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Effects of MCRS1 on proliferation, migration, invasion, and epithelial mesenchymal transition of gastric cancer cells by interacting with Pkmyt1 protein kinase

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ABSTRACT

Microspherule protein 1(MCRS1) is known to be an oncogene in several tumors. However, recent studies have shown that MCRS1 inhibits lymphatic metastasis in gastric cancer (GC) patients by inhibiting telomerase activity. Protein kinase, membrane associated tyrosine/threonine 1(Pkmyt1), a member of the WEE1 family, has been found to interact with MCRS1 by yeast two-hybrid assay; however, how these two proteins interact in GC is still unclear. Hence, this study aimed to investigate the effect of MCRS1 interaction with Pkmyt1 on GC cell proliferation, migration, and invasion. Initially, we observed increased expression of MCRS1 in GC SGC-7901 cells and decreased expression in GC BGC-823 cells. Hence, we down-regulated MCRS1 expression in SGC-7901 cells and up-regulated it in BGC-823 cells. Our results showed that overexpression of MCRS1 inhibits the growth, invasion and migration of GC cells, while downregulation of MCRS1 promotes the growth, invasion and migration of GC cells. When MK1775, an inhibitor of WEE1 kinase, was added after downregulation of MCRS1, phenotypic recovery effects were observed. Overexpression of MCRS1 also inhibited the expression of Pkmyt1 and vice versa. This indicated that there might be a possible interaction between MCRS1 and Pkmyt1. Furthermore, immunoprecipitation assay revealed the interaction between MCRS1 and Pkmyt1 *in virto*, and immunofluorescence experiments showed that the two proteins were co-localized in the cytoplasm. In conclusion, our study confirmed the specific tumor suppressive activity of MCRS1 in GC proliferation, invasion and migration and suggested that it might inhibit the progression of GC through its interaction with Pkmyt1.

1. Introduction

Gastric cancer (GC) is one of the most common malignant tumors of the digestive tract and is a serious threat to human health [1]. Most patients with GC are mainly diagnosed in the advanced stage since the early stages are clinically silent [2]. The five-year survival rate for advanced gastric cancer is 29.3% [3]. Furthermore, metastasis after surgical resection and a high frequency of tumor recurrence contribute to poor prognosis in GC patients. Therefore, it is important to understand the mechanisms that regulate invasion and migration of GC to develop successful therapeutic strategies to improve survival rate of GC patients [4].

Microspherule protein 1(MCRS1), also known as 58-kDa microspherule protein (MSP58), was found to be located at chromosome position 12q13.12 [5]. It interacts with p120, which is a proliferation-related protein [6]. MCRS1 plays an important role in various cellular activities including mitosis, DNA repair, transcriptional regulation, cell proliferation and oncogenic transformation [7,8].Studies have shown that MCRS1 is highly expressed in cells and tissues of breast, colon and esophageal cancers [9–12], and it promotes epithelial mesenchymal

Abbreviations: MCRS1, microspherule protein 1; GC, gastric cancer; Pkmyt1, Protein kinase, membrane associated tyrosine/threonine 1; MSP58, 58-kDa microspherule protein; EMT, Epithelial-mesenchymal transition; CDK1, cyclin-dependent kinase 1; FBS, fetal calf serum; DMSO, dimethyl sulfoxide; RIPA, radio immunoprecipitation assay; PDVF, polyvinylidene fluoride; BSA, bovine serum albumin; ECL, enhanced chemiluminescence

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Table 1
MCRS1 plasmid and MCRS1 siRNA sequence.

Gene	Sequence (5′-3′)
pcDNA3.1-myc-MCRS1	Sense:GGCGGAAGCTTCCACCATGGACAAAGATTCTCAGGGGC Anti-sense:GGCGGTCTAGACTGTGGTGTGATCTTGGCAGCC
MCRS1 siRNA 559	Sense: GCGUGUGAAGAAGAGUAAATT Anti-sense: UUUACUCUUCUUCACACGCTT
Control siRNA	Sense: UUCUCCGAACGUGUCACGUTT Anti-sense: ACGUGACACGUUCGGAGAATT

transition (EMT) and metastasis in non-small cell lung cancer [13]. Knockdown of MCRS1 significantly inhibited the proliferation of MGC-803 and BGC-823 GC cells [6]. However, a recent study showed that MCRS1 inhibits lymphatic metastasis in GC patients by inhibiting telomerase activity [14]. Thus, MCRS1 has both tumor-promoting and tumor-suppressive functions, but its expression pattern in GC is still unclear.

Protein kinase, membrane associated tyrosine/threonine 1(Pkmyt1), a member of the WEE1 family of protein kinases, is bispecific for phosphorylation of Tyr15 and Thr14. The binding of cyclin-dependent kinase 1 (CDK1) complexes by PKMYT1 sequesters them into the cytoplasm, thereby precluding entry into the nucleus and preventing cell cycle progression [15].Pkmyt1 promoted the growth and motility of hepatocellular carcinoma cells [16] and played an essential oncogenic role in colorectal cancer [17]. Previous studies have found that Pkmyt1 interacts with a variety of proteins, including CAMK1G, CLK3, CLK4, E4F1, RASAL2, and RPL27 [18]. Pkmyt1 has been found to interact with MCRS1 by a yeast two-hybrid assay (unpublished data), but its mechanism of action is still unclear.

In this study, we validated the inhibitory effect of MCRS1 on proliferation, invasion and migration of GC cells. MCRS1 interacted with Pkmyt1 in GC cells, suggesting that MCRS1 might regulate the progression of GC cells through its interaction with Pkmyt1.

