	n	Weak	Strong	P value
Age (years)				
<65	63	13	50	0.916
≥65	56	12	44	
Gender				
Male	66	10	56	0.081
Female	53	15	38	
Tumor size (cm)				
<5	56	12	44	0.916
≥5	63	13	50	
Histological grade				
Poorly	17	6	11	0.136
Moderately	88	17	71	
Well	14	2	12	
Depth of invasion (pT)				
T1,T2	18	6	12	0.165
T3,T4	101	19	82	
Lymph node metastasis (pN)				
No	76	21	55	**0.019***
Yes	43	4	39	
Distant metastasis (pM)				
No	108	24	84	0.310
Yes	11	1	10	
Pathological stage (pStage)				
Stages I, II	71	20	51	**0.020***
Stages III, IV	48	5	43	

Bold values indicate statistical significance, *P < 0.05.

TABLE 2 Expression of NDRG1 during colon cancer progression

Feature	n	Weak	Strong	P value
Age (years)				
<65	63	43	20	0.293
≥65	56	33	23	
Gender				
Male	66	42	24	0.954
Female	53	34	19	
Tumor size (cm)				
<5	56	38	18	0.395
≥5	63	38	25	
Histological grade				
Poorly	17	15	2	0.239
Moderately	88	51	37	
Well	14	10	4	
Depth of invasion (pT)				
T1,T2	18	14	4	0.184
T3,T4	101	62	39	
Lymph node metastasis (pN)				
No	76	41	35	**0.003***
Yes	43	35	8	
Distant metastasis (pM)				
No	108	67	41	0.195
Yes	11	9	2	
Pathological stage (pStage)				
Stages I, II	71	44	27	0.603
Stages III, IV	48	32	16	

Bold values indicate statistical significance. *P < 0.05.

of MORC2 on the *NDRG1* promoter (Figure 2D). The direct role of MORC2 in the negative regulation of the *NDRG1* gene was further evidenced by both overexpression and knockdown of MORC2 in CRC cells (Figure 1). These data suggest that *NDRG1* is a novel target gene of MORC2 in CRC cells.

Reversible acetylation is one of the best-characterized post-transcriptional modifications of the histone amino termini. In general, histone acetylation is correlated with a positive transcriptional regulation, whereas histone deacetylation is associated with transcriptional repression.[40] It has been reported that HDAC inhibitor trichostatin A promotes *NDRG1* mRNA expression,[41] indicating that histone deacetylation is involved in transcriptional silencing of *NDRG1*. Sirtuin 1 is a highly conserved NAD(+)-dependent HDAC that is upregulated in colon cancers.[26] Sirtuin 1 could promote cancer cell proliferation by enhancing expression of several epigenetic silenced tumor suppressor genes.[26,42] Sirtuin 1 is known to promote NDRG1 expression in hypoxic trophoblasts.[43] However, the influence of SIRT1 on NDRG1 expression in cancer cells remains unknown. Here, we found that sirtinol treatment inverted the inhibition of NDRG1 by MORC2 (Figure 3A,B), indicating that SIRT1 is involved in the MORC2-mediated *NDRG1* transcriptional inhibition. Moreover, we showed that SIRT1 downregulated *NDRG1* mRNA, protein level, and promoter activity (Figure 3C-E).

Sirtuin 1 interacts with many important transcription factors or cofactors to repress target gene transcription through deacetylation, which is involved in tumorigenesis and cancer progression.[44] In this study, we showed that SIRT1 interacted with MORC2 and inhibited *NDRG1* transcription independently and cumulatively with MORC2 (Figure 3F,G). Because SIRT1 is a member of the class III HDACs, we speculate that SIRT1 might contribute to MORC2-mediated *NDRG1* transcription inhibition through its histone deacetylation function. Further studies are needed to verify the speculation.

Based on previous knowledge, as well as findings from this study, we propose a hypothesized model (Figure 6D). In our model, MORC2 binds the −446 to −213 bp region of *NDRG1* promoter, and interacts with SIRT1, leading to a decrease in the histone H3 and H4 acetylation level of the *NDRG1* promoter and thus the transcriptional repression of the *NDRG1* gene (Figure 6D).

In conclusion, we recognize *NDRG1* as a novel target gene of MORC2 in CRC cells. MORC2 inhibits *NDRG1* transcription by binding the −446 to −213 bp region of *NDRG1* promoter and interacting with SIRT1. Moreover, MORC2 promotes the migration and invasion, as well as the pulmonic metastasis, of CRC cells through NDRG1. Notably, MORC2 expression correlates negatively with NDRG1 expression in CRC tissues and high expression of MORC2 is significantly associated with lymph node metastasis and poor pTNM stage. Our findings highlight a novel mechanism of MORC2 in the progression of CRC and provide MORC2 as a potential therapeutic target for CRC.

