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Original Articles

DEPDC1, negatively regulated by miR-26b, facilitates cell proliferation via the up-regulation of FOXM1 expression in TNBC

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ABSTRACT

Triple negative breast cancer (TNBC), characterized by lack of estrogen receptors, progesterone hormone receptors, and HER2 overexpression, is a more aggressive high grade tumor and not sensitive to current targeted drugs. The clinical prognosis of TNBC is poorer than other types of breast cancer, and there is no effective therapy strategy until now. Thus, it is necessary to determine important factors involved in regulating the progression of TNBC. In this study, we found DEPDC1 was up-regulated in the tissues of TNBC compared with their paired peritumoral tissues. DEPDC1 over-expression facilitated cell proliferation and tumor growth through increasing the expression of FOXM1 in TNBC cells. Conversely, knockdown of DEPDC1 had the opposite effects. Moreover, miR-26b, acting as a tumor suppressor in TNBC, directly repressed the expression of DEPDC1 and mitigated its promotive effects on cell growth and colony formation. These results indicate that DEPDC1, negatively regulated by miR-26b, promotes cell proliferation and tumor growth via up-regulating FOXM1 expression, implying an important underlying mechanism of regulating the progression of TNBC.

1. Introduction

Breast cancer is one of the most widespread carcinomas and can be classified into four different groups according to gene expression profiles: the luminal A subgroup, luminal B subgroup, HER2-over-expressing subgroup, and basal-like breast cancer (BLBC) subgroup [1–3]. Triple-negative breast cancer (TNBC), characterized by the absence of estrogen receptor (ER), progesterone receptor (PR) and HER2 expression, belongs to the BLBC subtype [4]. TNBC is a more aggressive high-grade tumor and often shows greater size and tumor burden, and patients with TNBC show greater susceptibility to developing metastases than the other three subgroups [5]. To date, the percentage of new TNBC diagnoses has been reported to range from 9 to 16%, with a higher frequency observed in young women [6]. However, TNBC is refractory to the targeted drugs currently used in clinical practice, and the main approach to TNBC treatment remains chemotherapy. Therefore, the identification of specific oncogenic drivers is a major issue for targeted therapy and improving the clinical therapeutic outcomes in TNBC.

DEPDC1, DEP (dishevelled, EGL-10, pleckstrin) domain-containing 1, is a highly conserved protein among many species from *Caenorhabditis elegans* to mammals [7]. Although there are many reports showing that proteins containing the DEP (dishevelled, EGL-10, pleckstrin) domain regulate several cellular functions, including a large number of signaling proteins [8–10], the pathophysiologic roles of DEPDC1 have not been thoroughly investigated. Since Kanehira et al. first identified and characterized DEPDC1 as a novel gene in bladder cancer and reported its essential role in the growth of bladder cancer cells [7], the critical functions of DEPDC1 and potential regulation pathways have been further elucidated. Mi et al. found that DEPDC1 is a novel cell cycle-related gene that regulates mitotic progression [9]. Additionally, other studies have shown that in other human cancers,

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such as hepatocellular carcinoma [11], colorectal cancer [12] and glioblastoma [13], DEPDC1 is associated with cell growth or apoptosis and may be a novel diagnostic marker or prognostic predictor. However, little is known about the functions of DEPDC1 in breast cancer.

The aim of the present study was to determine the role of DEPDC1 in TNBC progression and the corresponding molecular mechanisms. Our results showed that DEPDC1, which was frequently up-regulated in TNBC, facilitated cell growth and colony formation in TNBC cells as an oncogenic factor. Further mechanistic studies showed that DEPDC1 was negatively regulated by miR-26b in TNBC and that the promotive effects of DEPDC1 on cell proliferation were mediated by Forkhead Box M1 (FOXM1). These results may provide novel treatment targets for improving the therapeutic efficiency of TNBC.

2. Materials and methods

2.1. Cell culture and clinical samples

MDA-MB-231, MDA-MB-436, MDA-MB-468, MDA-MB-157, MDA-MB-435 and BT549 cells (human TNBC cell lines) were purchased from American Tissue Culture Collection (Manassas, USA). All cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) in a 5% CO_2 atmosphere at 37 °C.

2.2. CCK-8 assay

Cells were seeded in 96-well culture plates with 2×10^3 cells/well, and incubated at 37 °C with 5% CO_2. The cell viability assay was performed by using Cell Counting Kit-8 (CCK-8; Dojindo) at different time points according to the manufacturer's protocol. The absorbance at 450 nm was measured.

2.3. Bromodeoxyuridine incorporation

Cells were seeded at a density of 5000 cells/well in 96-well microplates and allowed to attach overnight. Cell proliferation was evaluated by analyzing 5′-bromo-2′-deoxyuridine (BrdU) incorporation into newly synthesized DNA using a cell proliferation enzyme-linked immunosorbent assay (ELISA) kit (Roche). Optical density was measured using an ELISA plate reader (Sinergy HT, BioTek) at 595 nm. Proliferation was expressed as a percentage of the control.

2.4. Western blot analysis

The cells were washed three times with cold PBS, harvested using cell lysis buffer (RIPA) and cell scrapes, and quantified with BCA methods. Equal amounts of protein extracts (50 μg) were loaded onto SDS/PAGE gels and ran at 80 mV for 90 min, followed by transfer to nitrocellulose membranes at 100 mV for 60 min at room temperature. The membranes were blocked in 5% non-fat milk and subsequently incubated with the indicated primary antibodies overnight. After washing five times with PBS-T for 30 min, the membranes were incubated with anti-rabbit or anti-mouse secondary antibodies (Santa Cruz Biotechnology) for 1 h. After washing with PBS-T for 30 min, the immune complexes were detected using the enhanced chemiluminescence (ECL) method.

2.5. RNA isolation and real-time RT-PCR

Total RNAs were extracted with TRIzol (Invitrogen) according to the protocol previously described, while microRNA was extracted using miRNA isolation kit from Ambion [14]. MiRNA reverse transcription and qRT-PCR were conducted using Taqman miRNA reverse transcription kit (Applied Bio-systems, Carlsbad, CA, USA) and Taqman premix (Takara, Shiga, Japan). The specific reverse primers and qRT-PCR Taqman probes for miR-26b and snRNA U6 (internal normalization control) were both purchased from Applied Biosystems. For mRNA analysis, total RNAs were reversed transcribed with Prime-Script RT kit from Takara, and amplified with SYBR Green Real-time PCR Master Mix (Applied Bio-systems). The mRNA level of beta-actin was used as an internal normalization control.

