European Review for Medical and Pharmacological Sciences 2020; 24: 2387-2400

Circular RNA circDENND4C facilitates proliferation, migration and glycolysis of colorectal cancer cells through miR-760/GLUT1 axis

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Abstract. – OBJECTIVE: Colorectal cancer is a common malignant tumor of the digestive tract, and its incidence is closely related to lifestyle inheritance and colorectal adenoma. Circular RNA (circRNA) has been proved to participate in the progression of colorectal cancer cells. Our study aimed to investigate the function and the underlying mechanism of circRNA circDENND4C in colorectal cancer cells.

PATIENTS AND METHODS: The expression of circDENND4C, glucose transporter 1 (GLUT1), and miR-760 was detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). Western blot was used to measure the protein levels of GLUT1, the proliferation-related protein (Cyclin D1) and matrix metallopeptidase 9 (MMP-9). Cell Counting Kit-8 (CCK-8) assay and transwell assay were performed to evaluate cell proliferation and migration. The glucose uptake and lactate production were detected by the corresponding kits. The targets between circDENND4C and miR-760 and miR-760 and GLUT1 were predicted by starBase 3.0 and TargetScan, and then confirmed by Dual-Luciferase reporter assay. Animal experiment revealed the effect of circDENND4C on colorectal cancer cells *in vivo*.

RESULTS: The expression of circDENND4C and GLUT1 was upregulated in colorectal cancer tissues and cells. Functionally, the knockdown of circDENND4C suppressed proliferation, migration, and glycolysis of colorectal cancer cells. Similarly, silence of GLUT1 also inhibited cell proliferation, migration, and glycolysis. Notably, the overexpression of GLUT1 reversed the functional effects of circDENND4C knockdown on colorectal cancer cells. More importantly, miR-760 acted as a direct target of circDENND4C, and miR-760 could bind to GLUT1, and circDENND4C regulated GLUT1 by sponging miR-760. Finally, circDENND4C knockdown decreased the growth of colorectal cancer cells *in vivo*.

CONCLUSIONS: CircRNA circDENND4C accelerated proliferation, migration, and glycolysis of colorectal cancer cells through regulating GLUT1 by sponging miR-760.

Key Words:
Colorectal cancer cells, CircDENND4C, MiR-760, GLUT1, Glycolysis.

Introduction

Colorectal cancer is a common malignancy that recurs after treatment in up to 40% of patients, usually with local tissue metastases to the liver or lung[1]. Colorectal cancer cells spread at the time of recurrence with a dormant period, although approximately 80% of recurrences occur in the first 3 years after radical surgery, but they may also recur several years after radical surgery[2]. Hence, improving the quality of effective screening for colorectal cancer can better prevent the occurrence of colorectal cancer and death[3]. Advances in molecular technology have made it possible for patients to choose better therapies, and molecular mutation analysis should be used as a guide in diagnosis[4]. Preventing recurrence is also very important to ensure the recovery of patients, but the mechanism for treating relapse is poorly understood, and different patients need specific treatment[5]. Therefore, it is of considerable significance to explore the molecular mechanism of colorectal cancer.

Circular RNAs (circRNAs) are a kind of non-coding RNAs with a stable ring structure, with Exonic circRNAs, Intronic circRNAs (ciRNAs) and exonic circRNAs with introns (EIciRNAs) three types. Sun et al[6] indicated the functional diversity of circRNAs in gastrointestinal tumors. It was reported that circRNA-100338 affected the mammalian target of rapamycin (mTOR) signaling pathway in liver cancer and it was associated with poor prognosis of cancer[7]. Meanwhile, a previous report indicated that circRNA_102171 accelerated the progression of papillary thyroid cancer through

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Wnt/β-catenin pathway activated by cell self-renewal by directly targeting catenin beta interacting protein 1 (CTNNBIP1)-dependent way[8]. Jin et al[9] also showed that circular RNA circRNA_100876 inhibited the proliferation of osteosarcoma cancer cells by interacting with microRNA-136. Furthermore, the silence of circDENND4C suppressing glycolysis, migration and invasion in breast cancer cells by regulating miR-200b and miR-200c in a hypoxic environment was also reported[10]. Verduci et al[11] investigated the interaction between circRNAs and miRNAs in cancer diagnosis and treatment. However, the precise mechanism by which circDENND4C affected the progression of colorectal cancer cells remains poorly defined. MicroRNAs (miRNAs) are a group of RNA sequences with the length of 21-23 ribonucleic acid nucleotides encoded by endogenous genes, which participate in the regulation of the post-transcriptional gene expression in animals and plants[12]. Abulizi et al[13] showed that circ_0071662 was regarded as a novel tumor suppressor and it could inhibit the proliferation and invasion of bladder cancer cells by directly targeting miR-146b-3p. Yang et al[14] indicated that increased expression of miR-760 could further suppress the proliferation of pancreatic cancer cells, while it could promote cell apoptosis and improve the sensitivity of pancreatic cancer cells to gemcitabine. However, the function and related signaling pathways of miR-760 in colorectal cancer cells need further research.