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CONFLICT OF INTEREST

Authors declare no conflict of interests for this article.

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REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin*. 2017;67:7-30.
2. Siegel RL, Miller KD, Fedewa SA, et al. Colorectal cancer statistics, 2017. *CA Cancer J Clin*. 2017;67:177-193.
3. Carethers JM, Jung BH. Genetics and genetic biomarkers in sporadic colorectal cancer. *Gastroenterology*. 2015;149:1177-1190.
4. Okugawa Y, Grady WM, Goel A. Epigenetic alterations in colorectal cancer: emerging biomarkers. *Gastroenterology*. 2015;149:1204-1225.
5. Shao Y, Li Y, Zhang J, et al. Involvement of histone deacetylation in MORC2-mediated down-regulation of carbonic anhydrase IX. *Nucleic Acids Res*. 2010;38:2813-2824.
6. Zhang Q, Song Y, Chen W, et al. By recruiting HDAC1, MORC2 suppresses p21 Waf1/Cip1 in gastric cancer. *Oncotarget*. 2015;6:16461-16470.
7. Tong Y, Li Y, Gu H, et al. Microchidia protein 2, MORC2, downregulates the cytoskeleton adapter protein, ArgBP2, via histone methylation in gastric cancer cells. *Biochem Biophys Res Commun*. 2015;467:821-827.
8. Tong Y, Li Y, Gu H, et al. HSF1, in association with MORC2, down-regulates ArgBP2 via the PRC2 family in gastric cancer cells. *Biochim Biophys Acta*. 2018;1864:1104-1114.
9. Li DQ, Nair SS, Ohshiro K, et al. MORC2 signaling integrates phosphorylation-dependent, ATPase-coupled chromatin remodeling during the DNA damage response. *Cell Rep*. 2012;2:1657-1669.
10. Sánchez-Solana B, Li DQ, Kumar R. Cytosolic functions of MORC2 in lipogenesis and adipogenesis. *Biochim Biophys Acta*. 2014;1843:316-326.
11. Wang G, Song Y, Liu T, et al. PAK1-mediated MORC2 phosphorylation promotes gastric tumorigenesis. *Oncotarget*. 2015;6:9877-9886.
12. Liao XH, Zhang Y, Dong WJ, et al. Chromatin remodeling protein MORC2 promotes breast cancer invasion and metastasis through a PRD domain-mediated interaction with CTNND1. *Oncotarget*. 2017;8:97941-97954.
13. Zhang FL, Cao JL, Xie HY, et al. Cancer-associated MORC2-mutant M276I regulates an hnRNPM-mediated CD44 splicing switch to promote invasion and metastasis in triple-negative breast cancer. *Cancer Res*. 2018;78:5780-5792.
14. Wang T, Qin ZY, Wen LZ, et al. Epigenetic restriction of Hippo signaling by MORC2 underlies stemness of hepatocellular carcinoma cells. *Cell Death Differ*. 2018. https://doi.org/10.1038/s41418-018-0095-6 [Epub ahead of print]
15. Tuupanen S, Hänninen UA, Kondelin J, et al. Identification of 33 candidate oncogenes by screening for base-specific mutations. *Br J Cancer*. 2014;111:1657-1662.

16. Sahni S, Krishan S, Richardson DR. NDRG1 as a molecular target to inhibit the epithelial-mesenchymal transition: the case for developing inhibitors of metastasis. *Future Med Chem*. 2014;6:1241-1244.

17. Sun J, Zhang D, Bae DH, et al. Metastasis suppressor, NDRG1, mediates its activity through signaling pathways and molecular motors. *Carcinogenesis*. 2013;34:1943-1954.

18. Mao Z, Sun J, Feng B, et al. The metastasis suppressor, N-myc downregulated gene 1 (NDRG1), is a prognostic biomarker for human colorectal cancer. *PLoS ONE*. 2013;8:e68206.

19. Mi L, Zhu F, Yang X, et al. The metastatic suppressor NDRG1 inhibits EMT, migration and invasion through interaction and promotion of caveolin-1 ubiquitylation in human colorectal cancer cells. *Oncogene*. 2017;36:4323-4335.

20. Liu F, Li X, Wang C, et al. Downregulation of p21-activated kinase-1 inhibits the growth of gastric cancer cells involving cyclin B1. *Int J Cancer*. 2009;125:2511-2519.

21. Berchuck A, Soisson AP, Clarke-Pearson DL, et al. Immunohistochemical expression of CA 125 in endometrial adenocarcinoma: correlation of antigen expression with metastatic potential. *Cancer Res*. 1989;49:2091-2095.

22. Zhu G, Li X, Guo B, et al. PAK5-mediated E47 phosphorylation promotes epithelial-mesenchymal transition and metastasis of colon cancer. *Oncogene*. 2016;35:1943-1954.