2.6. Cell cycle analysis

Cells (1×10^6) were collected by gentle trypsinization and re-suspended in PBS. After fixation in 70% cold ethanol at −20 °C for at least 1.5 h, the cells were stained with propidium iodide (PI) working solution (40 μg/ml PI and 100 μg/ml RNase A and 0.1% Triton X-100) at 37 °C for 1 h and subsequently analyzed for cell cycle distribution by flow cytometry. Flow cytometry was performed on an Epics Altra Flow Cytometer, and the results were analyzed using EXPO32 Multicomp and EXPO32 v1.2 analysis (Beckman Coulter) software.

2.7. Colony formation assay

The soft agar colony formation assay was used to detect cell growth ability. To this end, 0.4% agarose containing 3×10^3 suspended cells in 20% FBS DMEM medium was plated per well over a layer of 0.8% agarose on a 24-well plate. After 21 days, the colonies were viewed and counted under a microscope at × 40 magnification. Only clearly visible colonies (diameter > 50 μm) were counted.

2.8. Luciferase reporter assays

For the luciferase assay, approximately 1×10^5/well HEK-293T cells were seeded onto 24-well plates. The cells were co-transfected with 200 ng reporter vectors, 5 ng pRL-CMV (internal standard, Promega, Madison, WI, USA), and 5 nM miR-26b mimic or scrambled control (Ambion, Carlsbad, CA, USA) using Lipofectamine 2000 (Invitrogen) overnight. At 24 h after transfection, the luciferase activities were measured with the Dual-Luciferase Reporter Assay System (Promega). Reporter luciferase activity was normalized to the internal control Renilla luciferase activity in all samples.

2.9. Nude mouse xenograft model

Six-to eight-week-old female BALB/c nude mice (SLAC, Shanghai, China) were used to establish a breast cancer xenograft model. All experiments using animals were performed in accordance with a protocol approved by Shanghai Jiao Tong University Institutional Animal Care and Use Committee (IACUC). The mice were randomly divided into the indicated groups (n = 6), and 5×10^6 MDA-MB-436 and BT549 cells with DEPDC1 overexpression, or MDA-MB-231 and MDA-MB-468 cells with shDEPDC1 and shControl, respectively, were suspended in 100 μL of PBS and subcutaneously injected into the flanks of the nude mice. All mice were sacrificed at 30 days after injection. Subsequently, the tumor masses and sizes were measured. All animal studies were performed in accordance with the Renji Hospital Animal Care guidelines. All efforts were made to minimize animal suffering.

2.10. Statistical analysis

Statistical analysis was carried out with a GraphPad Prism version 5.0 for Windows (GraphPad Software Inc., San Diego, CA, USA). All data were represented as mean ± standard error of the mean (S.E.M.) from at least three independent experiments. Statistical analysis was performed with student's t-test (for comparisons of two groups) or one-way ANOVA followed by Dunnett's test (for multiple group comparisons) where appropriate. $P < 0.05$ was considered statistically significant.

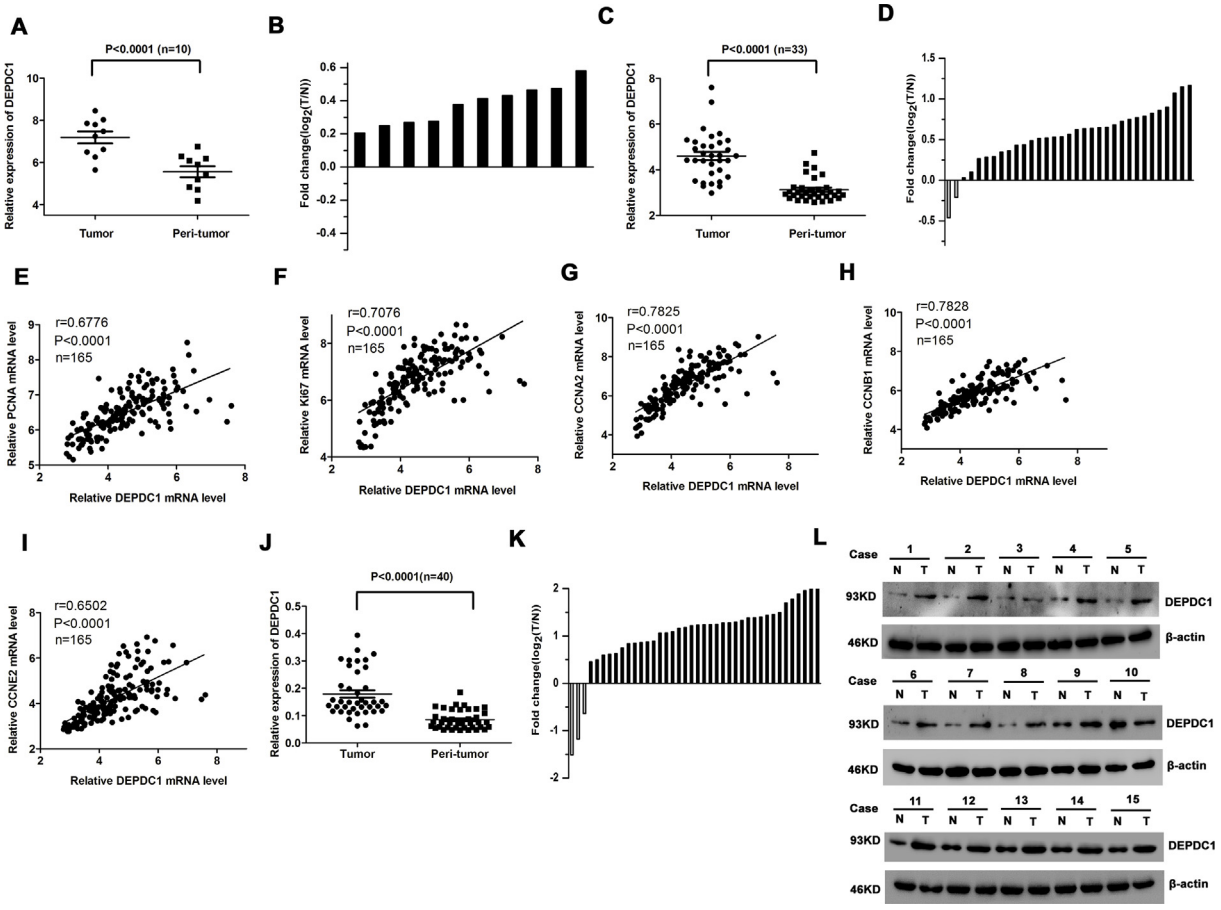

Fig. 1. DEPDC1 is up-regulated in TNBC as an oncogenic factor. A and B: DEPDC1 expression in 10 pairs of TNBC tissues and paired noncancerous tissues. C and D: DEPDC1 expression in 33 pairs of TNBC tissues and paired noncancerous tissues. E and F: Correlations of DEPDC1 and cell growth markers (PCNA and Ki67) in TNBC tissues. G–I: Correlations between levels of DEPDC1 and cell cycle related genes (CCNA2, CCNB1, and CCNE2) in TNBC tissues. Data are represented as means ± S.E.M. J and K: The DEPDC1 mRNA expression in 40 cases of TNBC and paired adjacent normal tissue samples. L: Representative western blot showing the expression of DEPDC1 in tumor and paired noncancerous tissues from 15 TNBC patients.

