International Journal of Biochemistry and Cell Biology 111 (2019) 27–36

Contents lists available at ScienceDirect

International Journal of Biochemistry and Cell Biology

journal homepage: www.elsevier.com/locate/biocel

miR-182 contributes to cell proliferation, invasion and tumor growth in colorectal cancer by targeting DAB2IP

Xiaoli Li[a], Xudong Zhang[b], Qiuge Zhang[a], Rui Lin[c,*]

[a] Department of Gerontology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, China
[b] Department of Oncology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, China
[c] Department of Gastroenterology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, China

ARTICLE INFO

Keywords:
miR-182
DAB2IP
CRC
Proliferation
Invasion
Tumor growth

ABSTRACT

miR-182 was revealed to be upregulated in colorectal cancer (CRC) and contributed to CRC development. However, the detailed molecular mechanism of miR-182 in the progression of CRC remains largely elusive. Herein, miR-182 was upregulated in CRC serum samples, CRC tissues and cells. miR-182 expression was evidently reduced in postoperative serum samples, compared with preoperative serum samples, whereas miR-182 expression was re-elevated in serum samples from CRC patients who developed postoperative recurrence. Exogenous miR-182 promoted the proliferation, colony formation, increased ki67 level and facilitated the invasion capability of CRC cells by enhancing the expressions of MMP-2 and MMP-9, while inhibition of miR-182 showed the opposite effects. Additionally, miR-182 was demonstrated to target DAB2IP and suppress its expression in CRC cells. Downregulation of miR-182 inhibited CRC tumor growth *in vivo* by upregulating DAB2IP. Moreover, restoration of DAB2IP attenuated miR-182-mediated activation of the PI3K/Akt/mTOR and Wnt/β-catenin pathways in CRC cells. Taken together, our findings showed that miR-182 exerted its oncogenic role in CRC by targeting DAB2IP, which may be involved in activating the PI3K/Akt/mTOR and Wnt/β-catenin pathways, shedding a novel light on the molecular mechanism of CRC tumorigenesis.

1. Introduction

Colorectal cancer (CRC) remains one of the most life-threatening cancers worldwide, particularly when it reaches the advanced stage (Brenner et al., 2014). The previous results of a survey have shown that new 93,090 patients are diagnosed with CRC and 49,700 patients die of the disease in the United states in 2015 (Siegel et al., 2015). Nowadays, although great improvements in diagnose and therapeutic strategies for CRC have been made, the prognosis for patients with advanced CRC remains dismal, which is mainly attributed to distant metastases or recurrence (Haggar and Boushey, 2009). Substantive studies have suggested that alterations in many oncogenes and tumor suppressor genes as well as deregulated signaling pathways contribute to the tumorgenesis and progression of CRC (Ogino et al., 2011). However, the molecular and genetic bases of colorectal carcinogenesis still remains poorly understood. Hence, there is a need to better explore the molecular mechanism of CRC metastasis.

microRNAs (miRNAs) are a class of small non-coding RNA molecules with length of 19–24 nucleotides and participate in down-regulating expression of target genes (Bartel, 2009).They are emerging as important regulators of broad biological processes, such as proliferation, apoptosis, invasion, migration and tumorigenesis (Mendell, 2005). More recently, literature has emerged that offers that miRNAs are capable of serving as either oncogenes or tumor suppressor genes, which is depending on the specific-tumor type or their target mRNAs (Croce, 2009). It has been proposed that aberrant miRNA expression undoubtedly contributed to the tumorigenesis and cancer progression in various tumors including CRC (Iorio and Croce, 2009; Xuan et al., 2015). miR-182, located on human chromosome 7q32.2, is transcribed from the cluster of the miR-183 family and highly expressed in the retina during embryo development (Xu et al., 2007). Recent reported research reveals that increased expression of miR-182 is observed in CRC, which is related to the poor prognosis of patients with CRC (Liu et al., 2013; Wang et al., 2014). Moreover, upregulated miR-182 has been revealed to contribute to CRC development by serving as an oncogene (Yang et al., 2014; Zhang et al., 2015).

DOC-2/DAB2 interactive protein (DAB2IP), also known as aspartokinase (ASK1)-interacting protein-1 (AIP1), a recently identified novel member of the Ras GTPase-activating protein (GAP) family, has been associated with cell growth inhibition and apoptosis (Wang et al., 2002;

* Corresponding author at: No. 1 Jianshe East Road, Zhengzhou, 450052, Henan, China.
E-mail address: zdyfylr@163.com (R. Lin).

https://doi.org/10.1016/j.biocel.2019.04.002
Received 7 January 2019; Received in revised form 4 April 2019; Accepted 7 April 2019
Available online 08 April 2019

Xie et al., 2009). Recent studies have reported that DAB2IP is frequently downregulated by altered epigenetic modification of its promoter and acts as a bona fide tumor suppressor in several types of tumor (Chen et al., 2003; Dote et al., 2004; Yano et al., 2005). Notably, DAB2IP has been reported to be downregulated in CRC tissues and suppressed proliferation, epithelial-mesenchymal transition (EMT), invasion and metastasis in CRC (Wang et al., 2015). Given the significance of both miR-182 and DAB2IP in the processes linked to CRC, it is necessary to illustrate the explicit modulatory relationship between miR-182 and DAB2IP in CRC.

In the present paper, we demonstrated that miR-182 was upregulated in serum samples from CRC patients, as well as CRC tissues and cells. In addition, we found that downregulation of miR-182 impeded cell proliferation and invasion in CRC cells *in vitro* and suppressed CRC tumor growth. Notably, DAB2IP was confirmed as a target of miR-182 and miR-182 suppressed DAB2IP expression. Moreover, restoration of DAB2IP attenuated miR-182-mediated activation of the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) and Wnt pathways. Therefore, it is possible that miR-182 contributed to CRC tumorigenesis by targeting DAB2IP through partly activating the PI3K/Akt/mTOR and Wnt/β-catenin pathways.

2. Materials and methods

2.1. Patients and samples

A total of 57 blood samples from consecutive CRC patients, including 25 preoperative CRC patients, 25 postoperative CRC patients and 7 CRC patients who developed postoperative recurrence of solitary liver metastasis during follow-up, were collected within 1 week of resection in the Department of Oncology Surgery of the First Affiliated Hospital of Zhengzhou University. Blood samples from 28 healthy individuals with no history of malignant disease were collected at the Medical Center of the First Affiliated Hospital of Zhengzhou University. The CRC patients were histopathologically diagnosed. Briefly, the blood sample processing was as followed: 1) Let the blood samples rest for about 30 min; 2) serum separation was performed by centrifugation at 900 g for 10 min; 3) The supernatant serum was collected and stored at −80 °C for further detection of miR-182 expression.

A total of 25 CRC tissue samples and paired adjacent normal tissues were collected from patients who underwent surgical operations at the First Affiliated Hospital of Zhengzhou University from August 2015 to July 2016. All tissue samples were snap frozen in liquid nitrogen immediately after surgical removal and stored at −80 °C for further analysis. This study was approved by the Research Ethics Committee of the First Affiliated Hospital of Zhengzhou University and written informed consent was obtained from all participants before surgery.

Clinical and pathologic data were extracted from the medical records and the detailed patient information is summarized in supplementary Table 1.

2.2. Cell culture and transfection

The human CRC cell lines and the normal colon epithelium cell line FHC were gained from the American Type Culture Collection (ATCC, Manassas, VA, USA). They were cultured in RPMI 1640 medium encompassing 10% fetal bovine serum (FBS), and 100 IU/mL penicillin (HyClone, Logan, Utah, USA), and 100 μg/mL streptomycin (HyClone) at 37 °C in a 5% CO_2 atmosphere.

miR-182 mimics (miR-182), miRNA scrambled control (miR-NC), miR-182 inhibitor (anti-miR-182), inhibitor control (anti-miR-NC) were purchased from Genepharma Co., Ltd. (Shanghai, China). SW480 and Caco-2 cells were transfected with the abovementioned oligonucleotides by Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA).