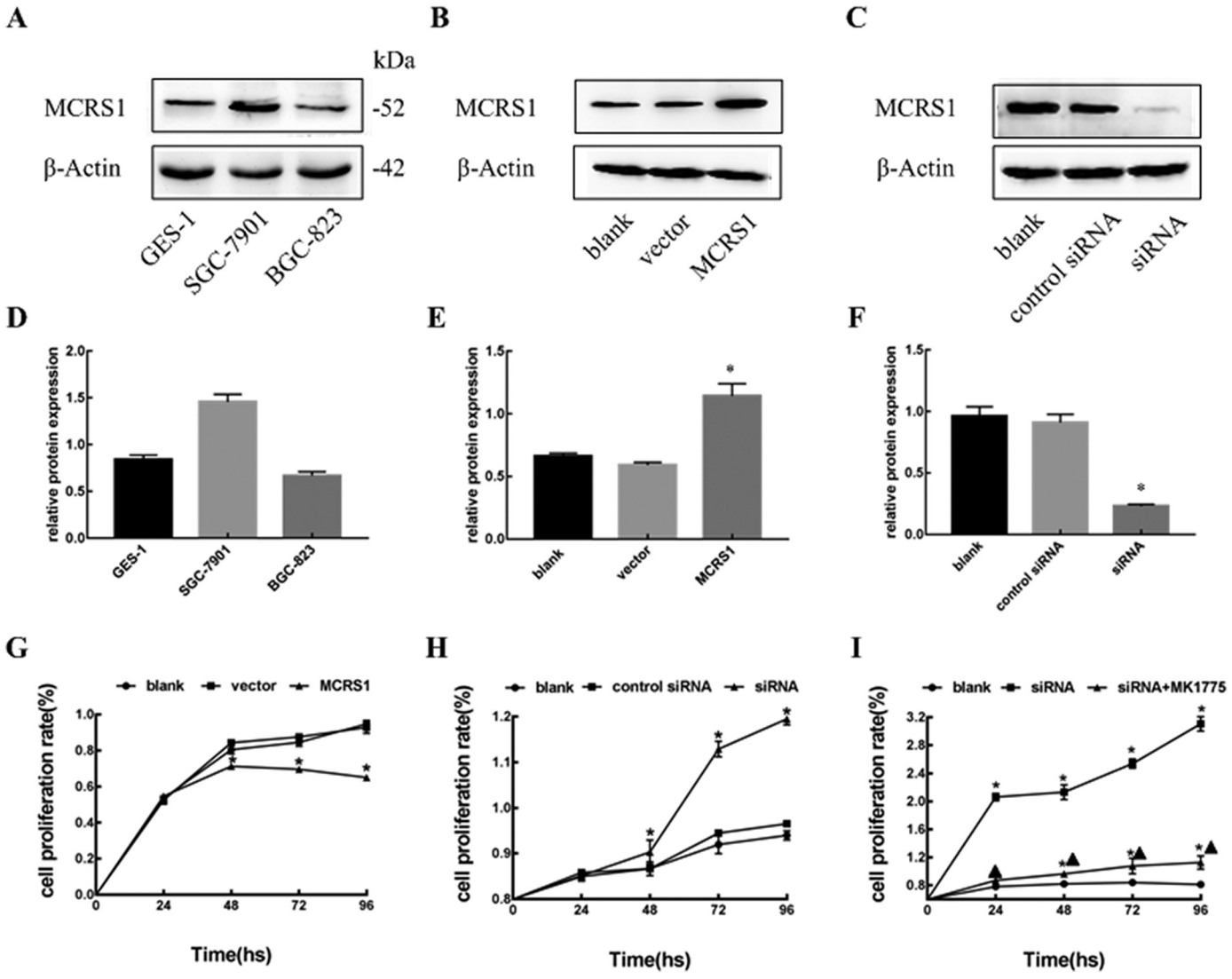

Fig. 1. The protein expression of MCRS1 in GES-1, BGC-823 and SGC-7901cells and the effects of MCRS1 on the growth of GC cells. A–C, the protein level of MCRS1 in cells was examined using western blotting; D–F, the protein expression analysis of corresponding A–C; G, the overexpression of MCRS1 inhibits the growth of BGC-823 cells using MTT assay; H, the down-regulation of MCRS1 promotes the growth of SGC-7901 cells using MTT assay; I, the effect of down-regulation of MCRS1 with MK1775 on the growth of SGC-7901 cells using MTT assay; *, $p < 0.05$ vs. the blank group; ▲, $p < 0.05$ vs. the siRNA group; the measurement data in figures were presented as sample mean ± standard deviation and analyzed by repeated measurement analysis of variance; the experiment was repeated at least 3 times.

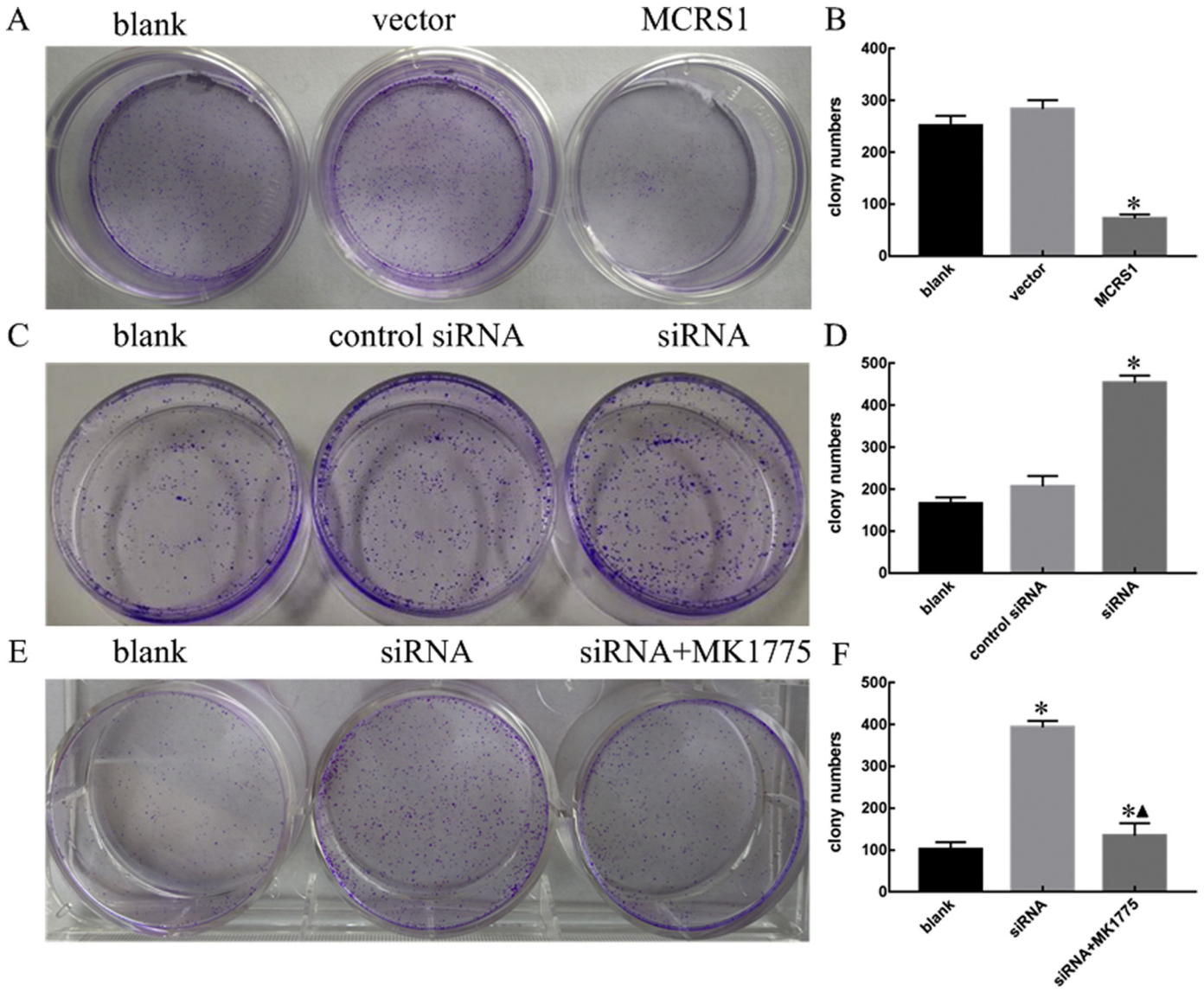

Fig. 2. The effects of MCRS1 on the proliferation of GC cells. A, the overexpression of MCRS1 inhibits the proliferation of BGC-823 cells using colony formation experiment; B, the colony numbers of corresponding A; C, the down-regulation of MCRS1 promotes the proliferation of SGC-7901 cells using colony formation experiment; D, the colony numbers of corresponding C; E, the effect of down-regulation of MCRS1 with MK1775 on the proliferation of SGC-7901 cells using colony formation experiment; F, the colony numbers of corresponding E; *, $p < 0.05$ vs. the blank group; ▲, $p < 0.05$ vs. the siRNA group; the measurement data in figures were presented as sample mean ± standard deviation and analyzed by one-way analysis of variance; the experiment was repeated at least 3 times.