Glucose transporter-1 (GLUT1) is a key glucose transporter involved in glycolysis in mammalian cells, and is often expressed at high levels in many tumors in order to maintain rapid cell growth, such as hepatocellular carcinoma (HCC) cells, colon cancer cells, and Human Papilloma Virus (HPV)-induced head and neck or cervical cancer[15-17]. Hu et al[15] showed that miR-455-5p could inhibit tumor growth and migration by damaging glycolysis through GLUT1 in HCC cells. In addition, circRNA hsa_circRNA_100290 acted as a ceRNA of miR-378a to affect oral squamous cell carcinoma cells progression through GLUT1 and glycolysis pathway[18]. These reserches suggested that GLUT1 acted as an essential factor in regulating the development of cancer.

In this study, the functional effects of circDENND4C on proliferation, migration, and glycolysis of colorectal cancer cells were researched by gain-of-function and loss-of-function experiments. The targeting relationship between circDENND4C and miR-760 or miR-760 and GLUT1 was firstly investigated.

Patients and Methods

Patients and Specimens

Colorectal cancer samples and normal tissues were obtained from the Shanghai Eighth People Hospital (Shanghai, China). Prior to this study, colorectal cancer patients were not treated with any other treatment. The written informed consent was signed by every colorectal cancer patient, and the research was approved from the Ethics Committee of Shanghai Eighth People Hospital.

Cell Culture and Cell Transfection

The normal human colorectal cell line HIEC6 and the colorectal cancer cell lines (SW480 and HCT116) were purchased from the Beijing Concorde Cell Library (Beijing, China). Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) was used to cultivate cells and the medium was added with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Waltham, MA, USA) for cell growth.

Short hairpin RNA (shRNA) targeting circDENND4C (sh-circDENND4C), sh-GLUT1, and shRNA negative control (sh-NC) were constructed by Sangon Biotechnology (Shanghai, China). The full length of GLUT1 (Sangon Biotechnology, Shanghai, China) was subcloned into pcDNA3.1 plasmid (Genomeditech, Shanghai, China) to establish the pcDNA-GLUT1 (GLUT1). Anti-miR-760 and the negative control Anti-NC were obtained from Biomics (Jiangsu, China). The transfection of colorectal cancer cells was using Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA).

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

The total RNA from colorectal cancer tissues and cells was extracted by the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Next, the reversed transcription was performed by RT-PCR kit (Invitrogen, Carlsbad, CA, USA). The ABI SYBR Green Master Mix (Invitrogen, Carlsbad, CA, USA) was carried out for the qRT-PCR. The primers used in this research were as follows: F-5'-GGGGCAGCAGTATTGTGAAA-3' and R-5'-AAGACTGTGTGCTCCCCATT-3' for circDENND4C. F-5'-TCATGAAGTGTGACGTGGACATC-3' and R-5'-CAGGAGGAGCAATGATCTTGATCT-3' for β-actin[10]. F-5'-TATTGCTTAAGAATACGCGTAG-3' and R-5'-AACTCCAGCAGGACCATGTGAT-3' for miR-760. F-5'-CTCGCTTCGGCAGCACA-3' and R-5'-AACGCTTCACGAATTTGCGT-3'

for U6[19]. F-5'-ATGGATCCCAGCAGCAAGAAG-GTGACGGGC-3' and R-5'-GATGCCAACGAC-GATTCCCAGCT-3' for GLUT1[20]. β-actin and U6 acted as the internal parameters.