2.3. Construction of plasmids and adenovirus preparation

The construction of recombinant adenoviral vectors expressing DAB2IP (Ad-DAB2IP) and its control vector (Ad) were performed as previously detailed (Li et al., 2005). Briefly, human DAB2IP cDNA were amplified by PCR and subcloned into pAdTrack-CMV vector, which contained cytomegalovirus promoters and a green fluorescent protein (GFP) gene. Subsequently, the recombinants were selected for kanamycin resistance and confirmed by restriction endonuclease analysis. Recombinant adenoviral vectors Ad-DAB2IP were produced by transfecting with linearized recombinant plasmids into 293 T cells. The control recombinant adenovirus Ad-GFP (Ad) was used as the control. Adenoviruses were injected into SW480 cells at a multiplicity of infection (MOI) of 40.

2.4. Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from serum, tissues and cultured cells using Trizol reagent (Invitrogen). For miRNA expression, 1 μg of total RNA was applied to produce cDNA using a One Step PrimeScript miRNA cDNA Synthesis kit (Takara Bio, Inc., Otsu, Japan). Mature miR-182 levels were analyzed by TaqMan miRNA Assay (Applied Biosystems, Foster City, CA, USA) on the ABI 7500 Fast Real-Time PCR system (Applied Biosystems). For mRNA expression, 1 μg of total RNA was applied to generate cDNA by a Reverse Transcription Kit (Takara Bio, Inc.) and DAB2IP mRNA expression was quantified by SYBR® Premix Ex Taq™ (Takara Bio, Inc.). U6 small RNA (snRNA) and GAPDH were used as the internal reference for miR-182 and DAB2IP mRNA, respectively.

2.5. MTT assay

MTT assay was applied to detect the capability of cell proliferation. Cells were plated at a density of 2×10^3 cells/well in 96-well plates. Following incubation for 0, 24, 48, or 72 h, 10 μL of dimethyl sulphoxide (MTT, 5 mg/mL; Sigma) was added to each well and the cells were incubated for another 4 h at 37 °C. After the cultural supernatant was discarded, 100 μL of DMSO was added to melt the formazan. The optical density at 490 nm was determined by a microplate reader (Molecular Devices, Sunnyvale, CA, USA).

2.6. Colony formation assay

At 24 h post-transfection, the collected SW480 and Caco-2 cells were resuspended in medium. Then the cells were seeded into 6-well plates at a density of 200 cells/well and cultured for 14 days with growth media being replaced every 3 days. The colonies were fixed with 4% formaldehyde and stained with 0.1% crystal violet for 30 min. The number of colonies containing more than 50 cells in each well was counted.

2.7. Transwell invasion assay

Cell invasion ability was assessed using a 24-well Transwell chamber (8 μm; Corning Inc., Corning, NY, USA) with a Matrigel-coated membrane. At 24 h post-transfection, 200 μL of SW480 and Caco-2 cell suspensions (2×10^5 cells/mL) were seeded into the upper chamber while the complete medium containing 10% FBS serving as the chemoattractant in the lower chamber. After incubation for 24 h, non-invading cells on the upper chamber were scraped off with a cotton swab and the invading cells attached to the lower chamber were fixed with methanol and stained with crystal violet. The invaded cells were counted in five random fields using Zeiss Axiophot Microscope (Carl Zeiss, Oberkochen, Germany).

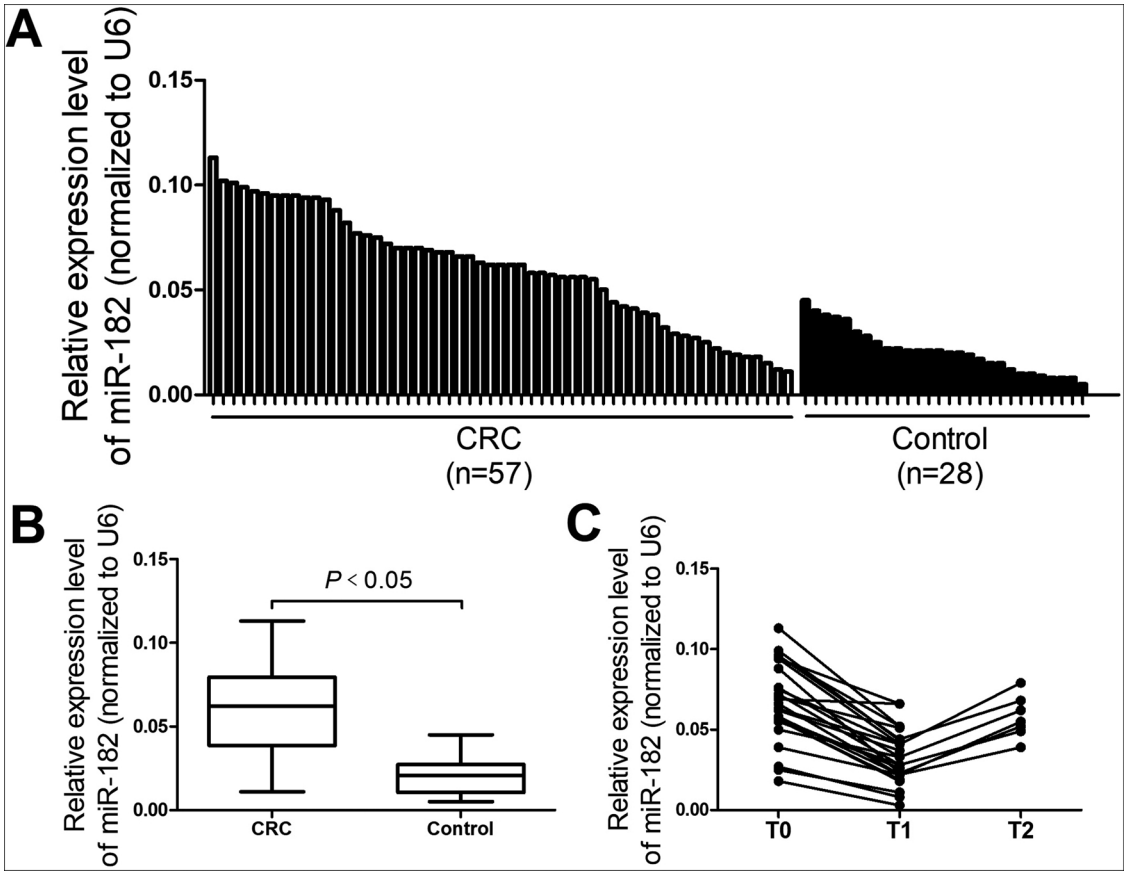

Fig. 1. The expression of miR-182 in serum samples from CRC patients. (A and B) The serum expression of miR-182 in 57 CRC patients and 28 healthy controls by qRT-PCR. (C) qRT-PCR analysis of the serum expression of miR-182 in preoperative serum samples (T0; n = 25), postoperative serum samples (T1; n = 25) and serum samples of postoperative recurrence (T2; n = 7). $P < 0.05$ *vs* control group.

2.8. Western blot analysis

Proteins were prepared from tissues and cells using RIPA lysis buffer (Sigma) with complete protease inhibitor Cocktail (Promega, Madison, WI, USA). The concentration of proteins was detected with the bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). 30 μg of protein samples were separated in 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a nitrocellulose membrane (Millipore, Bedford, MA, USA). The membranes were incubated with the following primary antibodies against ki67 (1:1000; Abcam, Cambridge, MA, USA), matrix metalloproteinase (MMP)-2 (1:2000; Abcam), MMP-9 (1:2000; Abcam), DAB2IP (1:2000; Abcam), PI3K (1:1000; Cell Signaling Technology, Beverly, MA, USA), p-Akt (Ser473) (1:2000; Cell Signaling Technology), mTOR (1:2000; Cell Signaling Technology), β-catenin (1:2000; Cell Signaling Technology) or β-actin (1:1000; Cell Signaling Technology) overnight at 4 °C, followed by incubation with the horseradish peroxidase (HRP)-conjugated secondary antibody anti-rabbit IgG (1 : 500; Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 h at room temperature. The protein signals were determined using the ECL detection kit (Pierce Biotechnology, Rockford, IL, USA).