2. Materials and methods

2.1. Cell culture

Human GC cell lines BGC-823, SGC-7901 and gastric mucosal epithelial cells GES-1 were obtained from GenePharma Co., Ltd. (Suzhou, Jiangsu, China). BGC-823 cells and GES-1 cells were cultured in RPMI 1640 (HyClone Laboratories, Inc., Logan, UT, USA) medium containing 10% fetal calf serum (FBS, HyClone). GC cells SGC-7901 cells were cultured in DMEM (HyClone) medium containing 10% FBS. Cells were cultured in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C, and the culture medium was changed every other day in all experiments.

2.2. Antibodies and siRNA

Recombinat rabbit monoclonal anti-MCRS1 antibody (ab124704; diluted 1:10000) was obtained from Abcam Biotechnology (Abcam, USA). Polyclonal rabbit anti-Pkmyt1 antibody (4282; diluted 1:1000) was obtained from Cell Signaling Technology (CST, USA). Polyclonal rabbit anti-N-cadherin antibody (22018–1-AP; diluted 1:1000) and polyclonal rabbit anti-ZO-1 antibody (21773–1-AP; diluted 1:1000) were obtained from Proteintech Biotechnology (Proteintech, USA). Polyclonal rabbit anti-E-cadherin antibody (A3044; diluted 1:1000), polyclonal rabbit anti-Snail1 antibody (A5243; diluted 1:1000), polyclonal rabbit anti-Snail2 antibody (A1057; diluted 1:1000), polyclonal rabbit anti-Twist antibody (A7314; diluted 1:1000), polyclonal rabbit anti-CCNB1 antibody (A2056; diluted 1:1000), polyclonal rabbit anti-CCND1 antibody (A1301; diluted 1:1000), phosphorylated rabbit anti-CDK1-Thr14 antibody (AP0015; diluted 1:1000), phosphorylated rabbit anti-CDK1-Tyr15 antibody (AP0016; diluted 1:1000), and polyclonal rabbit anti-β-Actin antibody (AC026; diluted 1:50000) were obtained from ABclonal Biotechnology (ABclonal, USA). MCRS1 siRNA and control siRNA were obtained from GenePharma Co., Ltd. (Table 1).

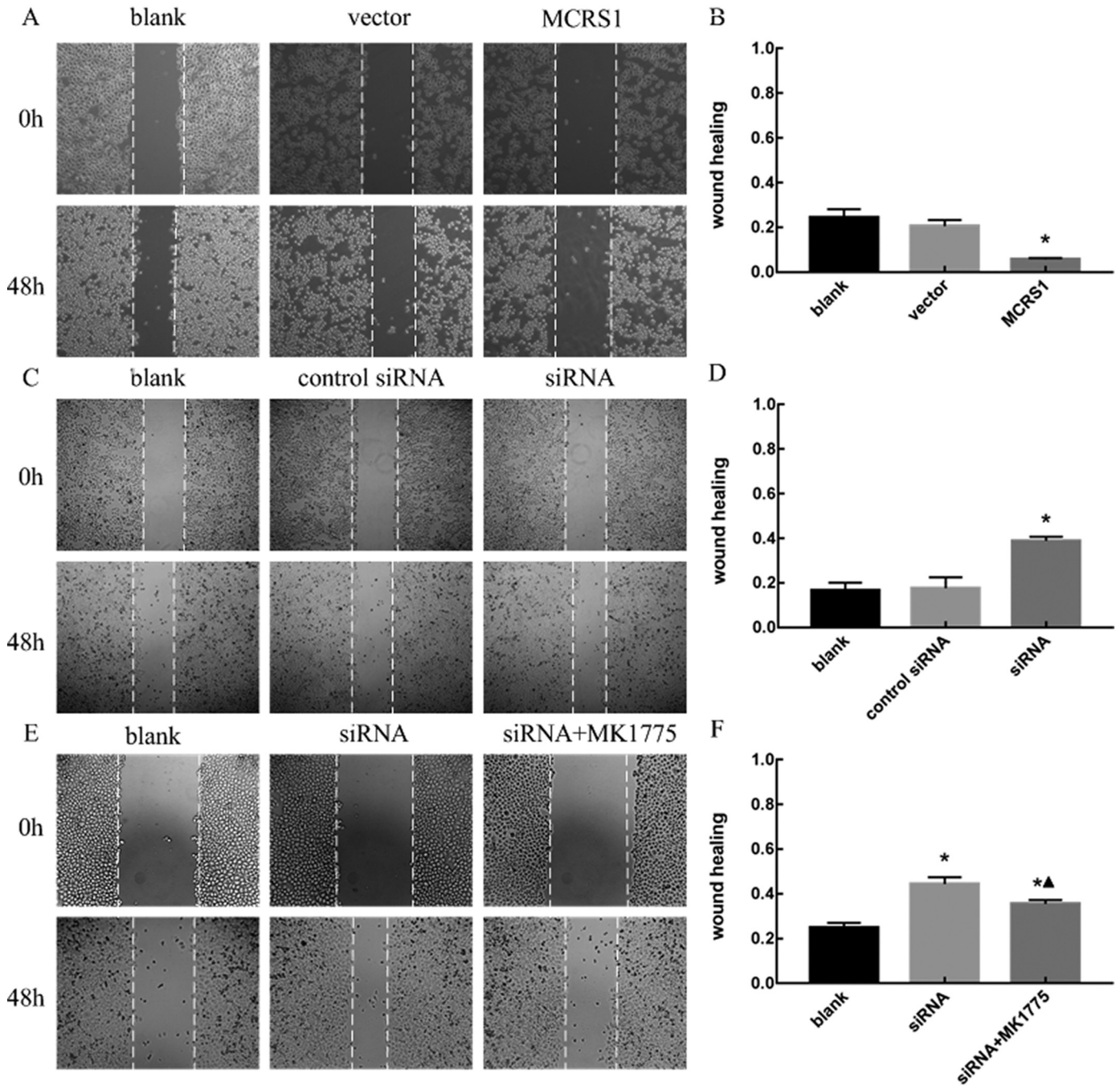

Fig. 3. The effects of MCRS1 on migration of GC cells. A, the overexpression of MCRS1 inhibits the migration of BGC-823 cells using cell scratch test; B, the migration of corresponding A; C, the down-regulation of MCRS1 promotes the migration of SGC-7901 cells using cell scratch test; D, the migration of corresponding C; E, the effect of down-regulation of MCRS1 with MK1775 on the migration of SGC-7901 cells using cell scratch test; F, the migration of corresponding E; *, $p < 0.05$ vs. the blank group; ▲, $p < 0.05$ vs. the siRNA group; the measurement data in figures were presented as sample mean ± standard deviation and analyzed by one-way analysis of variance; the experiment was repeated at least 3 times.