Western Blot Analysis

The RIPA lysis buffer (Sangon Biotech, Shanghai, China) was used to dissolve protein samples. Then, the proteins were separated and incubated with 5% skimmed milk, the membrane was incubated with primary anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (1:3000, ab8245, Abcam, Cambridge, MA, USA), anti-GLUT1 antibody (1:1000, ab40084, Abcam, Cambridge, MA, USA), anti-the proliferation-related protein (Cyclin D1) antibody (1:1000, ab134175, Abcam, Cambridge, MA, USA), anti-matrix metallopeptidase 9 (MMP-9) antibody (1:1000, ab38898, Abcam, Cambridge, MA, USA) at 4°C overnight. Finally, the goat anti-rabbit secondary antibody (1:5000, ab150077, Abcam, Cambridge, MA, USA) was mixed and the chemiluminescent image was detected by Kodak film developer (Fujifilm, Tokyo, Japan).

Cell Counting Kit 8 (CCK-8) Assay

The proliferation of SW480 cells and HCT116 cells was evaluated by using CCK-8 (ACMEC Biochemical, Shanghai, China), the cells were digested with trypsin after the SW480 cells and HCT116 cells were cultivated and collected. Then, 10 μL CCK-8 solution was used for the transfected cells and cultivated for 4 h. Finally, the microplate reader (Bio-Rad, Hercules, CA, USA) was employed to measure the optical density at 450 nm.

Transwell Assay

SW480 cells and HCT116 cells were collected 48 h after transfection and suspended in serum-free medium for cell migration detection, and the chambers (Corning, Corning, NY, USA) did not require any treatment. Then, SW480 cells and HCT116 cells were cultured into the upper chamber and incubated for 12 h. Next, cells were cultivated with 0.1% crystal violet (Corning, Corning, NY, USA) for 20 min, and then, the cells on the upper layer of the chamber were removed. Finally, the number of cells in three random regions was examined with an inverted optical microscope.

Glucose Uptake and Lactate Production

The SW480 cells and HCT116 cells (1×10^5/well) with transfection or non-transfection were cultivated into 6-well plates overnight. Next, the Glucose Assay Kit (Amyjet Scientific, Wuhan, China) and the Lactate Assay Kit (Amyjet Scientific, Wuhan, China) were used to detecting the glucose uptake and lactate production.

Dual-Luciferase Reporter Assay

The wild type circDENND4C sequences (WT-circDENND4C), mutant type circDENND4C sequences (MUT-circDENND4C), wild type 3'Untranslated Region (UTR) GLUT1 sequence (WT-GLUT1) or mutant type 3'UTR GLUT1 sequence (MUT-GLUT1) were obtained and then cloned into pGL-3 plasmid (Hedgehog Bio, Shanghai, China). Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA) was used to co-transfect the reporter plasmid and miR-760 or miR-NC. Finally, the Dual-Luciferase reporter assay system (Genomeditech, Shanghai, China) was used for measuring luciferase activity.

Animal Experiments

The 4-week-old BALB/c male nude mice (Guangdong Medical Lab Animal Center, Guangzhou, China) were raised in a sterile environment for operations. Then, the SW480 cells (2×10^6) transfected with sh-circDENND4C or sh-NC were suspended by phosphate buffer saline (PBS), and the cells inoculated subcutaneously into the nude mice. Finally, the xenograft colorectal tumor volume was detected every 5 d and the tumor weight was detected in 20 d. The animal experiment was approved by the Animal Experimentation Ethics Committee of Shanghai Eighth People Hospital.

Statistical Analysis

The data from three independent experiments were presented as mean ± standard deviation (SD). The software GraphPad Prism 7 (La Jolla, CA, USA) was performed for statistical analysis. The significant difference was calculated by Student's *t*-test or one-way analysis of variance (ANOVA) with Tukey's post-hoc test. $*p<0.05$ exhibited a statistical significance.

Results

CircDENND4C and GLUT1 Were Upregulated in Colorectal Cancer Cells

To begin with, our result found that the expression of circDENND4C was increased in colorectal cancer tissues (Figure 1A). Meanwhile, circDENND4C was significantly upregulated in colorectal cancer cells (SW480 cells and HCT116 cells) (Fig-

ure 1B). Furthermore, the level of GLUT1 was also measured by qRT-PCR and Western blot. Similarly, the GLUT1 was markedly upregulated in colorectal cancer tissues and cells (Figure 1C-F). These results indicated that circDENND4C and GLUT1 might play a crucial role in colorectal cancer cells.