2.9. Dual luciferase reporter assay

Fragments of wild-type (WT) or mutant type (MUT) DAB2IP 3′UTR containing the miR-182 binding sites were cloned into a pmirGLO control vector (Promega). To verify the binding specificity, SW480 and Caco-2 cells (1×10^5 cell/well) were seeded into 96-well plates and co-transfected with 20 nM miR-182 or miR-NC and 500 ng of 3′UTR DAB2IP-WT or 3′UTR DAB2IP-MUT. At 48 h post-transfection, the luciferase activity was examined.

2.10. Animal experiments

Male athymic BALB/C nude mice (4-to-6-week old, weighed 24–30 g) were purchased from Shanghai Laboratory Animal Center (Shanghai, China) and maintained in specific pathogen-free conditions. SW480 cells (1×10^6) stably transfected with Lv-anti-miR-182 or Lv-anti-miR-NC were inoculated subcutaneously into the armpit region of nude mice and constructed the xenograft models. The xenograft sizes were determined by vernier scale with a two day interval and xenograft volume was calculated using the formula: Volume = 0.5 × length × width^2. After 21 days of injection, the mice were killed and xenograft tumor tissues were isolated intactly, photographed and weighed. Subsequently, the excised tumor tissues were fixed in 10% formalin, paraffin embedded and cut into sections.

2.11. Immunohistochemistry (IHC) assay

The paraffin-embedded sections were used for examination of DAB2IP and ki67 expression. For IHC assay, sections of 3–4 μm were deparaffinized in xylene for 10 min, rehydrated in graded ethanol, and boiled in antigen retrieval solution for 3 min, followed by incubation with 3% hydrogen peroxide (H_2O_2) to block endogenous peroxidase for 10 min. After blocked with goat serum (Zymed, San Francisco, CA, USA), the sections were treated with primary antibodies against ki67 (1:1000) or DAB2IP (1:2000) overnight at 4 °C, followed by IgG (1 : 500) for 1 h at room temperature. After washing with water, sections were incubated with diaminobenzidine (DAB; Sigma), counterstained with hematoxylin (Richard Allan Scientific, Kalamazoo, MI, USA) and

examined and photographed with a fluorescence microscope (Olympus BX51, Shinjuku, Japan).

2.12. Statistical analysis

All data were analyzed by means of GraphPad Prism software ver. 5.0 (Inc., La Jolla, CA, USA). The differences between two groups were compared using two-tailed Student's *t*-test. The statistical analyses of more than two groups were conducted by one-way ANOVAs with multiple comparisons. All data were expressed as the mean ± standard deviation (SD). $P < 0.05$ was considered statistically significant difference.

3. Results

3.1. miR-182 was upregulated in CRC serum samples

We initially evaluated the level of miR-182 in serum of 57 patients with CRC and 28 healthy subjects by qRT-PCR. The results showed an aberrant upregulation of miR-182 in serum samples from CRC patients compared to that from healthy controls (Fig. 1A and B). Of note, miR-182 expression was evidently reduced in 25 postoperative serum samples (T1) when compared with that in 25 preoperative serum samples (T0), but miR-182 expression were found to be re-elevated in 7 serum samples from CRC patients who developed postoperative recurrence (T2) (Fig. 1C). These above results suggested that miR-182 expression might be closely associated with the development of CRC.

3.2. miR-182 was highly expressed in CRC tissues and cells

Next, we further validated the expression of miR-182 in 25 paired CRC tissues and the corresponding adjacent normal tissues. As shown in Fig. 2A, there was a marked rise in miR-182 expression in CRC tissues compared with adjacent normal tissues. Moreover, compared with the normal colon epithelium cell line FHC, expression of miR-182 was strikingly higher in CRC cells (SW480, LoVo, HCT116, Caco-2 and SW620) than (Fig. 2B). These data confirmed miR-182 was significantly upregulated in CRC tissues and cells.

3.3. Downregulation of miR-182 suppressed CRC cell growth

To investigate the functional effects of miR-182 on development of CRC, gain-of-function and loss-of-function assays were employed by transfecting SW480 cells with miR-182 and introducing Caco-2 cells with anti-miR-182. qRT-PCR results demonstrated that miR-182 mimic effectively increased miR-182 expression in SW480 cells and anti-miR-182 notably decreased miR-182 expression in Caco-2 cells (Fig. 3A). MTT assay manifested that the proliferation was remarkably promoted by exogenous miR-182 in SW480 cells, but distinctly hindered by miR-182 inhibitor (anti-miR-182) in Caco-2 cells (Fig. 3B). Consistent with MTT assay, colony formation assay showed that miR-182 overexpression dramatically increased the number of colonies in SW480 cells compared with miR-NC group. Conversely, downregulation of miR-182 triggered a substantial decline of the number of colonies in Caco-2 cells with respect to anti-miR-NC group (Fig. 3C). Moreover, results from western blot analysis implicated that the protein level of ki67, a biomarker of proliferation, was strikingly elevated after transfection with miR-182 in SW480 cells. On the contrary, anti-miR-182 conspicuously diminished ki67 level in Caco-2 cells relative to anti-miR-NC group (Fig. 3D). Collectively, these findings suggested that downregulation of miR-182 may function as a negative regulator on CRC cell growth.

3.4. Downregulation of miR-182 repressed the invasion capability of CRC cells by suppressing MMP-2 and MMP-9 expression

As shown in Fig. 4A and C, ectopic expression of miR-182 caused a remarkable promotion of the invasion ability of SW480 cells, while inhibition of miR-182 resulted in an obvious repression of the invasion ability of Caco-2 cells. Numerous studies have demonstrated that MMP-2 and MMP-9 are extracellular membrane-degrading enzymes associated with tumor invasiveness (Dong et al., 2013). Herein, western blot analysis demonstrated that miR-182-transfected SW480 cells exhibited a significant increase of the protein levels of MMP-2 and MMP-9, while anti-miR-182 elicited the opposite effects in Caco-2 cells (Fig. 4B and D). Taken together, these results proved that inhibition of miR-182 impeded invasion of CRC cells by suppressing MMP-2 and MMP-9 expression.

3.5. DAB2IP was a target of miR-182 in CRC cells

Prediction programs (Targetscan, MIRDB, and PicTar) were applied to predict the probable target genes of miR-182. Results of analysis hinted that 3′UTR of DAB2IP encompassed the complementary binding sites for miR-182, as shown in Fig. 5A. Moreover, spearman correlation analysis indicated a negative correlation between miR-182 and DAB2IP expression in CRC tissues (Fig. 5B). The results from luciferase reporter assay demonstrated that luciferase activity of 3′UTR DAB2IP-WT was considerably reduced after miR-182 was overexpressed in SW480 and Caco-2 cells, while the reporter containing the mutant binding sites was unaffected in the luciferase activity (Fig. 5C), confirming the authentic binding between miR-182 and DAB2IP. Furthermore, western blot analyses revealed that exogenous miR-182 successfully repressed the protein level of DAB2IP in SW480 cells (Fig. 5D), while decrement of