2.3. Construction of recombinant plasmid pcDNA3.1-myc-MCRS1

According to the sequence of human MCRS1 (Genbank ID: NM_001012300), specific PCR primers were designed, the upstream primer was with *Hind* III restriction site, and the downstream primer was with *Xba* I restriction site and stop codon. The primers were synthesized by Taihe Biotechnology Co., Ltd. (Beijing, China) (Table 1). The PCR amplification product was recovered by 1% agarose gel electrophoresis and purified. The plasmid was extracted with a plasmid extraction kit (Axygen, USA), and then digested with *Hind* III and *Xba* I. Positive clones with the expected size of the fragment were selected and then sequenced. The recombinant plasmid with the correct sequence was amplified, and endotoxin-free DNA was isolated.

2.4. Cell grouping and transfection

Cells were pre-cultured in medium containing 10% FBS until they were 70–80% confluent and then transfected with MCRS1 plasmid or MCRS1 siRNA using Lipofectamine2000 (Invitrogen, USA) for 40 h. The transfection efficiency of each group was confirmed by western blot analysis. Overexpression experiments were performed in three groups: blank group, pcDNA3.1 empty vector transfected group and pcDNA3.1-

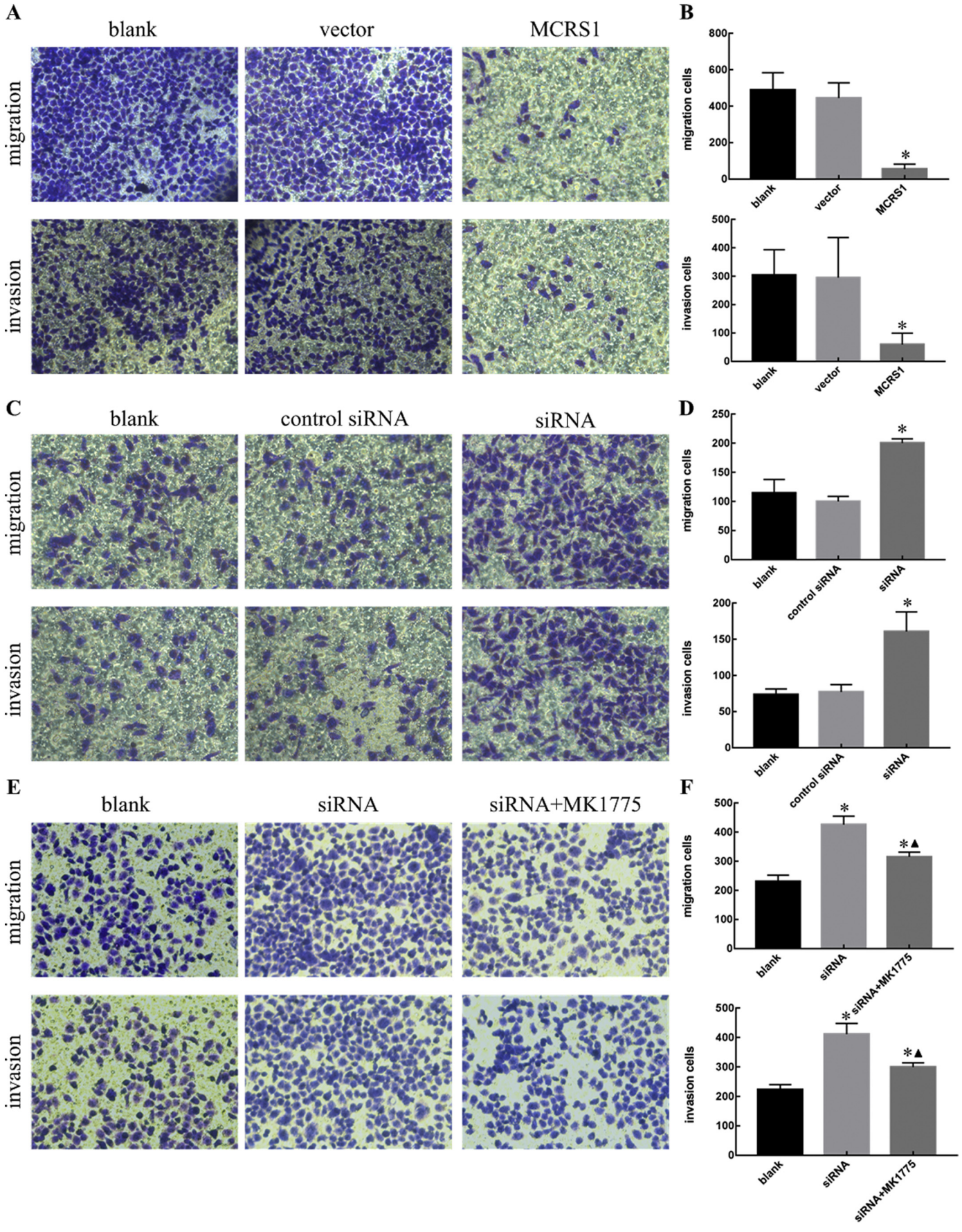

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Fig. 4. The effects of MCRS1 on migration and invasion of GC cells. A, the overexpression of MCRS1 inhibits the migration and invasion of BGC-823 cells using transwell assay; B, the migration and invasion numbers of corresponding A; C, the down-regulation of MCRS1 promotes the migration and invasion of SGC-7901 cells using transwell assay; D, the migration and invasion numbers of corresponding C; E, the effect of down-regulation of MCRS1 with MK1775 on the migration and invasion of SGC-7901 cells using transwell assay; F, the migration and invasion numbers of corresponding E; *, $p < 0.05$ vs. the blank group; ▲, $p < 0.05$ vs. the siRNA group; the measurement data in figures were presented as sample mean ± standard deviation and analyzed by one-way analysis of variance; the experiment was repeated at least 3 times.

myc-MCRS1 transfected group (overexpression group); siRNA interference experiments were performed in three groups: blank group, control siRNA transfected group and MCRS1 siRNA transfected group (downregulation group); rescue experiments were performed in three groups: blank group, MCRS1 siRNA transfected group and MCRS1 siRNA with 40 nmoL of MK1775 (WEE1 inhibitor) transfected group.

2.5. 3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay

Cells were seeded in 96-well plates at a density of 2×10^3 cells in each well (200 μL/well). At 24, 48, 72 and 96 h, cells were incubated with 15 μL of 0.5 mg/mL sterile MTT for 4 h at 37 °C, after which the culture medium was removed and 150 μL of dimethyl sulfoxide (DMSO) was added. The absorbance values were measured at a wavelength of 490 nm. The experiment was repeated 3 times.

2.6. Colony formation experiment

Cells were plated in 6-well plates, cultured for 7–10 days then treated with 4% formaldehyde for 15 min, followed by staining using crystal violet for 15 min before counting the number of colonies.