Knockdown of CircDENND4C Inhibited Cell Proliferation, Migration and Glycolysis

Based on these reports, we further explored the functions of circDENND4C in colorectal cancer cells. Firstly, the sh-circDENND4C was established and qRT-PCR was used to detect the transfection efficiency. The expression of circDENND4C was decreased in SW480 cells and HCT116 cells transfected with sh-circDENND4C (Figure 2A). Then, CCK-8 assay showed that the proliferation of SW480 cells and HCT116 cells transfected with sh-circDENND4C were markedly inhibited (Figure 2B). In addition, the cell migration was detected by transwell assay and the results showed that the migration cell number of SW480 cells and HCT116 cells transfected with sh-circDENND4C were less than control (Figure 2C and D). Meanwhile, the SW480 cells and HCT116 cells transfected with sh-circDENND4C generated a reduction in the levels of Cyclin D1 and MMP-9 (Figure 2E and F). Finally, the glucose uptake Assay Kit and the lactate production Assay Kit were performed. The glucose uptake and the lactate production in SW480 cells and HCT116 cells transfected with sh-circDENND4C were decreased (Figure 2G and H). These data suggested that circDENND4C promoted the proliferation, migration, and glycolysis of SW480 cells and HCT116 cells.

Knockdown of GLUT1 Inhibited Cell Proliferation, Migration and Glycolysis

To investigate the functions of GLUT1 in SW480 cells and HCT116 cells, we constructed the sh-GLUT1; the transfection efficiency of sh-GLUT1 in W480 cells and HCT116 cells was detected by Western blot (Figure 3A). Subsequently, the proliferation of W480 cells and HCT116 cells transfected with sh-GLUT1 was detected by CCK-8, and the results indicated that the cell proliferation was downregulated (Figure 3B). Similarly, transwell assay also showed that the migration of W480 cells and HCT116 cells transfected with sh-GLUT1 was inhibited (Figure 3C). To further determine the effects of GLUT1 on cell proliferation and migration, Western blot assay was performed to detect the protein levels of Cyclin D1 and MMP-9. Results showed that the expression of Cyclin D1 and MMP-9 was decreased in W480 cells and HCT116 cells transfected with sh-GLUT1 (Figure 3D). Besides, we also detected that the glucose uptake and the lactate production in SW480 cells and HCT116 cells transfected with sh-GLUT1 were inhibited (Figure 3E and F).

Overexpression of GLUT1 Reversed the Effects of CircDENND4C Knockdown on Cell Proliferation, Migration and Glycolysis

We next explored the functional relationship between circDENND4C and GLUT1; firstly, the transfection efficiency of pcDNA-GLUT1 (GLUT1) in SW480 cells and HCT116 cells was detected by Western blot (Figure 4A). Then, cells were co-transfected with sh-circDENND4C and GLUT1 to investigate the proliferation, migration, and glycolysis of SW480 cells and HCT116 cells. The data indicated that overexpression of GLUT1 reversed the inhibition effects caused by sh-circDENND4C on cell proliferation and migration (Figure 4B and C). Meanwhile, the expression of Cyclin D1 and MMP-9 was higher in SW480 cells and HCT116 cells transfected with sh-circDENND4C + GLUT1 than cells transfected with sh-circDENND4C (Figure 4D and E). Finally, the reduction of the glucose uptake and the lactate production in SW480 cells and HCT116 cells transfected with sh-circDENND4C was also reversed by sh-circDENND4C + GLUT1 (Figure 4F and G).

MiR-760 Acted as a Target of CircDENND4C and MiR-760 Could Bind to GLUT1

MiR-760 was predicted to have potential binding sites of WT-circDENND4C and WT-GLUT1-3'UTR by the starBase 3.0 and TargetScan (Figure 5A). Then, the Dual-Luciferase reporter assay was used to confirm the relationship between WT-circDENND4C and miR-760, WT-GLUT1-3'UTR and miR-760, and the results showed that miR-760 strongly reduced the Luciferase activity of WT-circDENND4C and WT-GLUT1 in SW480 cells and HCT116 cells, but the Luciferase activity of MUT-circDENND4C and MUT-GLUT was not affected (Figure 5B). Notably, qRT-PCR was performed to detect the expression of miR-760. The results showed that miR-760 was upregulated in W480 cells and HCT116 cells transfected with sh-circDENND4C (Figure 5C). Next, the Anti-miR-760 was obtained and transformed into