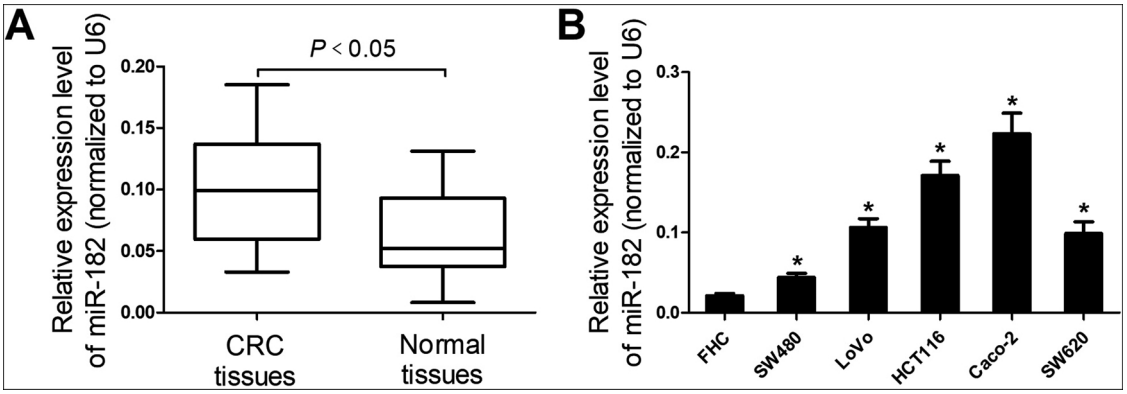

Fig. 2. miR-182 expression in CRC tissues and cells. (A) qRT-PCR analysis of miR-182 expression in 25 paired CRC tissues and the corresponding adjacent normal tissues. (B) qRT-PCR analysis of miR-182 expression in the normal colon epithelium cell line FHC and CRC cells (SW480, LoVo, HCT116, Caco-2 and SW620). *$P <$ 0.05 *vs* control group.

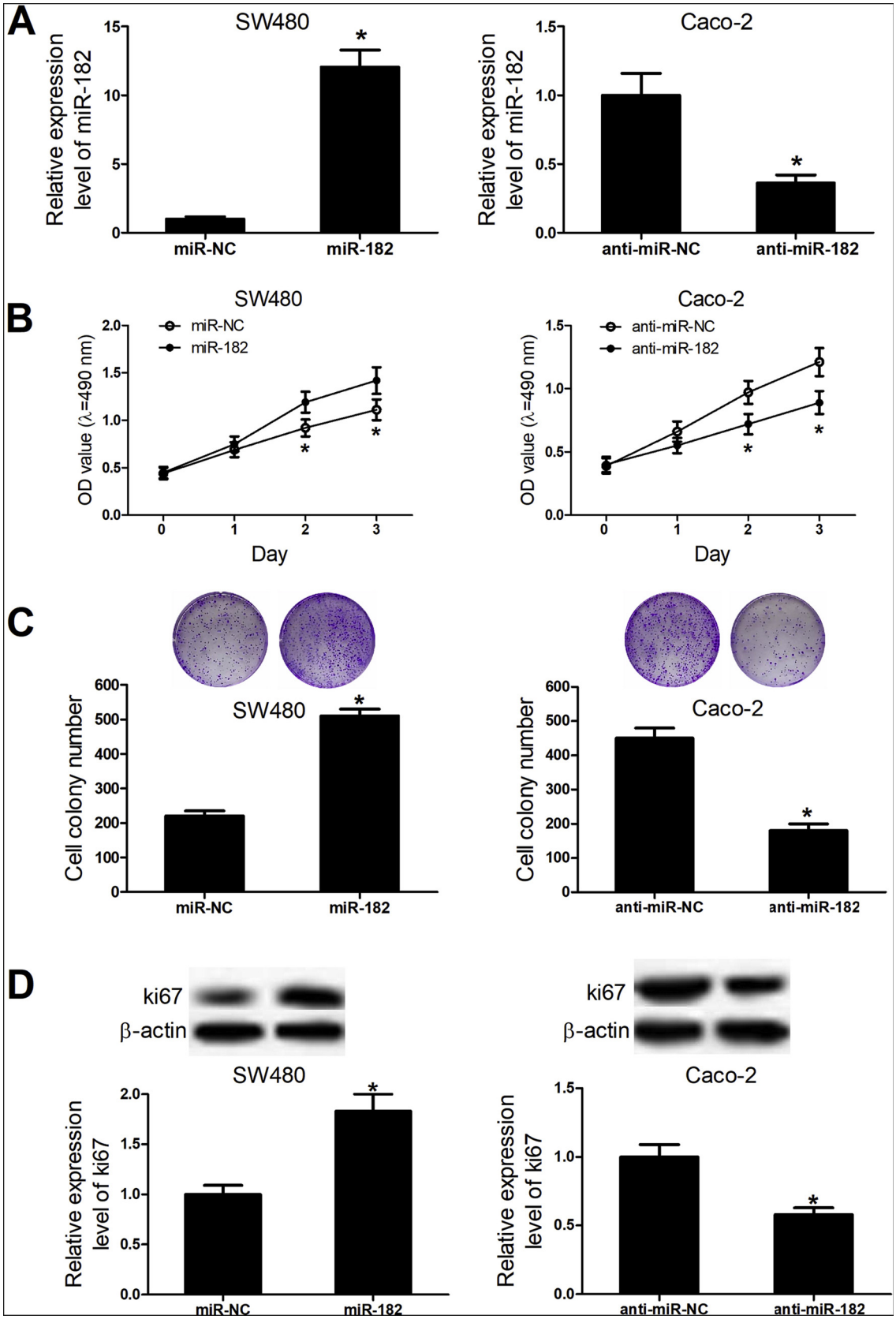

Fig. 3. The effects of miR-182 on CRC cell growth. SW480 cells were transfected with miR-182 or miR-NC and Caco-2 cells were introduced with anti-miR-182 or anti-miR-NC, and subjected to the subsequent assessments at 48 h post-transfection. (A) qRT-PCR analysis was performed to measure miR-182 expression in the treated SW480 and Caco-2 cells. (B) MTT assay was performed to determine cell proliferation at 0, 24, 48, and 72 h in the transfected SW480 and Caco-2 cells. (C) The colony formation was evaluated by colony formation assay in the transfected SW480 and Caco-2 cells. (D) The protein level of ki67 was determined in the transfected SW480 and Caco-2 cells by western blot. *$P < 0.05$ *vs* control group.