23. Vaes N, Schonkeren SL, Brosens E, et al. A combined literature and in silico analysis enlightens the role of the NDRG family in the gut. *Biochim Biophys Acta*. 2018;1862:2140-2151.

24. Fang BA, Kovačević Ž, Park KC, et al. Molecular functions of the iron-regulated metastasis suppressor, NDRG1, and its potential as a molecular target for cancer therapy. *Biochim Biophys Acta*. 2014;1845:1-19.

25. Li D, Mei H, Qi M, et al. FOXD3 is a novel tumor suppressor that affects growth, invasion, metastasis and angiogenesis of neuroblastoma. *Oncotarget*. 2013;4:2021-2044.

26. Stünkel W, Peh BK, Tan YC, et al. Function of the SIRT1 protein deacetylase in cancer. *Biotechnol J*. 2007;2:1360-1368.

27. Wangpu X, Lu J, Xi R, et al. Targeting the metastasis suppressor, N-Myc downstream regulated gene-1, with Novel Di-2-Pyridylketone Thiosemicarbazones: suppression of tumor cell migration and cell-collagen adhesion by inhibiting focal adhesion kinase/paxillin signaling. *Mol Pharmacol*. 2016;89:521-540.

28. Ding QS, Zhang L, Wang BC, et al. Aberrant high expression level of MORC2 is a common character in multiple cancers. *Hum Pathol*. 2018;76:58-67.

29. Strzelczyk B, Szulc A, Rzepko R, et al. Identification of high-risk stage II colorectal tumors by combined analysis of the NDRG1 gene expression and the depth of tumor invasion. *Ann Surg Oncol*. 2009;16:1287-1294.

30. Bandyopadhyay S, Pai SK, Gross SC, et al. The Drg-1 gene suppresses tumor metastasis in prostate cancer. *Cancer Res*. 2003;63:1731-1736.

31. Sun B, Chu D, Li W, et al. Decreased expression of NDRG1 in glioma is related to tumor progression and survival of patients. *J Neurooncol*. 2009;94:213-219.

32. Mao XY, Fan CF, Wei J, et al. Increased N-myc downstream-regulated gene 1 expression is associated with breast atypia-to-carcinoma progression. *Tumour Biol*. 2011;32:1271-1276.

33. Chua MS, Sun H, Cheung ST, et al. Overexpression of NDRG1 is an indicator of poor prognosis in hepatocellular carcinoma. *Mod Pathol*. 2007;20:76-83.

34. Wang D, Tian X, Jiang Y. NDRG1/Cap43 overexpression in tumor tissues and serum from lung cancer patients. *J Cancer Res Clin Oncol*. 2012;138:1813-1820.

35. Kovacevic Z, Richardson DR. The metastasis suppressor, Ndrg-1: a new ally in the fight against cancer. *Carcinogenesis*. 2006;27:2355-2366.

36. Akiba J, Ogasawara S, Kawahara A, et al. N-myc downstream regulated gene 1 (NDRG1)/Cap43 enhances portal vein invasion and intrahepatic metastasis in human hepatocellular carcinoma. *Oncol Rep*. 2008;20:1329-1335.

37. Ellen TP, Ke Q, Zhang P, Costa M. NDRG1, a growth and cancer related gene: regulation of gene expression and function in normal and disease states. *Carcinogenesis*. 2008;29:2-8.

38. Li J, Kretzner L. The growth-inhibitory Ndrg1 gene is a Myc negative target in human neuroblastomas and other cell types with over-expressed N- or c-myc. *Mol Cell Biochem*. 2003;250:91-105.

39. Masuda K, Ono M, Okamoto M, et al. Downregulation of Cap43 gene by von Hippel-Lindau tumor suppressor protein in human renal cancer cells. *Int J Cancer*. 2003;105:803-810.

40. Kuo MH, Allis CD. Roles of histone acetyltransferases and deacetylases in gene regulation. *BioEssays*. 1998;20:615-626.

41. Li Q, Chen H. Transcriptional silencing of N-Myc downstream-regulated gene 1 (NDRG1) in metastatic colon cancer cell line SW620. *Clin Exp Metastasis*. 2011;28:127-135.

42. Pruitt K, Zinn RL, Ohm JE, et al. Inhibition of SIRT1 reactivates silenced cancer genes without loss of promoter DNA hypermethylation. *PLoS Genet*. 2006;2:e40.

43. Chen B, Nelson DM, Sadovsky Y. N-myc down-regulated gene 1 modulates the response of term human trophoblasts to hypoxic injury. *J Biol Chem*. 2006;281:2764-2772.

44. Michan S, Sinclair D. Sirtuins in mammals: insights into their biological function. *Biochem J*. 2007;404:1-13.

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