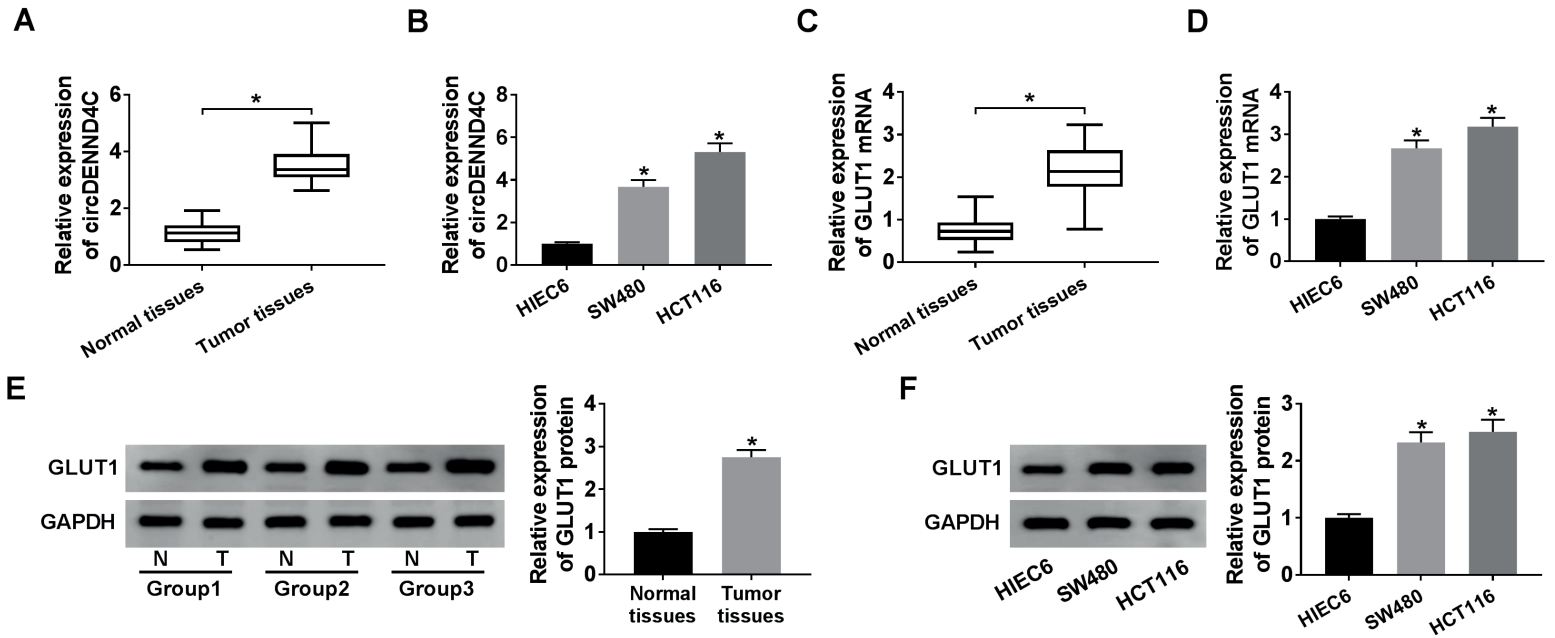

Figure 1. The levels of circDENND4C and GLUT1 were increased in colorectal cancer cells. **A,** and **C,** The expression of circDENND4C and GLUT1 in colorectal cancer tissues and adjacent normal tissues was detected by qRT-PCR. **B,** and **D,** The levels of circDENND4C and GLUT1 in colorectal cancer cells (SW480 cells and HCT116 cells) and normal cells (HIEC6 cells) were measured by qRT-PCR. **E,** and **F,** Western blot was used to detect the levels of GLUT1 in colorectal cancer tissues and cells. *$p < 0.05$.