2.7. Cell scratch test

Cells at the logarithmic growth phase exhibiting good growth were conventionally detached and counted. Then, the cells were resuspended in culture medium, and evenly inoculated into a 6-well plate (5×10^5 cells/well). The next day, wounds were created using a sterile pipette tip. PBS solution was used to gently wash the cells in order to remove the floating cells, after which the cells were incubated with the medium at 37 °C in a 5% CO_2 incubator. At 0 h and 48 h time points, cells were observed and photographed under an inverted microscope. Finally, wound healing of cells was measured and recorded.

2.8. Transwell assay

Transwell chambers (BD, USA) with or without diluted martrigel (BD, USA) were placed in a 37 °C incubator for 1 h. Cells (5×10^4) were added to the chamber and seeded with serum-free medium. Complete medium was added to the lower chamber. After incubation for 24 h, tumor cells that failed to invade the stroma were removed with using cotton swabs. The cells were then fixed by 4% paraformaldehyde, stained with crystal violet for 15 min. The cells were observed under a 200-fold zoom microscope and 5 fields were randomly selected to calculate the total number of cells, and the average value was quantified as the experimental result.

2.9. Western blot analysis

Total protein from cells was extracted using radio immunoprecipitation assay (RIPA) buffer and separated by SDS-PAGE. The separated proteins were transferred onto polyvinylidene fluoride (PDVF) membranes. After blocking with 1% bovine serum albumin (BSA) solution, the membranes were incubated with primary antibodies over-night at 4 °C. The membranes were washed with TBST solution and incubated with secondary antibodies for 1.5 h at room temperature. The proteins were visualized using enhanced chemiluminescence (ECL, DingGuo Biotechnology Co., Ltd., Beijing, China) kit. Image J software (National Institutes of Health, USA) was employed for the gray value analysis of the target bands.

2.10. Immunoprecipitation

Protease inhibitors were added to the transfected SGC-7901 cells and disrupted by sonication on ice. After shaken well, the supernatant was taken, A + G beads were added and shaken at 4 °C for 1 h. After shaken well, 50 μL aliquots of the supernatant was distributed as negative and positive control and shaken at 4 °C for 3 h. A + G beads were added and shaken at 4 °C for 2 h. After shaken well, the supernatant was discarded, the beads were washed several times. Finally loading buffer was added, the sample was shaken well, and used for western blotting.

2.11. Immunofluorescence

SGC-7901 cells were fixed with paraformaldehyde and washed with PBS. Cells were blocked by PBS containing 1% BSA at room temperature. Primary antibody was added to the cells and incubated overnight at 4 °C. Cells were washed with PBS, secondary antibody was added and incubated at room temperature in the dark. Then, the cells were washed again with PBS, stained with DAPI, and observed under a fluorescence microscope.

2.12. Statistical analysis

Statistical analyses were conducted using GraphPad prism 7.0 software. The experiments were conducted at least three times in each group. Data were expressed as mean ± standard deviation. Comparisons between two groups were analyzed using un-paired *t*-test. One-way analysis of variance was used for comparing data among multiple groups. Enumeration data were expressed as percentages. A value of $p < 0.05$ was indicative of statistical significance.

3. Results

3.1. MCRS1 expression in GC cell lines

To study the expression of MCRS1 in GC, we first examined the protein expression level of MCRS1 in BGC-823, SGC-7901 and GES-1 cells. As shown in Fig. 1A & D, MCRS1 expression was low in BGC-823 cells, but high in SGC-7901 cells compared to GES-1 cells. Therefore, BGC-823 cells were selected for subsequent up-regulation experiments, and SGC-7901 cells were selected for down-regulation experiments.

After transfection, the cells from each group were collected for western blotting analysis. The results showed that the expression of MCRS1 protein in pcDNA3.1-myc-MCRS1 transfected group was significantly higher than that in blank group and pcDNA3.1 empty vector transfected group (Fig. 1B & E) ($p < 0.05$). Compared to the blank group and control siRNA transfected group, the expression of MCRS1 protein in MCRS1 siRNA transfected group was significantly decreased (Fig. 1C & F) ($p < 0.05$).

3.2. MCRS1 inhibited the proliferation of GC cells

The proliferation rate of BGC-823 cells was decreased when MCRS1 was overexpressed, the proliferation rate of SGC-7901 cells was