3. Results

3.1. DEPDC1 is up-regulated in TNBC

We first analyzed the expression of DEPDC1 on Oncomine (a public dataset, www.oncomine.org) and found that DEPDC1 was up-regulated in most types of cancers, especially breast cancer, esophageal cancer and lung cancer (Figure S1A). To examine roles of DEPDC1 in TNBC, we analyzed two sets of microarray data (GSE76250 and GSE81838) from the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo) and validated the expression of DEPDC1 in TNBC tissues and paired non-cancerous tissues. As shown in Fig. 1A and B, the expression of DEPDC1 was significantly increased in TNBC tissues compared with that in the paired peritumoral tissues. Similar results were also obtained in the other dataset. In the 33 paired TNBC and noncancerous tissue samples, we found that the expression of DEPDC1 in most tissues of TNBC was higher than that in the adjacent normal tissues (Fig. 1C and D). Moreover, the expression levels of PCNA (proliferating cell nuclear antigen) and Ki67 (a proliferation marker), important indicators of the growth of cancer cells, were positively correlated with DEPDC1 expression in 165 cases of TNBC tissues (Fig. 1E and F), and significant correlations between the levels of DEPDC1 and cell cycle-related genes (CCNA2, CCNB1, and CCNE2) were also observed in TNBC tissues (Fig. 1G–I). Furthermore, we also examined the mRNA and protein levels of DEPDC1 in 40 cases of TNBC and paired adjacent normal tissue samples and found that the expression of DEPDC1 was significantly increased in TNBC tissues (Fig. 1J–L). The IHC results showed that although DEPDC1 was both expressed by tumor cells and by stromal cells in TNBC, DEPDC1 was mainly expressed by tumor cells and the expression of DEPDC1 can only be detected in a few stromal cells (Figure S1B). These results imply that DEPDC1 is likely to be associated with regulating the progression of TNBC.

3.2. DEPDC1 overexpression promotes cell proliferation and colony formation in TNBC cells

We next examined the mRNA expression of DEPDC1 in a panel of TNBC cell lines (Figure S2A). Based on the endogenous expression of DEPDC1 in these cells, stable DEPDC1-overexpression cell lines were established in MDA-MB-436 cells and BT549 cells. The mRNA and protein expression of DEPDC1 were detected by real-time PCR and western blotting, respectively, to ensure the transfection efficiency (Figures S2B- S2E). As shown in Fig. 2A, the results of the CCK-8 assay showed that cell viability was significantly increased by DEPDC1 overexpression. Both BrdU incorporation and PCNA expression were investigated to measure cell proliferation. The overexpression of DEPDC1 enhanced BrdU incorporation into newly synthesized DNA and induced PCNA expression (Fig. 2B and C). Moreover, we also studied cell cycle distribution via flow cytometry, and the results showed that DEPDC1 overexpression decreased the number of cells at the G0/G1 phase from 70.21% to 49.82%, while the proportion of cells at the S phase was increased by DEPDC1 overexpression from 20.44% to 36.55% in MDA-MB-436 cells (Figure S3). Similar results were obtained for BT549 cells, showing that DEPDC1 overexpression attenuated the