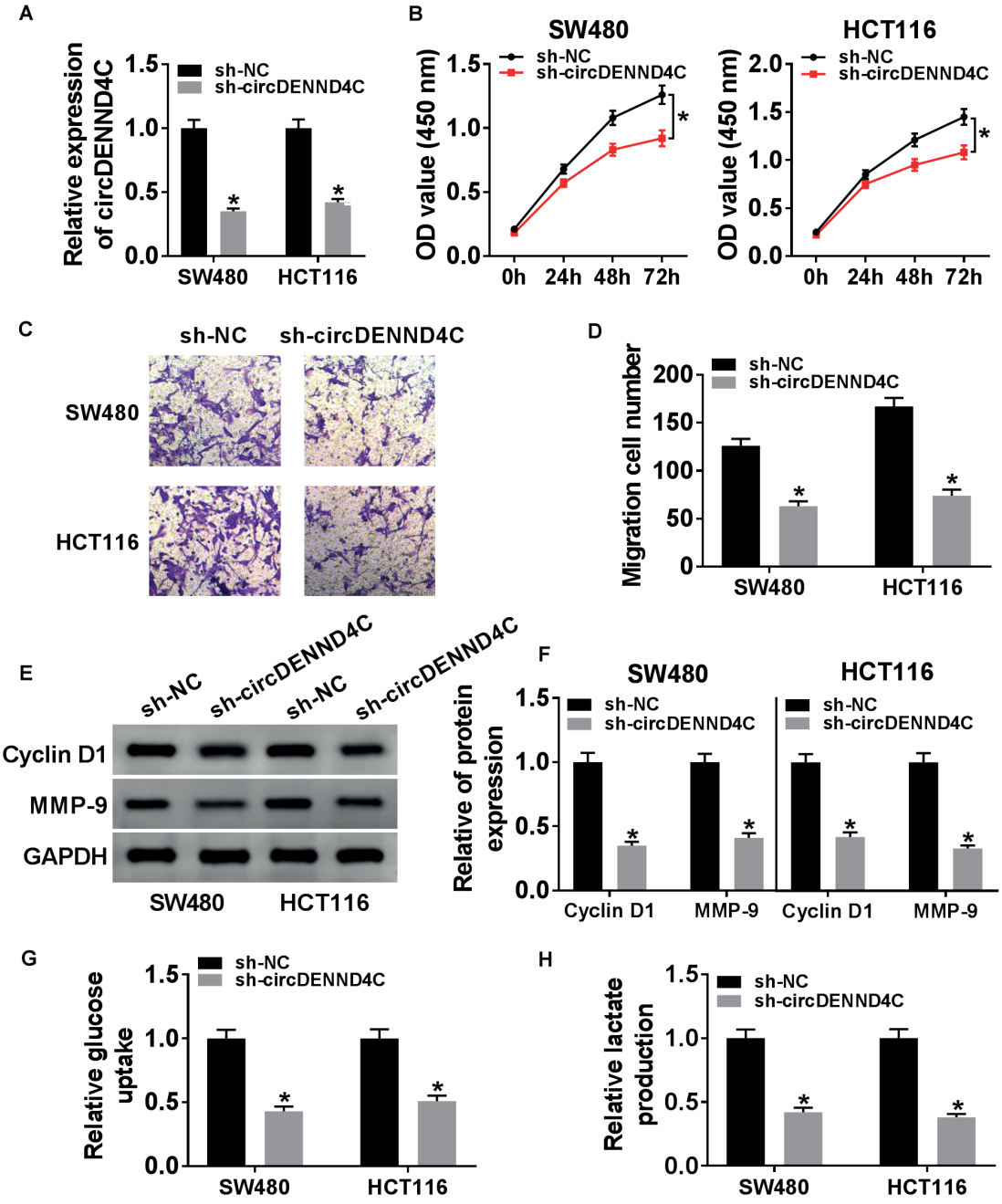

Figure 2. Knockdown of circDENND4C suppressed proliferation, migration and glycolysis in colorectal cancer cells. **A,** QRT-PCR was used to detect the transfection efficiency of sh-circDENND4C in SW480 cells and HCT116 cells. **B,** CCK-8 assay was used for measuring the proliferation of SW480 cells and HCT116 cells transfected with sh-NC or sh-circDENND4C. **C,** and **D,** Transwell assay revealed the migration of SW480 cells and HCT116 cells transfected with sh-NC or sh-circDENND4C (x200). **E,** and **F,** The protein levels of Cyclin D1 and MMP-9 in SW480 cells and HCT116 cells transfected with sh-NC or sh-circDENND4C were measured by Western blot. **G,** and **H,** The special commercial kits were used to detect the glucose uptake and lactate production of SW480 cells and HCT116 cells transfected with sh-NC or sh-circDENND4C. *$p < 0.05$.

SW480 cells and HCT116 cells, and the transfection efficiency of Anti-miR-760 was detected by qRT-PCR (Figure 5D). Notably, the protein and mRNA levels of GLUT1 were measured by Western blot and qRT-PCR. The result indicated that GLUT1 was increased in W480 cells and HCT116 cells transfected with Anti-miR-760 (Figure 5E and F).