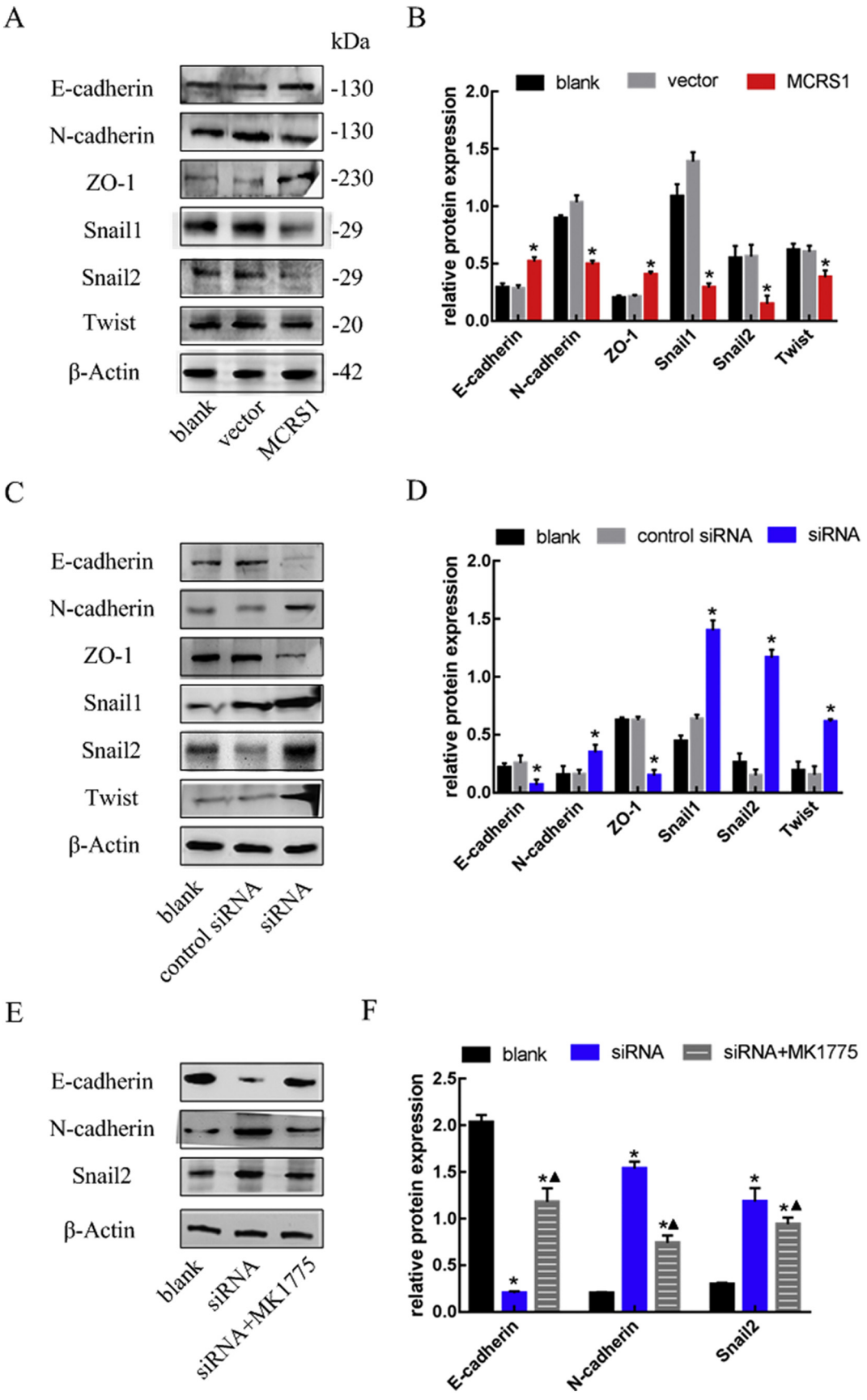

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Fig. 5. The effects of MCRS1 on the EMT markers of GC cells. A, the effects of overexpression of MCRS1 on the expression of E-cadherin, N-cadherin, ZO-1, Snail1, Snail2 and Twist protein in GC cells were examined using western blotting; B, the protein expression analysis of corresponding A; C, the effects of down-regulation of MCRS1 on the expression of E-cadherin, N-cadherin, ZO-1, Snail1, Snail2 and Twist protein in GC cells were examined using western blotting; D, the protein expression analysis of corresponding C; E, the effects of down-regulation MCRS1 with MK1775 on the expression of E-cadherin, N-cadherin and Snail2 protein in SGC-7901 cells were detected using western blotting; F, the protein expression analysis of corresponding E; *, $p < 0.05$ vs. the blank group; ▲, $p < 0.05$ vs. the siRNA group; the measurement data in figures were presented as sample mean ± standard deviation and analyzed by one-way analysis of variance; the experiment was repeated at least 3 times.

increased when MCRS1 was down-regulated. When transfected SGC-7901 cells were treated with MK1775, an inhibitor of WEE1 kinase, a phenotypic recovery effect was observed, the proliferation rate of SGC-7901 cells was decreased again (Fig. 1G–I) ($p < 0.05$). The number of cell colonies was decreased when MCRS1 was overexpressed (Fig. 2A & B) and the number of cell colonies was increased when MCRS1 was down-regulated (Fig. 2C & D). When MK1775 was added to SGC-7901 cells, the phenotypic recovery effect was again observed, the number of cell colonies was decreased (Fig. 2E & F) ($p < 0.05$).

3.3. MCRS1 inhibited the invasion and migration of GC cells

Overexpression of MCRS1 inhibited the migration of GC cells and decreased the migration ability of GC cells (Fig. 3A & B), while down-regulation of MCRS1 promoted the migration of gastric cancer cells and enhanced the migration ability of gastric cancer cells (Fig. 3C & D). When MK1775 was added to transfected SGC-7901 cells, the phenotypic recovery effect was observed, the migration ability of SGC-7901 cells was decreased (Fig. 3E & F) ($p < 0.05$).

In transwell assay, when MCRS1 was overexpressed, the migration and invasion number of BGC-823 cells were reduced (Fig. 4A & B). The migration and invasion number of SGC-7901 cells were increased when MCRS1 was down-regulated (Fig. 4C & D). When MK1775 was added to transfected SGC-7901 cells, the phenotypic recovery effect was observed again, the migration and invasion number of SGC-7901 cells were reduced (Fig. 4E & F) ($p < 0.05$).

3.4. MCRS1 affected the expression of EMT markers of GC cells

Overexpression of MCRS1 increased the protein levels of E-cadherin and ZO-1, but decreased the protein levels of N-cadherin, Snail1, Snail2 and Twist in BGC-823 cells (Fig. 5A & B). Down-regulation of MCRS1 inhibited the protein levels of E-cadherin and ZO-1 and promoted the protein expression of N-cadherin, Snail1, Snail2 and Twist in SGC-7901 cells (Fig. 5C & D). Furthermore, when MK1775 was added to transfected SGC-7901 cells, the phenotypic recovery effect was observed again (Fig. 5E & F) ($p < 0.05$).

3.5. MCRS1 interacted with Pkmyt1 in GC cells

To verify whether MCRS1 and Pkmyt1 interact, we detected the changes of Pkmyt1 protein expression upon up-regulation and down-regulation of MCRS1. The results showed that when MCRS1 expression was increased, Pkmyt1 expression was decreased, and when MCRS1 expression was decreased, Pkmyt1 expression was increased (Fig. 6A–D) ($p < 0.05$). Next, we confirmed the interaction between MCRS1 and Pkmyt1 in cells by immunoprecipitation assay (Fig. 6E & F) ($p < 0.05$). Further, immunofluorescence experiment showed that MCRS1 and Pkmyt1 were localized in the cytoplasm of SGC-7901 cells (Fig. 6G).

3.6. MCRS1 affected the cycle of GC cells

As shown in Fig. 7A & B, overexpression of MCRS1 increased the protein levels of CCNB1, Phospho-CDK1-T14 and Phospho-CDK1-Y15 and decreased the protein level of CCND1. Down-regulation of MCRS1 decreased the protein level of CCNB1, Phospho-CDK1-T14 and Phospho-CDK1-Y15 and increased the protein expression of CCND1 (Fig. 7C & D) ($p < 0.05$).