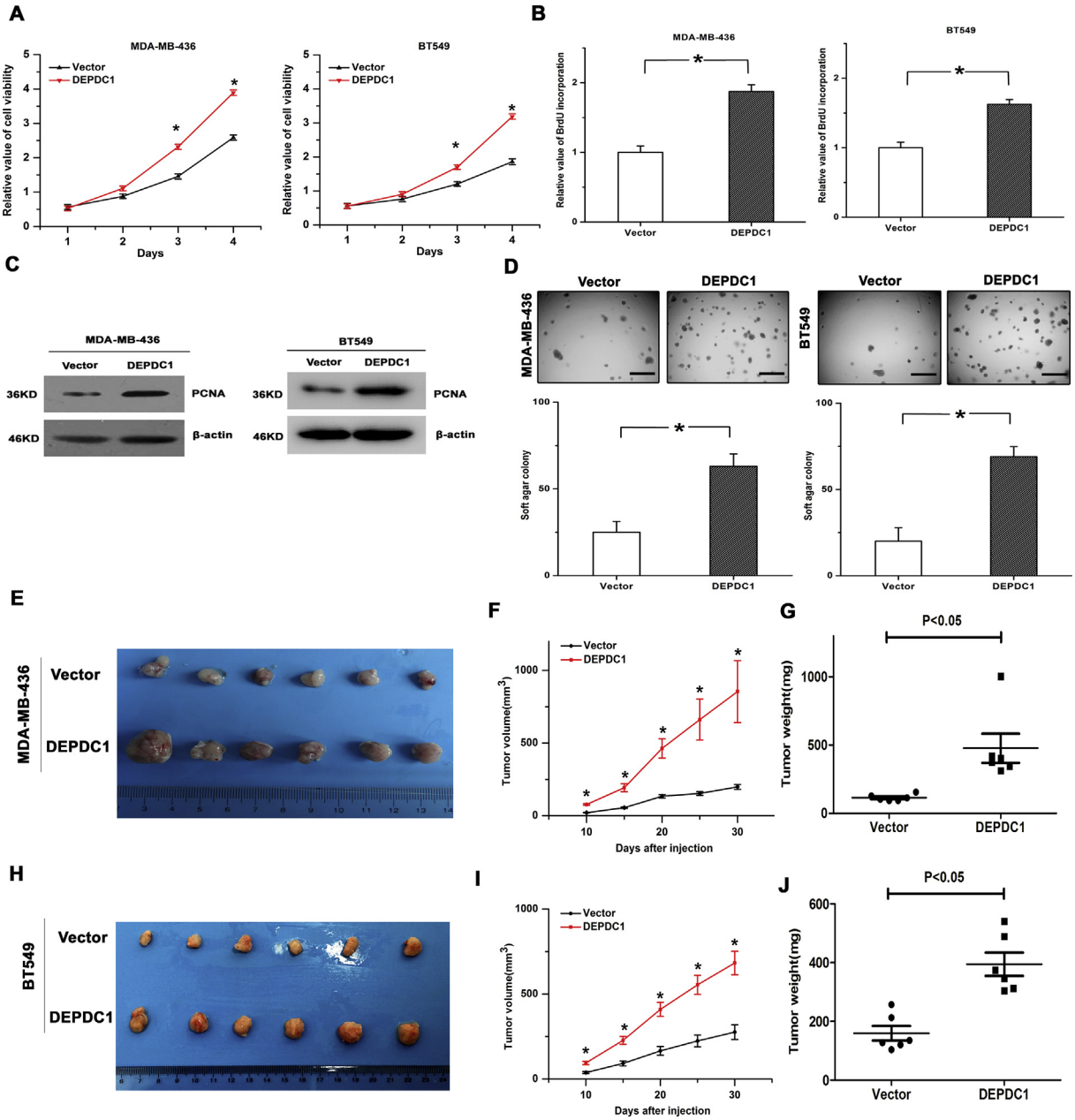

Fig. 2. Overexpression of DEPDC1 promotes cell proliferation and tumorigenesis in TNBC. A: DEPDC1 overexpression resulted in the increased cell viability in MDA-MB-436 cells and BT549 cells. B: BrdU incorporation into synthesized DNA was enhanced by DEPDC1 overexpression. C: DEPDC1 overexpression induced the protein levels of PCNA as compared with the vector group. D: DEPDC1 overexpression promoted the colony formation of MDA-MB-436 cells and BT549 in vitro. Scale bars: 1 mm. E: Representative tumors isolated from nude mice from the indicated groups. F and G: Overexpression of DEPDC1 in MDA-MB-436 increased the volumes (F) and weights (G) of the xenograft tumors. H: Representative tumors isolated from nude mice from the indicated groups. I and J: DEPDC1 overexpression in BT549 cells enhanced the volumes (I) and weights (J) of the xenograft tumors.

number of cells at the G0/G1 phase from 76.01% to 57.67% (Figure S3). And DEPDC1 overexpression promoted colony formation in vitro in both MDA-MB-436 and BT549 cells (Fig. 2D). Furthermore, we established subcutaneous tumors in nude mice and examined the roles of DEPDC1 in tumor growth in vivo. The results showed that overexpression of DEPDC1 significantly enhanced the tumorigenic ability of MDA-MB-436 and BT549 cells and increased both tumor volumes and weights compared with those of the control group (Fig. 2E–J). We also examined the roles of DEPDC1 in proliferation and apoptosis in vivo and found that the overexpression of DEPDC1 significantly increased the percentage of Ki67-positive cells and inhibited cell apoptosis (Figure S3B and S3C). Collectively, these results indicate that DEPDC1 overexpression promotes cell growth and tumor growth by facilitating cell cycle progression in TNBC.

3.3. Cell proliferation is attenuated by knockdown of DEPDC1 in TNBC

Additionally, to determine the physiological function of DEPDC1 in TNBC, we knocked down DEPDC1 expression in MDA-MB-231 and MDA-MB-468 cells. Knockdown efficiency was confirmed by real-time PCR and western blotting (Figures S4A-S4D). As shown in Fig. 3A, the knockdown of DEPDC1 suppressed cell growth in MDA-MB-231 and MDA-MB-468 cells. Both BrdU incorporation and the protein level of PCNA were decreased by DEPDC1 knockdown (Fig. 3B and C). The cell cycle distribution analysis showed that knocking down the expression of DEPDC1 increased the population of cancer cells arrested at the G0/