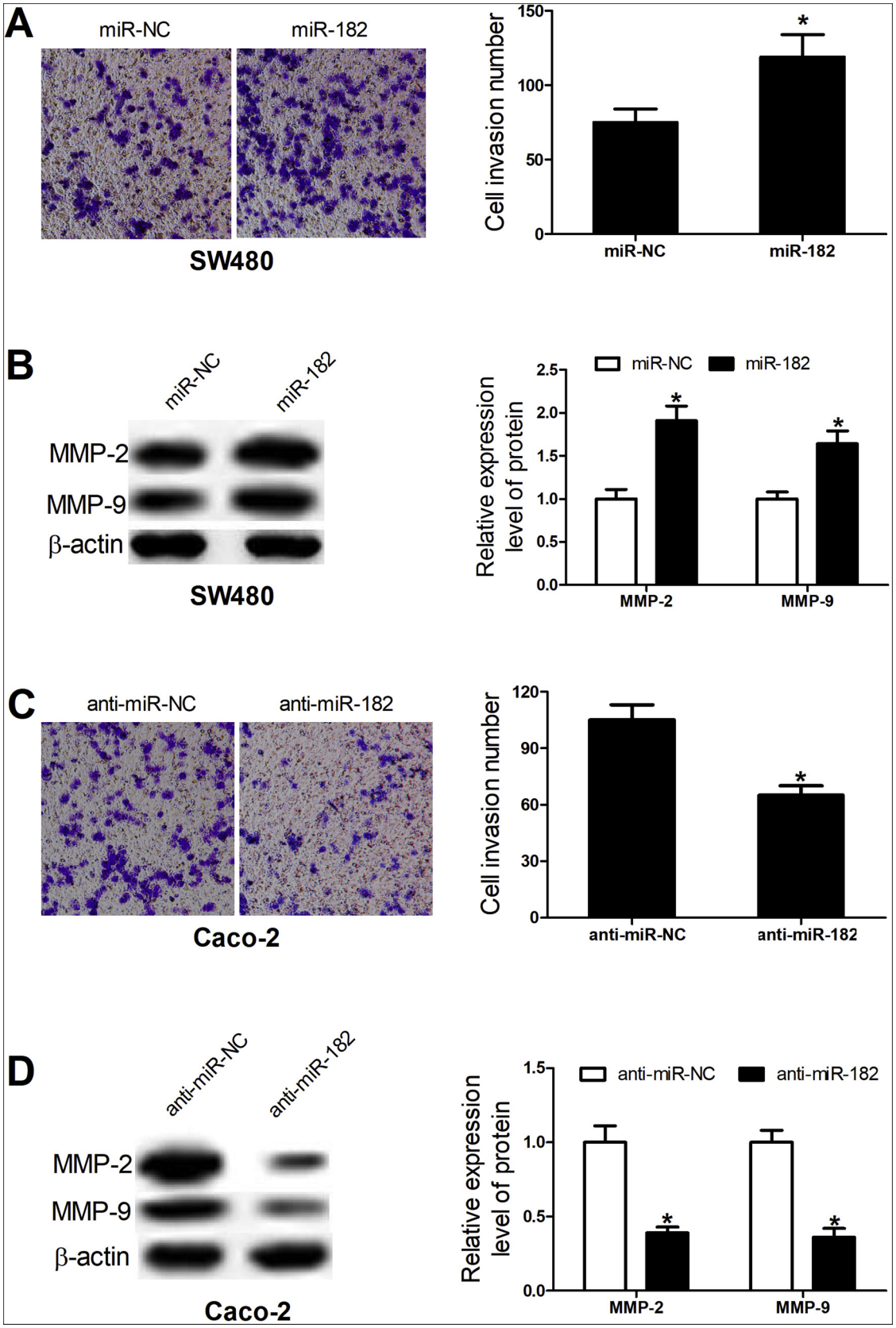

Fig. 4. The effects of miR-182 on CRC cell invasion. SW480 cells were transfected with miR-182 or miR-NC and Caco-2 cells were introduced with anti-miR-182 or anti-miR-NC, and subjected to the next analyses at 48 h post-transfection. (A and C) Transwell assay was applied to investigate the invasion ability of the transfected SW480 and Caco-2 cells. (B and D) Western blot was conducted to test the protein levels of MMP-2 and MMP-9 in the introduced SW480 and Caco-2 cells. *$P < 0.05$ *vs* control group.

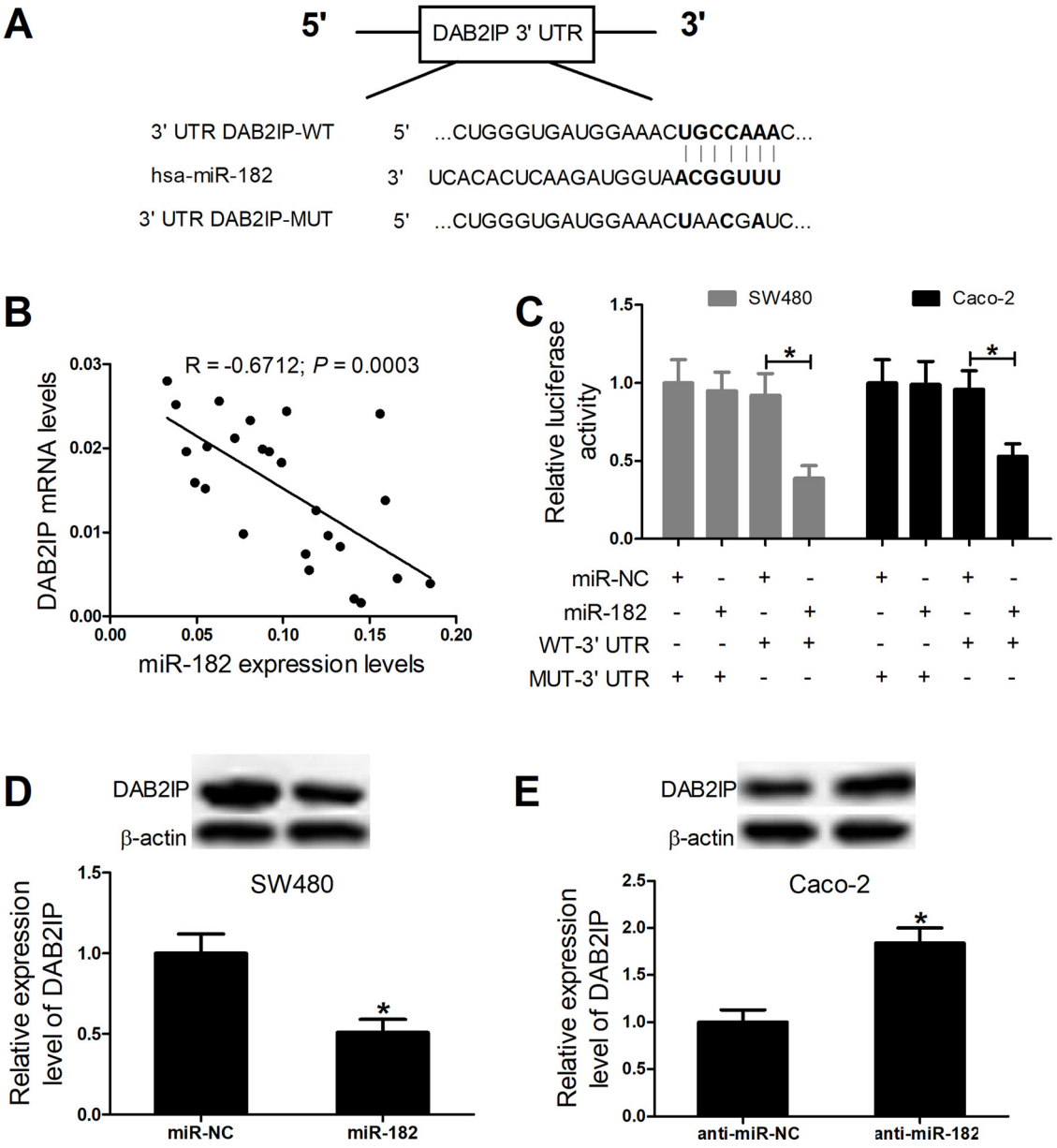

Fig. 5. The interaction between miR-182 and DAB2IP in CRC cells. (A) The predicted miR-182 binding sites in the 3′UTR of DAB2IP and its mutant containing altered nucleotides in the 3′UTR. (B) The correlation between miR-182 and DAB2IP expression in CRC tissues. (C) Luciferase activity was detected by luciferase reporter assays in SW480 and Caco-2 cells after cotransfection with miR-182 or mR-NC and 3′UTR DAB2IP-WT or 3′UTR DAB2IP-MUT. (D) Western blot analysis was carried out to detect DAB2IP level in SW480 cells transfected with miR-182 or miR-NC and Caco-2 cells introduced with anti-miR-182 or anti-miR-NC. *$P < 0.05$ *vs* control group.

miR-182 greatly enhanced DAB2IP level in Caco-2 cells (Fig. 5E). Collectively, these data demonstrated the specificity of miR-182 targeting DAB2IP mRNA.