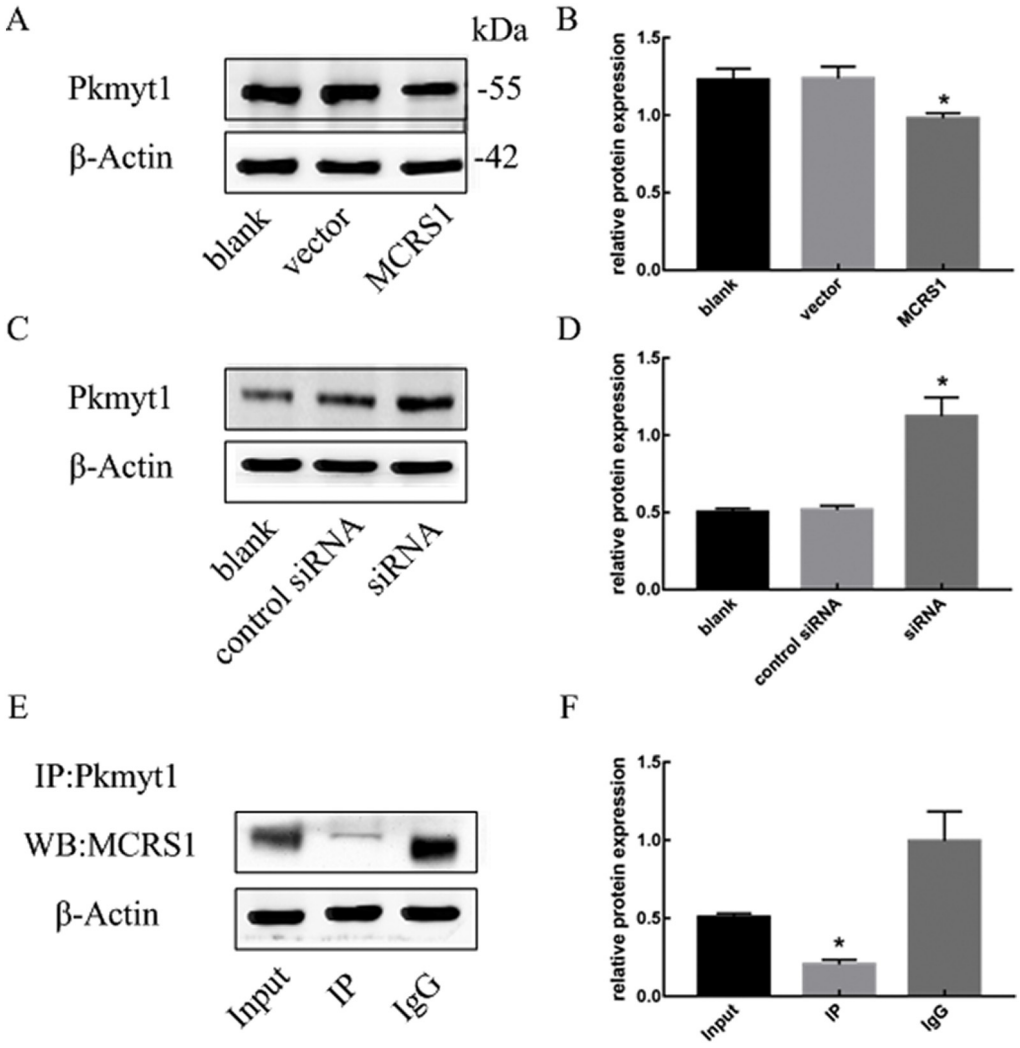
4. Discussion

GC is among the leading causes of cancer related death worldwide [19]. The exact reasons leading to GC are not clearly understood. Due to high frequency of multiple metastasis and tumor recurrence after resection of gastric cancer, it is necessary to explore the mechanisms regulating the invasion and migration of gastric cancer to improve the survival rate of gastric cancer patients. The occurrence of cancer is closely related to the imbalance in the expression of cancer-related genes [20]. Our research group has been studying the WEE1 family of protein kinases. MCRS1 is a protein that has been found to interact with WEE1 by a yeast two-hybrid screen, and the protein kinase Pkmyt1 is a member of WEE1 family. The aim of the current study was to elucidate the effects and mechanism of the interaction between MCRS1 and Pkmyt1 in gastric cancer cells.

Initially, results of this study demonstrated that MCRS1 was highly expressed in SGC-7901 cells and its expression was decreased in BGC-823 cells. We successfully constructed the pcDNA3.1-MCRS1 over-expressing plasmid and MCRS1 siRNA construct and transfected them into cells, separately. Through MTT and colony formation assays, we found that MCRS1 inhibited GC cell proliferation. It has been reported that increased expression of MCRS1 promotes cell proliferation of BGC-823 and MGC-803 cells [6]. This difference in expression may be due to the fact that MCRS1 has different substrates and complex interactions in different cells, different genotypes and specific environments. But it is certain that MCRS1 is an important regulator in the development of tumorigenesis. By performing scratch tests and transwell assays we revealed that MCRS1 inhibited the invasion and migration of GC cells. A recent study showed that MCRS1 inhibits lymphatic metastasis in GC patients [14]. This finding was strongly consistent with the observations from our study.

EMT was first observed during embryonic development. During tumor development, EMT causes tumor cells to lose some of the characteristics of epithelial cells and obtain some characteristics of stromal cells, so that tumor cells gain a stronger ability to invade and migrate [21]. EMT is the first and most important step in the invasion and metastasis of malignant tumors [22]. The process of EMT is regulated by a series of transcription factors [23–26]. The results of this study also demonstrated that MCRS1 up-regulated the protein expression levels of E-cadherin and ZO-1 while it down-regulated the protein levels of N-cadherin, Snail1, Snail2 and Twist, thereby subsequently inhibiting EMT.

Pkmyt1, a member of the WEE1 family, acts as a cell cycle-regulating kinase [27,28]. It is known to inhibit precocious entry of cells into mitosis by phosphorylating CDK1 at Thr14 and Tyr15 residues. Pkmyt1 promoted the growth and motility of hepatocellular carcinoma cells [16] and played an essential oncogenic role in colorectal cancer [17]. In this study, when MCRS1 was overexpressed, the protein expression of Pkmyt1 decreased, and vice versa. MCRS1 interacts with Pkmyt1 in SGC-7901 cells and both proteins localized in the cytoplasm of these cells. The operation of cell cycle is an extremely orderly process, which is closely related to oncogene, tumor suppressor gene, growth factor, cell proliferation and differentiation. Cyclin is a kind of protein that appears and disappears with cell cycle, its regulation of cell



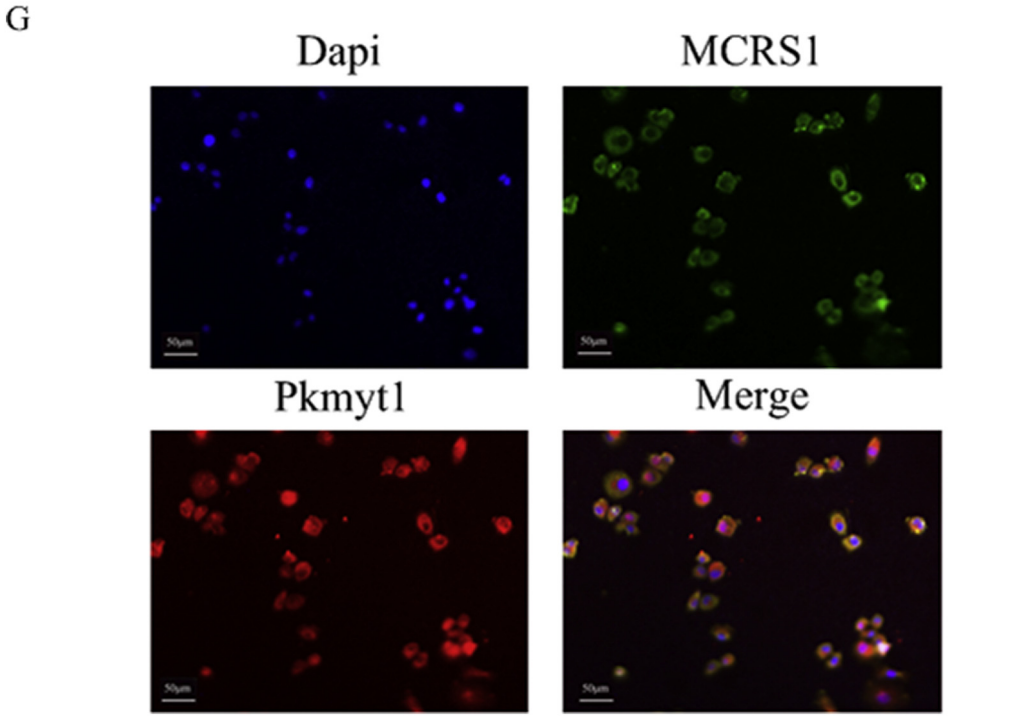


Fig. 6. MCRS1 interacted with PKMYT1 in GC cells. A, the protein expression of Pkmyt1 after overexpression MCRS1 in GC cells were examined using western blotting; B, the protein expression analysis of corresponding A; C, the protein expression of Pkmyt1 after down-regulation MCRS1 in GC cells were examined using western blotting; D, the protein expression analysis of corresponding C; E, the endogenous interaction between MCRS1 and Pkmyt1 in SGC-7901 cells was examined using immunoprecipitation assay; F, the protein expression analysis of corresponding E; G, the colocalization of MCRS1 and Pkmyt1 in the cytoplasm was examined using immunofluorescence($N = 25$, scare bar = 50 μm); *, $p < 0.05$ vs. the blank group; ▲, $p < 0.05$ vs. the siRNA group; the measurement data in figures were presented as sample mean ± standard deviation and analyzed by one-way analysis of variance; the experiment was repeated at least 3 times.