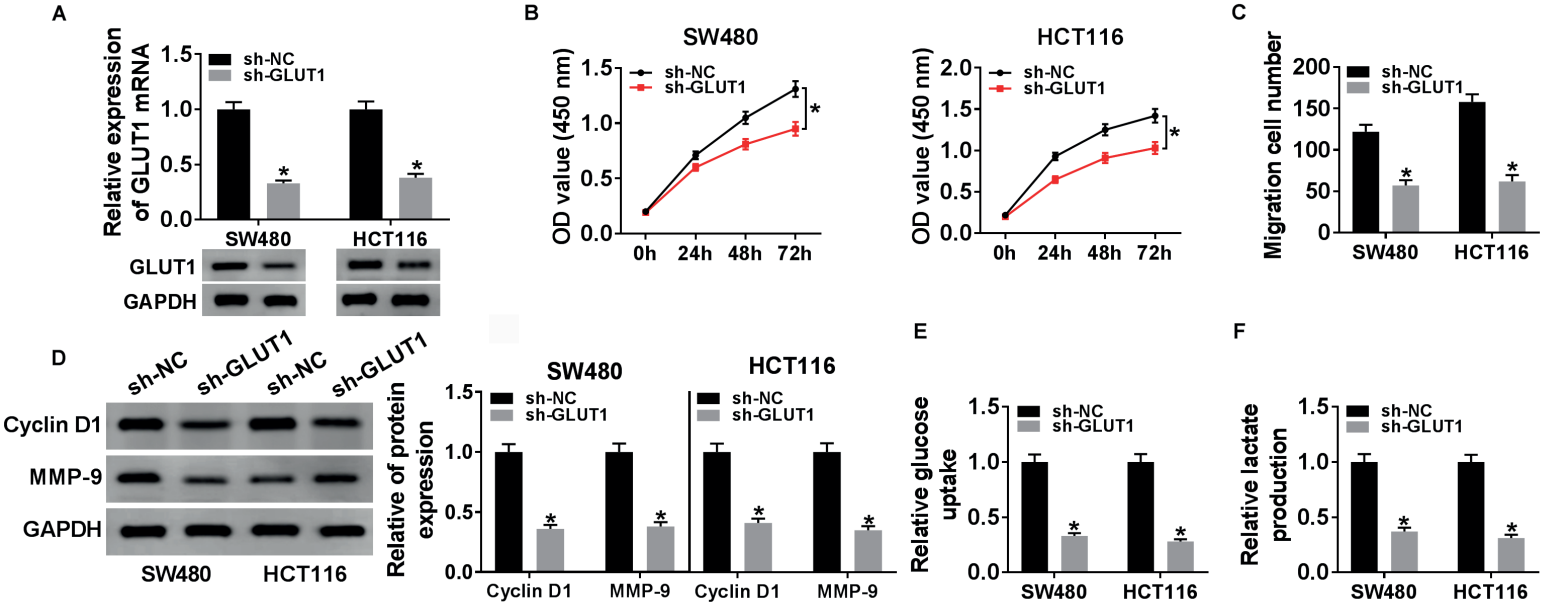

Figure 3. Silence of GLUT1 inhibited colorectal cancer cells proliferation, migration and glycolysis. **A,** The transfection efficiency of sh-GLUT1 in SW480 cells and HCT116 cells was detected by Western blot. **B,** CCK-8 assay was performed to measure the proliferation of SW480 cells and HCT116 cells transfected with sh-NC or sh-GLUT1. **C,** The migration of SW480 cells and HCT116 cells transfected with sh-NC or sh- GLUT1 was detected by transwell assay. **D,** The levels of Cyclin D1 and MMP-9 in SW480 cells and HCT116 cells transfected with sh-NC or sh-GLUT1 were detected by Western blot. **E,** and **F,** The glucose uptake and lactate production of SW480 cells and HCT116 cells transfected with sh-NC or sh-circDENND4C were measured by the special commercial kits. *$p < 0.05$.