3.6. Downregulation of miR-182 inhibited CRC tumor growth by upregulating DAB2IP in vivo

To explore the effect of miR-182 on tumorigenesis of CRC *in vivo*, CRC mouse xenograft models were established by injecting SW480 cells with Lv-anti-miR-182 or Lv-anti-miR-NC into the nude mice. IHC analysis showed that ki67 level was obviously decreased and DAB2IP expression was distinctly elevated in the Lv-anti-miR-182 group versus Lv-anti-miR-NC group (Fig. 6A). Moreover, consistently with the *in vitro* findings, miR-182 inhibitor (anti-miR-NC) led a marked suppression of tumor growth when compared with control group (Fig. 6B). Also, the tumor size and weight in the anti-miR-182 group were evidently lower than those in control groups (Fig. 6C and 6D). In addition, western blot analysis manifested that the protein level of DAB2IP was dramatically enhanced in xenograft tumor tissues resulting from the Lv-anti-miR-182 SW480 cell injection compared with that from the Lv-anti-miR-NC SW480 cell injection (Fig. 6E and 6 F). These above results demonstrated that downregulation of miR-182 inhibited CRC tumor growth by targeting DAB2IP *in vivo*.

3.7. Restoration of DAB2IP attenuated miR-182-mediated activation of the PI3K/Akt/mTOR and Wnt/β-catenin pathways in CRC cells

The PI3K/Akt/mTOR and Wnt pathways have been demonstrated to be involved in CRC progression (Liu et al., 2015). Thus, we characterized the effects of miR-182 and DAB2IP on the PI3K/Akt/mTOR and

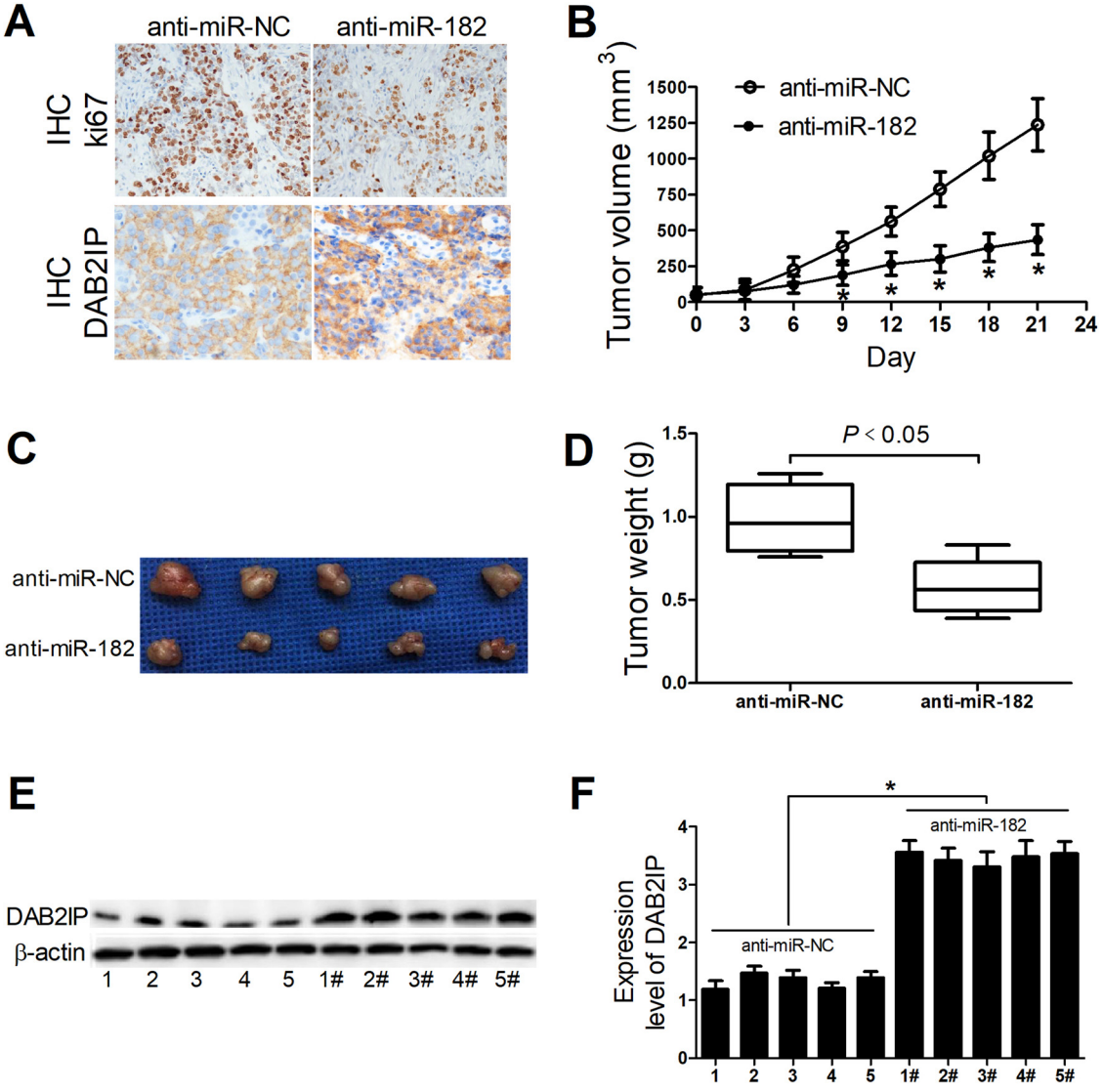

Fig. 6. Downregulation of miR-182 inhibited CRC tumor growth *in vivo* by upregulating DAB2IP. SW480 cells stably transfected with LV-anti-miR-182 or LV-anti-miR-NC were subcutaneously inoculated into the nude mice. (A) IHC analyses of ki67 and DAB2IP expressions in the resected tumor tissues. (original magnification, ×200) (B) After 3 days of injection, xenograft size was measured every 3 days. (C and D) After 21 days, the mice were sacrificed and tumors were photographed and weighted. (E and F) Western blot analysis was conducted to measure DAB2IP expression in the resected tumor tissues. *$P < 0.05$ *vs* control group.

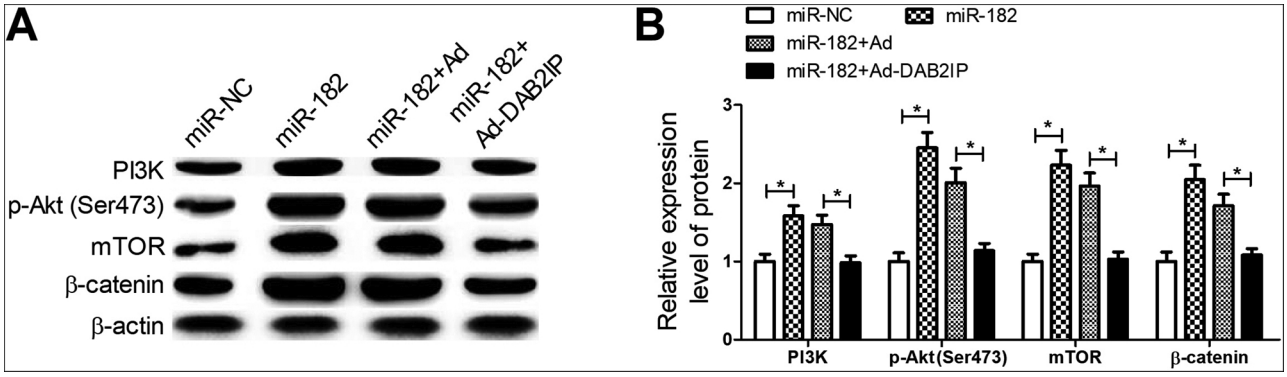

Fig. 7. miR-182/DAB2IP axis activated the PI3K/Akt/mTOR and Wnt pathways in CRC cells. (A and B) Western blot analysis was used to examine the expressions of PI3K, p-Akt (Ser473), mTOR, and β-catenin in SW480 cells transfected with miR-182, miR-NC, miR-182 + Ad, or miR-182 + Ad-DAB2IP. *$P < 0.05$ *vs* control group.