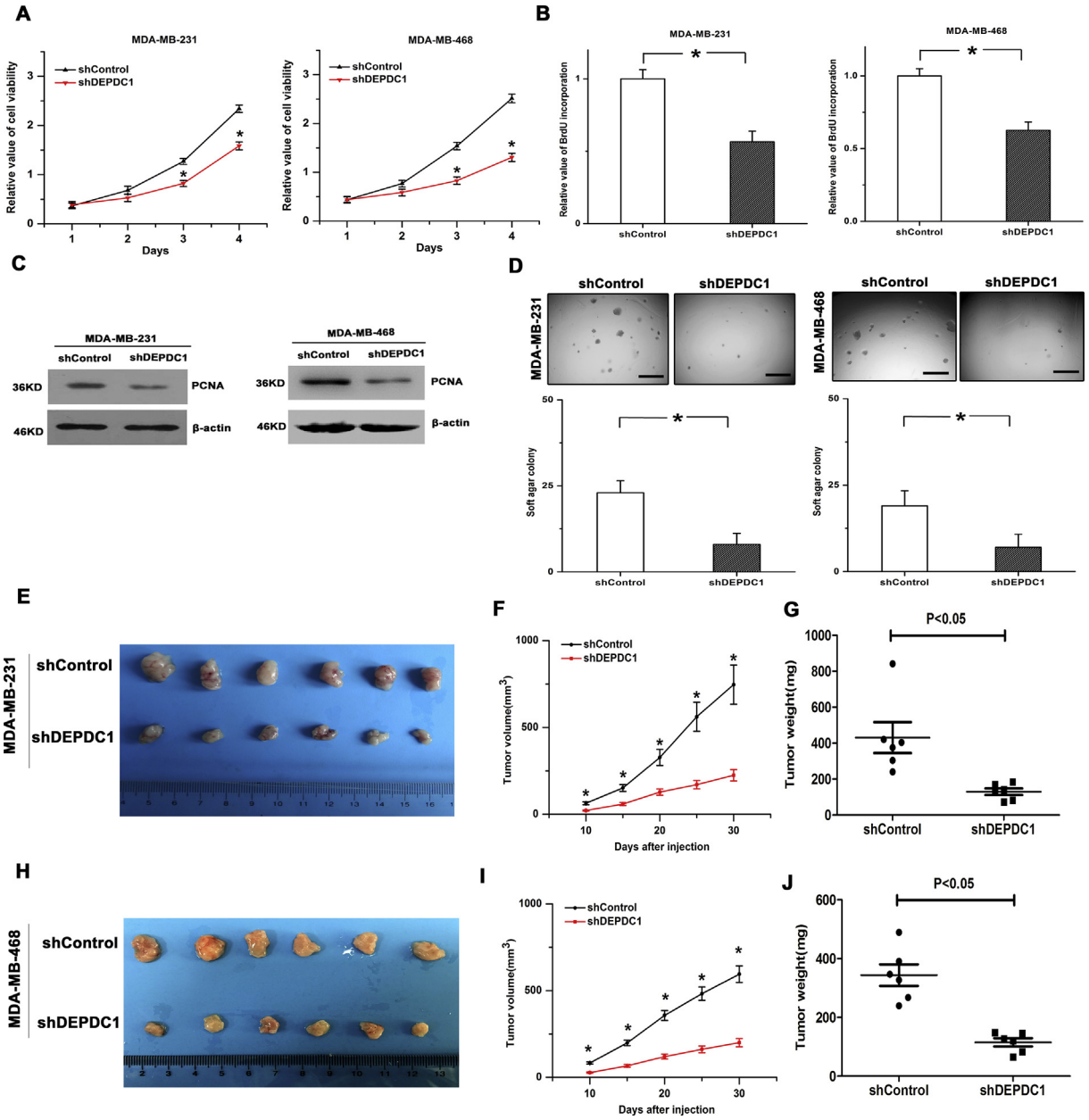

Fig. 3. Proliferation and colony formation were inhibited by DEPDC1 knockdown in TNBC cells. A: Cell growth was significantly repressed by knockdown of DEPDC1. B: Knockdown of DEPDC1 inhibited the BrdU incorporation into synthesized DNA in MDA-MB-231 and MDA-MB-468. C: Expression of PCNA was mitigated by DEPDC1 knockdown. D: Colony forming ability was attenuated by DEPDC1 depletion. Scale bars: 1 mm. E: Representative tumors isolated from nude mice from the indicated groups. F and G: Knockdown of DEPDC1 in MDA-MB-231 cells lessened the volumes (F) and weight (G) of the subcutaneous tumors. H: Representative tumors isolated from nude mice from the indicated groups. I and J: Knockdown of DEPDC1 in MDA-MB-468 cells decreased the volumes (I) and weight (J) of the subcutaneous tumors.

G1 phase (from 56.31% to 78.52%), which was accompanied by a decrease in the number of S phase cells from 32.41% to 16.31% in MDA-MB-231 cells. In MDA-MB-468 cells, knockdown of DEPDC1 increased cells at the G0/G1 phase (from 61.61% to 80.17%) and decreased cells at S phase (from 28.51% to 13.52%) (Figure S5). As shown in Fig. 3D, colony formation was also inhibited by DEPDC1 depletion (Fig. 3D). Moreover, the results obtained in nude mice with subcutaneous implanted tumors suggested that knockdown of DEPDC1 remarkably inhibited the tumor growth of MDA-MB-231 and MDA-MB-468 cells (Fig. 3E and H). Consistently, both tumor volumes and weights were significantly decreased by DEPDC1 depletion (Fig. 3F–J). Besides, the knockdown of DEPDC1 significantly inhibited cell proliferation and elicited cell apoptosis in vivo (Fiugre S5B and S5C). These results suggest that DEPDC1 deletion inhibits cell proliferation and tumor growth in TNBC cells.

3.4. *DEPDC1 expression is negatively regulated by miR-26b in TNBC*

MicroRNAs negatively regulate the expression of target genes by binding to their 3' untranslated region (UTR). To determine the upstream regulator of DEPDC1 in TNBC, we used TargetScan (http://www.targetscan.org/) and Miranda (http://www.microrna.org) to predict the potential binding microRNAs of DEPDC1. Based on the microRNA expression in TNBC tissues (GSE76250), miR-26b, whose expression was down-regulated and negatively correlated with DEPDC1 expression in the same TNBC tissues, was considered a functional regulator of DEPDC1 (Fig. 4A–C). We next examined whether DEPDC1 was directly modulated by miR-26b. As shown in Fig. 4D, there is a