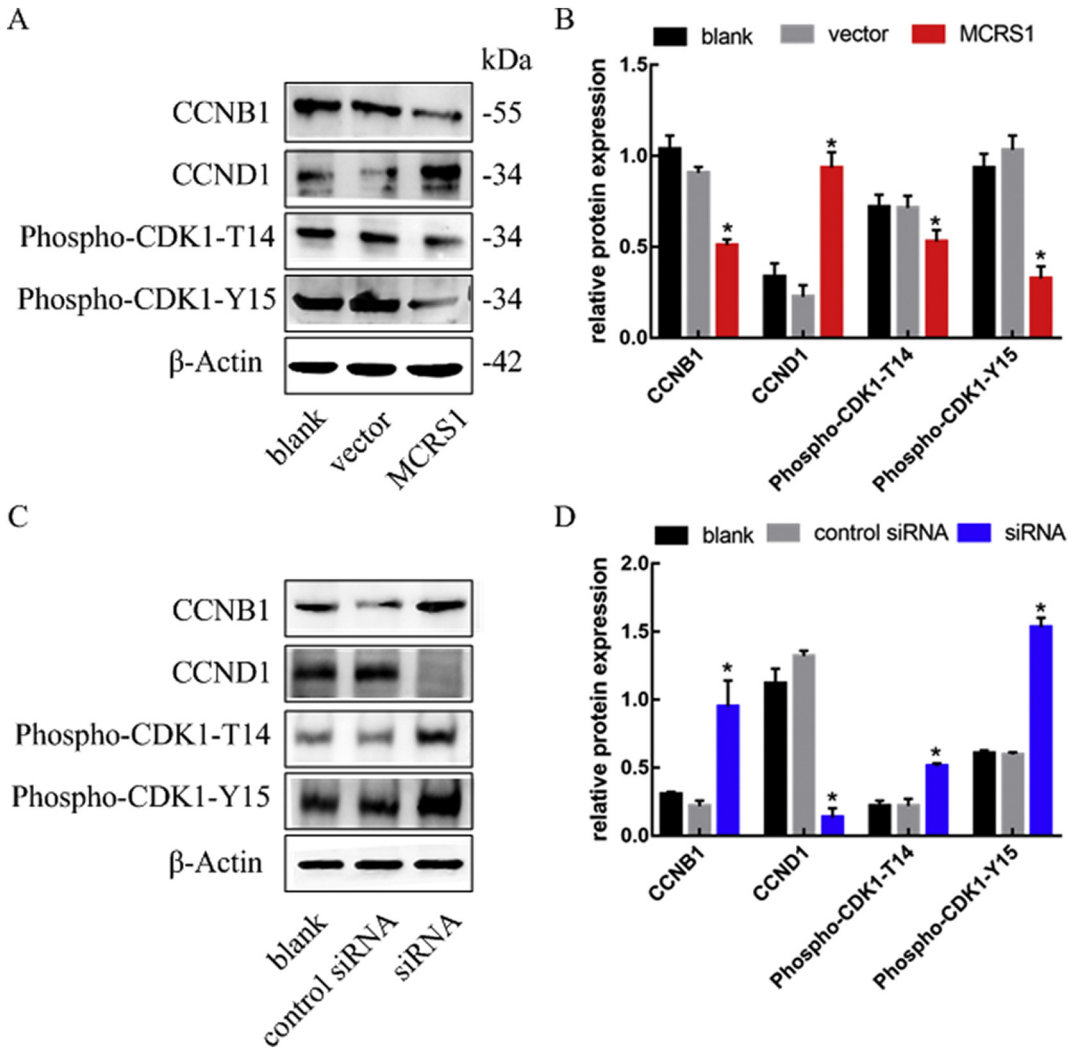


Fig. 7. The effects of MCRS1 on the cell cycle related proteins of GC cells. A, the effects of overexpression of MCRS1 on the expression of CCNB1, CCND1, T14 and Y15 protein in GC cells were examined using western blotting; B, the protein expression analysis of corresponding A; C, the effects of down-regulation of MCRS1 on the expression of CCNB1, CCND1, T14 and Y15 protein in GC cells were examined using western blotting; D, the protein expression analysis of corresponding C; *, $p < 0.05$ vs. the blank group; ▲, $p < 0.05$ vs. the siRNA group; the measurement data in figures were presented as sample mean ± standard deviation and analyzed by one-way analysis of variance; the experiment was repeated at least 3 times.

cycle is mainly in G1 phase and S/M phase. CCND1 is a protein that regulates cell cycle progression from G1 phase to S phase, its overexpression can make the cells pass through the G1/S checkpoint quickly and shorten the S phase, which is a potential target for tumor therapy [29]. CCNB1 is a protein that regulates cell cycle progression from G2 to M. When the cells exit M phase, cyclinB degrades and CDK1 kinase activity is inactivated, which can promote chromosome agglutination, nuclear membrane nucleolus remodeling, and guide cells to the next cell cycle [30]. Overexpression of MCRS1 increased the protein level of CCND1 and decreased the protein level of CCNB1 and phosphorylation status of CDK1-Thr14 and Tyr15. Down-regulation of MCRS1 inhibited the protein level of CCND1 and promoted the protein expression of CCNB1 and phosphorylation status of CDK1-Thr14 and Tyr15. Furthermore, when MK1775 was added, the phenotypic recovery effect was observed. The above results indicated that MCRS1 interacted with Pkmyt1 and was involved in the regulation of cell cycle in the GC.

5. Conclusions

Taken together, our study demonstrates that MCRS1 could inhibit the invasion, migration and proliferation of GC cells, via interacting with Pkmyt1 and regulating cell cycle progression. These data provide a better understanding of the molecular mechanisms by which MCRS1 is involved in the development and progression of GC.

Conflict of interest

The authors have declared that there are no conflicts of interest in this work.

Authors' contributions

C. Liu and J.Y. Xiao supervised the experiment. L.L. Ren guided the immunofluorescence co-localization experiment. Y.M. Liu was responsible for the early yeast two-hybrid experiment. T.S. Wang helped to complete the analysis and drawing of experimental data. X.M. Wang completed all the remaining experiments and wrote the manuscript alone. Q.Y. Li, T.C. Mu and S. Fu were members of our research group and participated in the study of the WEE1 family. All authors approved the final manuscript.

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