Wnt/β-catenin pathways. Western blot analysis further revealed that ectopic expression of miR-182 triggered a substantial elevation of PI3K, p-Akt (Ser473), mTOR, and β-catenin levels in SW480 cells compared to miR-NC group, which was considerably ameliorated by DAB2IP overexpression (Fig. 7A and B), suggesting that miR-182/DAB2IP axis activated the PI3K/Akt/mTOR and Wnt/β-catenin pathways in CRC cells.

4. Discussion

The application of miRNAs in diagnosis and treatment of cancer is attracting more and more attention due to their frequent dysregulation and involvement of the malignant phenotypes of various tumors, including CRC (Shibuya et al., 2010). Increasing evidence has exhibited that deregulation of miRNAs boosts the pathogenesis of cancers via different molecular mechanisms. And tremendous studies have expounded the controversial roles of dysregulated miR-182 in human cancers. For example, researchers have reported that miR-182 is upregulated and acts as an oncogene in most human cancers, such as prostate cancer (Hirata et al., 2013), where miR-182 knockdown hinders proliferation, migration and invasion of prostate cancer cells (Hirata et al., 2013). In melanoma, upregulated miR-182 promotes survival, invasion and migration by directly repressing microphthalmia-associated transcription factor-M and FOXO3 (Segura et al., 2009). Conversely, miR-182 exerts tumor suppressive effects on lung cancer, where miR-182 inhibits the proliferation and invasion through decreasing human cortical actin-associated protein expression (Zhang et al., 2011). In glioma, miRNA-182 inhibits cell proliferation and migration capacity through the induction of neuritin expression (Feng et al., 2015). In gastric cancer, miR-182 suppresses the proliferation and colony formation by targeting oncogenic ANUBL1 (Tang et al., 2015). As reported by the recent study of miR-182 (Liu et al., 2018), miR-182 is significantly upregulated in patients with early-stage CRC, which is consistent with our findings. Of note, we first found that miR-182 expression was reduced in postoperative serum samples, but was re-elevated in the serum samples from CRC patients who developed postoperative recurrence, suggesting that the elevated serum miR-182 expression was markedly correlated with tumor recurrence in CRC patients. Moreover, our results also confirmed the high expression of miR-182 in CRC tissues and cells, which was consistent with the previous studies (Liu et al., 2013; Wang et al., 2014). Functional analyses manifested that exogenous miR-182 boosted the proliferation and colony formation and the invasion of CRC cells by inhibiting the expressions of MMP-2 and MMP-9, while downregulation of miR-182 elicited the opposite effects, indicating that miR-182 functioned as an oncogene to contribute to CRC cell growth and invasion, in line with the previous studies (Yang et al., 2014; Zhang et al., 2015).

DAB2IP, a well-known tumor-suppressor, plays an important role in cancer cell growth and metastasis during tumor progression (Liu et al., 2016). For instance, downregulation of DAB2IP is an independent prognostic factor of disease recurrence and promoted the proliferation, migration and invasion of bladder cancer (Shen et al., 2014). Low expression of DAB2IP has been reported to be related to poor prognosis in hepatocellular carcinoma (HCC) patients and promote the proliferation and invasion in HCC (Zhang et al., 2012). Downregulation of DAB2IP also promotes EMT and metastasis of prostate cancer (Wang et al., 2016). More importantly, the inhibitory roles of DAB2IP in CRC progression have also been well-documented (Wang et al., 2015). Also, it has been previously reported that downregulation of DAB2IP promotes CRC invasion and metastasis by translocating heterogeneous nuclear ribonucleoprotein K (hnRNPK) into nucleus to enhance the transcription activity of MMP2 (Zhu et al., 2017). Our results showed that DAB2IP was negatively correlated with miR-182 expression in CRC patients. As expected, DAB2IP was identified as a functional target of miR-182 and suppressed by miR-182 in CRC cells. *In vivo* experiments demonstrated that inhibition of miR-182 suppressed CRC tumor growth *in vivo* by upregulating DAB2IP. These aforementioned results demonstrated that miR-182 contributed to cell growth and invasion via targeting DAB2IP.

Growing evidence has revealed that the PI3K/Akt/mTOR pathway controls malignant phenotypes including proliferation, invasion, angiogenesis and tumorgenesis in a wide range of tumors, including CRC (Engelman, 2009). Moreover, abnormally activating the Wnt/β-catenin pathway could stimulate the initiation and progression of CRC (Clevers and Nusse, 2012). The PI3K/Akt/mTOR and Wnt/β-catenin pathways have been identified as the potential targets for the prevention of CRC (Pandurangan, 2013). It has been proposed that DAB2IP acts as a signaling adaptor to negative modulate other oncogenic pathways, including PI3K/Akt and Wnt/β-catenin (Bellazzo et al., 2017). DAB2IP could suppress cell survival through inhibiting the PI3K/AKT signaling (Xie et al., 2009). The effects of miR-182 on CRC have been reported by (Jia et al., 2017) and they have found that miR-182 can promote tumorigenesis and invasiveness of CRC cells via targeting ST6GALNAC2 and PI3K/AKT pathway, which are similar to our study. However, it is well known that cancer is a complex disease and a miRNA has different target genes which function in cancer development. There are a number of important differences in target gene and its action pathway of miR-182 between our study and the previous one (Jia et al., 2017). Our study manifested that restoration of DAB2IP attenuated miR-182-mediated activation of the PI3K/Akt/mTOR and Wnt/β-catenin pathways, which suggested that miR-182 exerted its oncogenic role by targeting DAB2IP via partly activating the PI3K/Akt/mTOR and Wnt/β-catenin pathways.

In summary, our study demonstrated that miR-182 was upregulated in CRC and significantly correlated with tumor recurrence in CRC patients. Moreover, we provided the first evidence that miR-182 promoted the malignant progression in CRC cells by targeting DAB2IP, which might be involved in facilitating the activation of the PI3K/Akt/mTOR and Wnt/β-catenin pathways, contributing to better understanding the molecular mechanism of CRC tumorigenesis. Our study provided a theoretical basis for its application in the treatment of patients with CRC.

Conflict of interest

No potential conflicts of interest were disclosed.

Acknowledgments

No.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biocel.2019.04.002.

References

Bartel, D.P., 2009. MicroRNAs: target recognition and regulatory functions. Cell. 136 (2), 215.
Bellazzo, A., Di Minin, G., Collavin, L., 2017. Block one, unleash a hundred. Mechanisms of DAB2IP inactivation in cancer. 24 (1), 15.
Brenner, H., Kloor, M., Pox, C.P., 2014. Colorectal cancer. Lancet 383 (9927), 1490.
Chen, H., Toyooka, S., Gazdar, A.F., Hsieh, J.T., 2003. Epigenetic regulation of a novel tumor suppressor gene (hDAB2IP) in prostate cancer cell lines. J. Biol. Chem. 278 (5), 3121.
Clevers, H., Nusse, R., 2012. Wnt/beta-catenin signaling and disease. Cell. 149 (6), 1192.
Croce, C.M., 2009. Causes and consequences of microRNA dysregulation in cancer. Nat. Rev. Genet. 10 (10), 704.
Dong, Q.Z., Wang, Y., Tang, Z.P., Fu, L., Li, Q.C., Wang, E.D., et al., 2013. Derlin-1 is overexpressed in non-small cell lung cancer and promotes cancer cell invasion via EGFR-ERK-mediated up-regulation of MMP-2 and MMP-9. Am. J. Pathol. 182 (3), 954.
Dote, H., Toyooka, S., Tsukuda, K., Yano, M., Ouchida, M., Doihara, H., et al., 2004. Aberrant promoter methylation in human DAB2 interactive protein (hDAB2IP) gene in breast cancer. Clin. Cancer Res. 10 (6), 2082.
Engelman, J.A., 2009. Targeting PI3K signalling in cancer: opportunities, challenges and