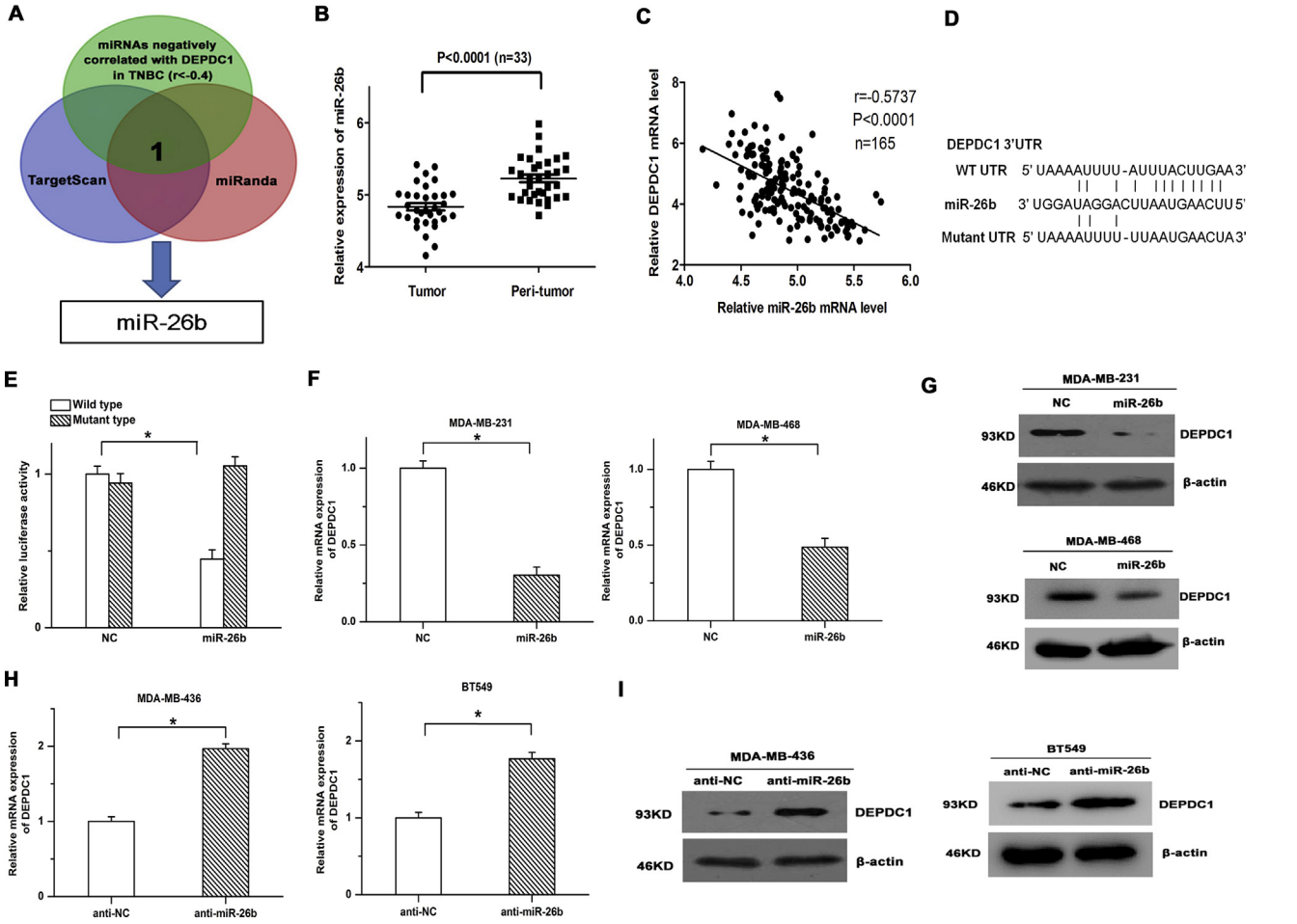

Fig. 4. DEPDC1 is directly down-regulated by miR-26b in TNBC cells. A: A schematic diagram of the protocol used to search for a candidate functional regulator of DEPDC1. B: miR-26b expression in TNBC tissues and paired peri-tumor tissues. C: Correlation of miR-26b and DEPDC1 expression in the same TNBC tissues. D: The potential binding site of miR-26b conserved in the 3′UTR of DEPDC1. E: Luciferase reporter assay showed that miR-26b significantly decreased the luciferase activity of the plasmid with WT 3′UTR, whereas the luciferase activity of the MT plasmid was not altered. F and G: miR-26b repressed the mRNA (F) and protein (G) levels of DEPDC1 in MDA-MB-231 cells and MDA-MB-468 cells. H and I: mRNA (H) and protein (I) levels of DEPDC1 were increased by the treatment with anti-miR-26b in both MDA-MB-436 cells and BT549 cells. All values are denoted as means ± S.E.M. from three independent experiments (*P < 0.05).

potential binding site conserved in the 3′UTR of DEPDC1. The luciferase reporter assay showed that miR-26b significantly decreased the luciferase activity of the WT (wild type) 3′UTR plasmid, whereas the luciferase activity of the MT (mutant type) plasmid was not altered, implying that miR-26b directly bound to the predicted binding site in the 3′UTR of DEPDC1 (Fig. 4E). Moreover, the mRNA and protein levels of DEPDC1 were significantly repressed by miR-26b, while treatment with a miR-26b inhibitor (anti-miR-26b) increased the expression of DEPDC1 in TNBC cells (Fig. 4F–I). These results indicate that miR-26b serves as a negative regulator of DEPDC1 and decreases its expression in TNBC.

3.5. MiR-26b suppresses the proliferation of TNBC cells by targeting DEPDC1

We further studied the effects of miR-26b on cell proliferation in TNBC cells. As shown in Fig. 5A, the inhibition of miR-26b with a specific inhibitor led to an increase in cell viability. BrdU incorporation into synthesized DNA and PCNA protein expression were facilitated by miR-26b inhibition in MDA-MB-436 and BT549 cells (Fig. 5B and C). Moreover, the colony formation of cancer cells was enhanced by anti-miR-26b (Fig. 5D). In contrast, cell viability was decreased by miR-26b treatment in MDA-MB-231 and MDA-MB-468 cells, and this effect was rescued by the reintroduction of DEPDC1 (Fig. 5E). Moreover, miR-26b inhibited BrdU incorporation, mitigated PCNA expression and repressed colony formation, and the inhibitory effect of miR-26b on cell proliferation and colony formation was attenuated by the restoration of DEPDC1 protein expression (Fig. 5F–H). These results indicate that miR-26b inhibits the proliferation of cancer cells and TNBC progression by decreasing the expression of DEPDC1.

3.6. DEPDC1 positively regulates the expression of FOXM1 in TNBC

Previous studies have revealed an interaction between DEPDC1 and FOXM1, showing that FOXM1 functions as an important mediator in regulating cell cycle progression [15,16]. To illuminate the mechanisms underlying the promotive effects of DEPDC1 on cell proliferation and cell cycle progression, we next investigated the potential association between DEPDC1 and FOXM1 in TNBC. As shown in Fig. 6A and B, the expression of FOXM1 in TNBC tissues was significantly up-regulated compared with that in the paired noncancerous tissue samples, and the expression of FOXM1 was positively correlated with DEPDC1 expression in the same TNBC tissues (Fig. 6C). Moreover, the overexpression of DEPDC1 augmented FOXM1 expression, while FOXM1 expression was decreased by DEPDC1 knockdown in TNBC cells (Fig. 6D and E). Furthermore, co-immunoprecipitation showed that DEPDC1 overexpression enhanced the endogenous interaction between DEPDC1 and FOXM1 in MDA-MB-436 and BT549 cells (Fig. 6F). These results imply

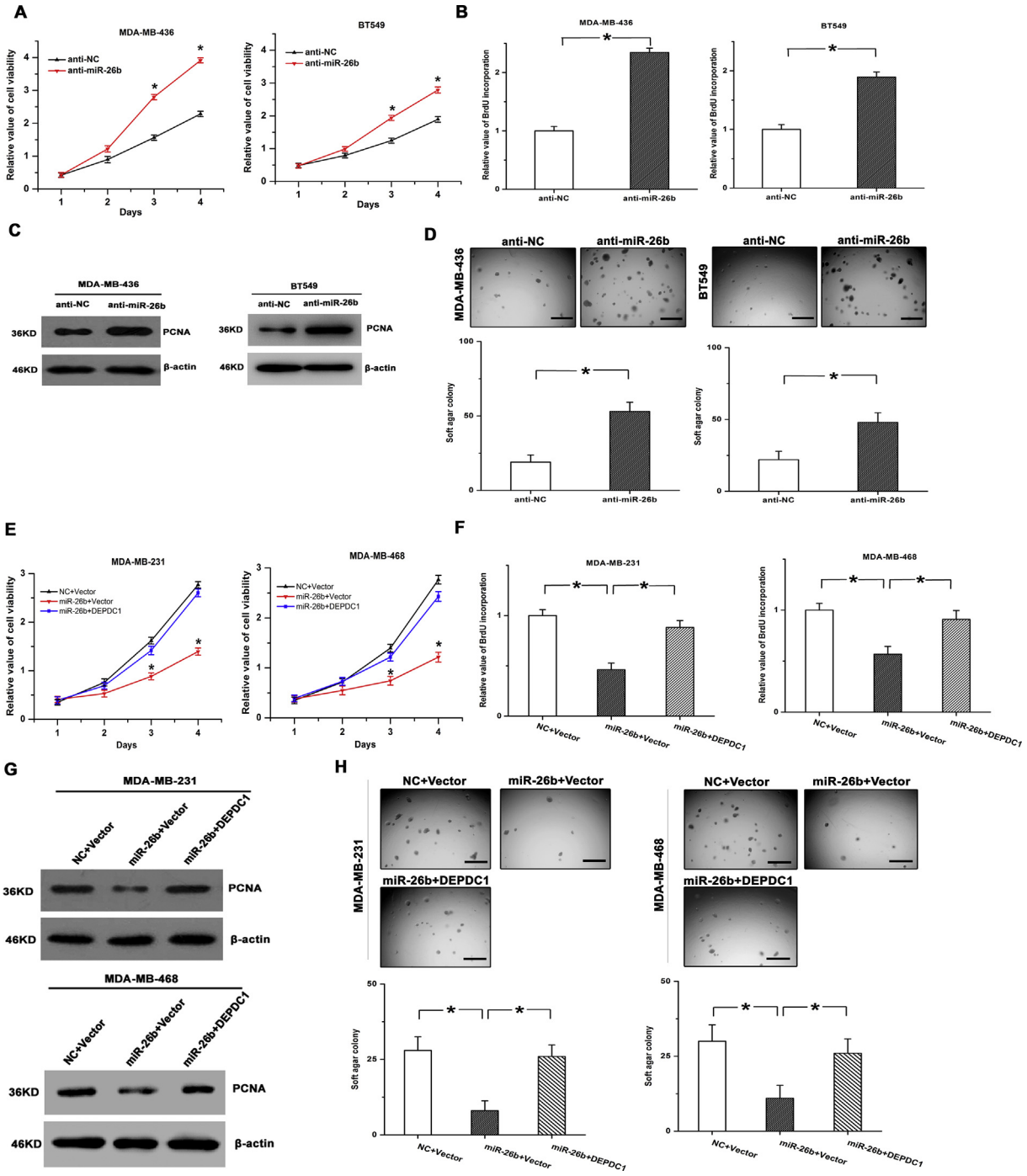

Fig. 5. MiR-26b-suppressed cell proliferation and colony formation was eliminated by DEPDC1 overexpression in TNBC cells. A: Inhibition of miR-26b led to an increase in cell viability in MDA-MB-436 cells and BT549 cells. B: BrdU incorporation was facilitated by miR-26b inhibition. C: miR-26b inhibition enhanced the protein level of PCNA. D: Colony formation of cancer cells was promoted by anti-miR-26b. Scale bars: 1 mm. E: miR-26b-depressed cell growth was eliminated by reintroduction of DEPDC1 in both MDA-MB-231 cells and MDA-MB-468 cells. F and G: Inhibitory roles of miR-26b on BrdU incorporation (F) and PCNA expression (G) were antagonized by overexpression of DEPDC1. H: Colony formation was also inhibited by miR-26b treatment in MDA-MB-231 and MDA-MB-468, which were reversed by DEPDC1 overexpression. Scale bars: 100 μm. All values are denoted as means ± S.E.M. from three independent experiments (*P < 0.05).

that FOXM1 expression is positively regulated by DEPDC1 in TNBC.

3.7. DEPDC1-promoted cell growth is mitigated by knockdown of FOXM1

To explore the effects of FOXM1 in DEPDC1-facilitated cell proliferation, we utilized FOXM1 siRNA to repress its expression. Real-time PCR and western blotting were used to validate the knockdown efficiency (Fig. 6G and H). We found that the increased cell viability induced by DEPDC1 overexpression was weakened by FOXM1 siRNA (Fig. 6I), and the cell proliferation induced by DEPDC1 overexpression was antagonized by FOXM1 knockdown (Fig. 6J and K). These results show that the promotive effects of DEPDC1 on cell proliferation are, at least in part, mediated by FOXM1.