limitations. Nat. Rev. Cancer 9 (8), 550.
Feng, Y.A., Liu, T.E., Wu, Y., 2015. microRNA-182 inhibits the proliferation and migration of glioma cells through the induction of neuritin expression. Oncol. Lett. 10 (2), 1197.
Haggar, F.A., Boushey, R.P., 2009. Colorectal cancer epidemiology: incidence, mortality, survival, and risk factors. Clin. Colon Rectal Surg. 22 (4), 191.
Hirata, H., Ueno, K., Shahryari, V., Deng, G., Tanaka, Y., Tabatabai, Z.L., et al., 2013. MicroRNA-182-5p promotes cell invasion and proliferation by down regulating FOXF2, RECK and MTSS1 genes in human prostate cancer. PLoS One 8 (1), e55502.
Iorio, M.V., Croce, C.M., 2009. MicroRNAs in cancer: small molecules with a huge impact. J. Clin. Oncol. 27 (34), 5848.
Jia, L., Luo, S., Ren, X., Li, Y., Hu, J., Liu, B., et al., 2017. miR-182 and miR-135b mediate the tumorigenesis and invasiveness of colorectal Cancer cells via targeting ST6GALNAC2 and PI3K/AKT pathway. Dig. Dis. Sci. 62 (12), 3447.
Li, H.W., Gao, Y.X., Raizada, M.K., Sumners, C., 2005. Intronic enhancement of angiotensin II type 2 receptor transgene expression in vitro and in vivo. Biochem. Biophys. Res. Commun. 336 (1), 29.
Liu, H., Du, L., Wen, Z., Yang, Y., Li, J., Wang, L., et al., 2013. Up-regulation of miR-182 expression in colorectal cancer tissues and its prognostic value. Int. J. Colorectal Dis. 28 (5), 697.
Liu, X., Ji, Q., Fan, Z., Li, Q., 2015. Cellular signaling pathways implicated in metastasis of colorectal cancer and the associated targeted agents. Future Oncol. 11 (21), 2911.
Liu, L., Xu, C., Hsieh, J.T., Gong, J., Xie, D., 2016. DAB2IP in cancer. Oncotarget. 7 (4), 3766.
Liu, X., Xu, T., Hu, X., Chen, X., Zeng, K., Sun, L., et al., 2018. Elevated circulating miR-182 acts as a diagnostic biomarker for early colorectal cancer. Cancer Manag. Res. 10, 857.
Mendell, J.T., 2005. MicroRNAs: critical regulators of development, cellular physiology and malignancy. Cell Cycle 4 (9), 1179.
Ogino, S., Chan, A.T., Fuchs, C.S., Giovannucci, E., 2011. Molecular pathological epidemiology of colorectal neoplasia: an emerging transdisciplinary and interdisciplinary field. Gut. 60 (3), 397.
Pandurangan, A.K., 2013. Potential targets for prevention of colorectal cancer: a focus on PI3K/Akt/mTOR and Wnt pathways. Asian Pac. J. Cancer Prev. 14 (4), 2201.
Segura, M.F., Hanniford, D., Menendez, S., Reavie, L., Zou, X., Alvarez-Diaz, S., et al., 2009. Aberrant miR-182 expression promotes melanoma metastasis by repressing FOXO3 and microphthalmia-associated transcription factor. Proc. Natl. Acad. Sci. U.S.A. 106 (6), 1814.
Shen, Y.J., Kong, Z.L., Wan, F.N., Wang, H.K., Bian, X.J., Gan, H.L., et al., 2014. Downregulation of DAB2IP results in cell proliferation and invasion and contributes to unfavorable outcomes in bladder cancer. Cancer Sci. 105 (6), 704.
Shibuya, H., Iinuma, H., Shimada, R., Horiuchi, A., Watanabe, T., 2010. Clinicopathological and prognostic value of microRNA-21 and microRNA-155 in colorectal cancer. Oncology 79 (3-4), 313.
Siegel, R.L., Miller, K.D., Jemal, A., 2015. Cancer statistics, 2015. CA Cancer J. Clin. 65 (1), 5.
Tang, L., Chen, F., Pang, E.J., Zhang, Z.Q., Jin, B.W., Dong, W.F., 2015. MicroRNA-182 inhibits proliferation through targeting oncogenic ANUBL1 in gastric cancer. Oncol. Rep. 33 (4), 1707.
Wang, Z., Tseng, C.P., Pong, R.C., Chen, H., McConnell, J.D., Navone, N., et al., 2002. The mechanism of growth-inhibitory effect of DOC-2/DAB2 in prostate cancer. Characterization of a novel GTPase-activating protein associated with N-terminal domain of DOC-2/DAB2. J. Biol. Chem. 277 (15), 12622.
Wang, S., Yang, M.H., Wang, X.Y., Lin, J., Ding, Y.Q., 2014. Increased expression of miRNA-182 in colorectal carcinoma: an independent and tissue-specific prognostic factor. Int. J. Clin. Exp. Pathol. 7 (6), 3498.
Wang, J., Zhu, X., Hu, J., He, G., Li, X., Wu, P., et al., 2015. The positive feedback between Snail and DAB2IP regulates EMT, invasion and metastasis in colorectal cancer. Oncotarget 6 (29), 27427.
Wang, B., Huang, J., Zhou, J., Hui, K., Xu, S., Fan, J., et al., 2016. DAB2IP regulates EMT and metastasis of prostate cancer through targeting PROX1 transcription and destabilizing HIF1alpha protein. Cell. Signal. 28 (11), 1623.
Xie, D., Gore, C., Zhou, J., Pong, R.C., Zhang, H., Yu, L., et al., 2009. DAB2IP coordinates both PI3K-Akt and ASK1 pathways for cell survival and apoptosis. Proc. Natl. Acad. Sci. U. S. A. 106 (47), 19878.
Xu, S., Witmer, P.D., Lumayag, S., Kovacs, B., Valle, D., 2007. MicroRNA (miRNA) transcriptome of mouse retina and identification of a sensory organ-specific miRNA cluster. J. Biol. Chem. 282 (34), 25053.
Xuan, Y., Yang, H., Zhao, L., Lau, W.B., Lau, B., Ren, N., et al., 2015. MicroRNAs in colorectal cancer: small molecules with big functions. Cancer Lett. 360 (2), 89.
Yang, M.H., Yu, J., Jiang, D.M., Li, W.L., Wang, S., Ding, Y.Q., 2014. microRNA-182 targets special AT-rich sequence-binding protein 2 to promote colorectal cancer proliferation and metastasis. J. Transl. Med. 12, 109.
Yano, M., Toyooka, S., Tsukuda, K., Dote, H., Ouchida, M., Hanabata, T., et al., 2005. Aberrant promoter methylation of human DAB2 interactive protein (hDAB2IP) gene in lung cancers. Int. J. Cancer 113 (1), 59.
Zhang, L., Liu, T., Huang, Y., Liu, J., 2011. microRNA-182 inhibits the proliferation and invasion of human lung adenocarcinoma cells through its effect on human cortical actin-associated protein. Int. J. Mol. Med. 28 (3), 381.
Zhang, X., Li, N., Li, X., Zhao, W., Qiao, Y., Liang, L., et al., 2012. Low expression of DAB2IP contributes to malignant development and poor prognosis in hepatocellular carcinoma. J. Gastroenterol. Hepatol. 27 (6), 1117.
Zhang, Y., Wang, X., Wang, Z., Tang, H., Fan, H., Guo, Q., 2015. miR-182 promotes cell growth and invasion by targeting forkhead box F2 transcription factor in colorectal cancer. Oncol. Rep. 33 (5), 2592.
Zhu, X.H., Wang, J.M., Yang, S.S., Wang, F.F., Hu, J.L., Xin, S.N., et al., 2017. Down-regulation of DAB2IP promotes colorectal cancer invasion and metastasis by translocating hnRNPK into nucleus to enhance the transcription of MMP2. Int. J. Cancer 141 (1), 172.