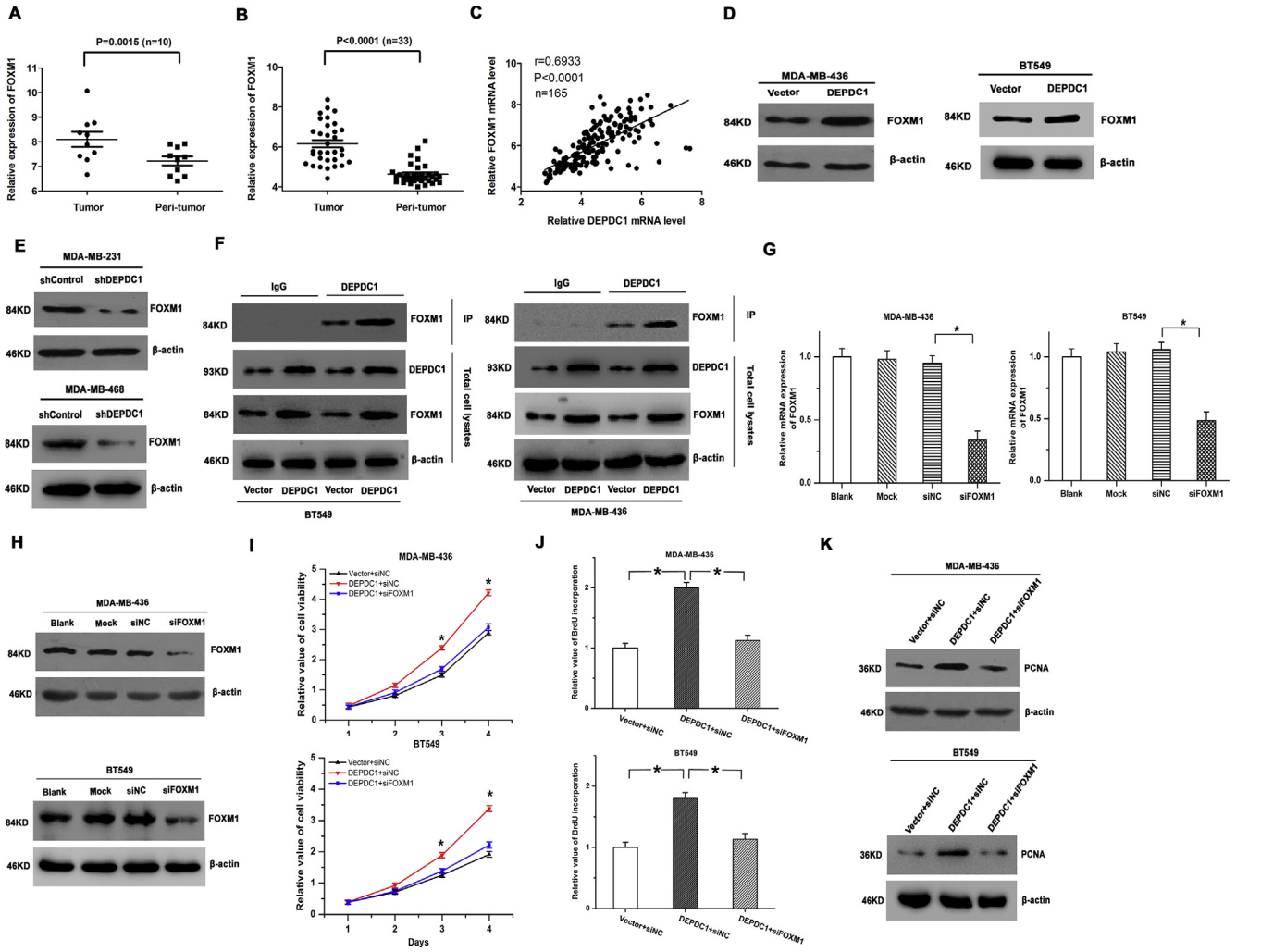

Fig. 6. DEPDC1 promotes cell growth and proliferation via up-regulating FOXM1 expression in TNBC. A and B: Expression of FOXM1 in TNBC tissues was significantly up-regulated as compared with their paired noncancerous tissues. C: Correlation between the expression of FOXM1 and DEPDC1 expression in the same TNBC tissues. D: Overexpression of DEPDC1 induced FOXM1 expression in TNBC MDA-MB-436 and BT549 cells. E: Protein level of FOXM1 was repressed by DEPDC1 knockdown in MDA-MB-231 cells and MDA-MB-468 cells. F: Co-immunoprecipitation was used to examine the endogenous interaction between DEPDC1 and FOXM1 in MDA-MB-436 and BT549 cells. IgG was used as a negative control. G and H: mRNA expression and protein level of FOXM1 were utilized to validate the knockdown efficiency of siFOXM1. I: Increased cell viability induced by DEPDC1 overexpression was weakened by siFOXM1. J: DEPDC1-promoted BrdU incorporation into synthesized DNA was antagonized by siFOXM1. K: Promotive effects of DEPDC1 overexpression on PCNA expression were attenuated by siFOXM1. All values are denoted as means ± S.E.M. from three independent experiments (*P < 0.05).

4. Discussion

TNBC is an aggressive type of breast cancer with a poor clinical prognosis. As the pathogenesis of TNBC remains largely unknown, it is necessary to identify the factors involved in regulating the progression of TNBC. In the present study, we found that DEPDC1, which was frequently up-regulated in TNBC tissues, promoted cell growth and proliferation via FOXM1, and the effects of DEPDC1 on cell proliferation were negatively regulated by miR-26b in TNBC.

The overgrowth of cancer cells, which largely results from defects in cell cycle regulation, leads to the development and progression of tumors. Hence, cancer is frequently considered a cell cycle disease [17,18]. Previous studies have shown that some cell cycle-related genes (including cyclins, cyclin-dependent kinases (CDKs), and CDK inhibitors) are up-regulated and/or down-regulated in breast cancer tissues and cells and participate in modulating the progression of cancers, and the upstream regulators of the cell cycle and their related genes are also considered tumor-related genes [19–21]. DEPDC1, a highly conserved protein, has been identified as a novel cell cycle-related gene that regulates cell cycle progression and mitosis [9]. In recent years, accumulating studies have been shown that DEPDC1 plays important roles in regulating the development of some cancers. Additional studies showed that DEPDC1 positively regulates the growth of bladder cancer cells and thus represents a promising molecular target for improving the clinical management of bladder cancer patients [22,23]. In nasopharyngeal carcinoma, DEPDC1 plays important roles in regulating cell cycle progression and motility [24]. Besides, it is reported that DEPDC1 was significantly up-regulated in TNBC tissues when compared to hormone receptor-positive tumor tissues [25]. However, evidence for the function of DEPDC1 in human malignancies remains limited. In the present study, the results showed that expression of DEPDC1 was significantly up-regulated in TNBC tissues compared to that in paired peritumoral tissue samples. Moreover, DEPDC1 overexpression promoted cell proliferation and cell cycle transition and enhanced tumor growth in TNBC cells. Conversely, cell proliferation and colony formation were mitigated by the knockdown of DEPDC1. These results indicate that DEPDC1 regulates the progression of TNBC as an oncogenic factor.

MicroRNAs, a class of small non-coding RNAs, play important roles in modulating cellular physiological functions, such as cell growth,

apoptosis, differentiation and metastasis, via regulating the translation of many genes [26–28]. Previous studies have shown that miR-26b regulates the progression and development of various types of cancers. For instance, miR-26b decreases the proliferation and metastasis of lung cancer cells by targeting CDC6, and downregulation of miR-26b is associated with poorer prognosis in patients with lung cancer [29]. In hepatocellular carcinoma, miR-26b inhibits the activation of NF-κB signaling by decreasing TAK1 and TAB3 expression, leading to increased chemosensitivity of HCC cells [30]. In ER-positive breast cancers, expression of miR-26b is repressed in cancer-associated fibroblasts, and cell migration and invasion are facilitated by reduced miR-26b expression [31]. However, the roles of miR-26b in TNBC remain to be explored. In the present study, miR-26b was down-regulated in TNBC tissues and mitigated the growth and proliferation of TNBC cells. Consistently, the antagonism of miR-26b led to increased proliferation in TNBC cells. Moreover, miR-26b depressed DEPDC1 expression via directly binding to its 3′UTR, and the inhibitory roles of miR-26b in cell proliferation were attenuated by DEPDC1 in TNBC. These results imply that miR-26b acts as a tumor suppressor and inhibits the development of TNBC by targeting DEPDC1.

FOXM1 is a transcription factor that regulates the cell cycle transition and DNA replication during normal cell growth and carcinogenesis [32–34]. A growing body of evidence suggests that the transcriptional activity of a number of genes, including cyclin B, cyclin A, and polo-like kinase 1, is positively regulated by FOXM1, and FOXM1 protein can repress the nuclear levels of $p27^{kip1}$ and $p21^{cip1}$ (the CDK inhibitor proteins) via their degradation [35,36]. In contrast, knockdown of FOXM1 expression leads to cell cycle arrest, mitotic catastrophe and growth inhibition, indicating the critical roles of FOXM1 in cell growth and cell cycle transition [37,38]. DEP domains commonly function via interactions with other proteins, and the interaction between DEPDC1 and FOXM1 has previously been reported [15]. Thus, we further examined whether the effects of DEPDC1 on cell proliferation and tumor growth were mediated by FOXM1 in TNBC. In the present study, we found that the expression of DEPDC1 was positively correlated with the expression of FOXM1 in TNBC tissues. Additionally, DEPDC1 overexpression led to increased expression of FOXM1, while the expression of FOXM1 was suppressed by knockdown of DEPDC1. Moreover, the cell proliferation induced by DEPDC1 was attenuated by FOXM1 knockdown. These results indicate that the roles of DEPDC1 in cell growth and proliferation are, at least in part, mediated by FOXM1 in TNBC. However, whether FOXM1 is directly regulated by DEPDC1 in TNBC is still uncertain, we will explore the regulatory mechanisms in the further studies.

In summary, the present study showed that DEPDC1 was up-regulated in TNBC tissues and promoted the growth and proliferation of TNBC cells by increasing the expression of FOXM1. Moreover, DEPDC1 was negatively regulated by miR-26b. These results demonstrate a critical role for DEPDC1 in regulating the growth of TNBC cells, and the corresponding molecular mechanisms may provide new potential treatment targets for the molecular therapy of TNBC.

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Conflicts of interest statement

None.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.canlet.2018.11.003.

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