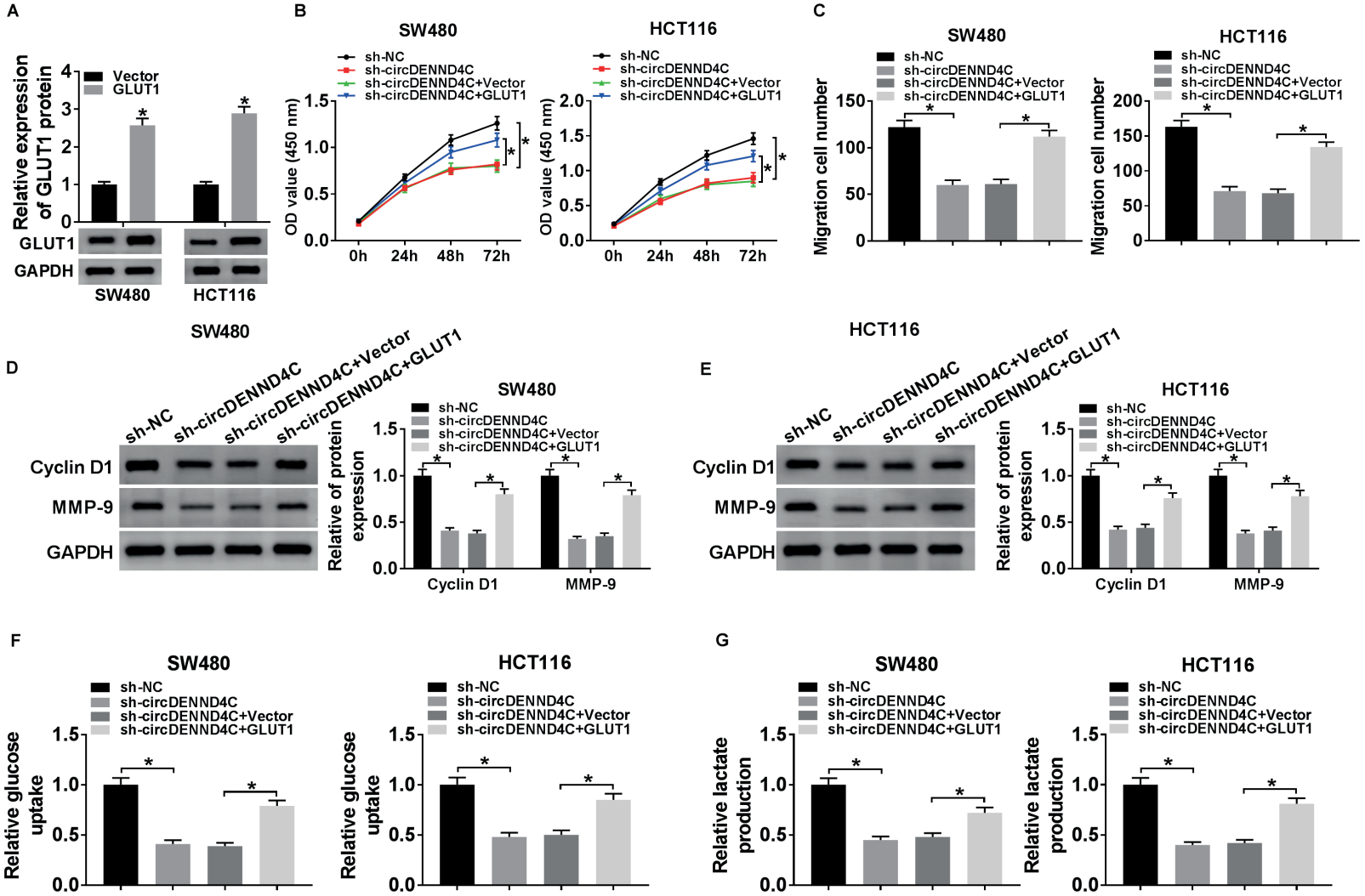

Figure 4. Overexpression of GLUT1 reversed the effects of circDENND4C knockdown on cell proliferation, migration and glycolysis. **A,** Western blot was used to detect the transfection efficiency of pcDNA-GLUT1 (GLUT1) in colorectal cancer cells. **B,** The proliferation of SW480 cells and HCT116 cells transfected with sh-circDENND4C + GLUT1 was detected by CKK-8 assay. **C,** Transwell assay was used to detect the migration of SW480 cells and HCT116 cells transfected with sh-circDENND4C + GLUT1. **D,** and **E,** The protein levels of Cyclin D1 and MMP-9 in SW480 cells and HCT116 cells transfected with sh-circDENND4C + GLUT1 were measured by Western blot. **F,** and **G,** The glucose uptake and lactate production of SW480 cells and HCT116 cells transfected with sh-circDENND4C + GLUT1 were measured by the special commercial kits. *$p < 0.05$.

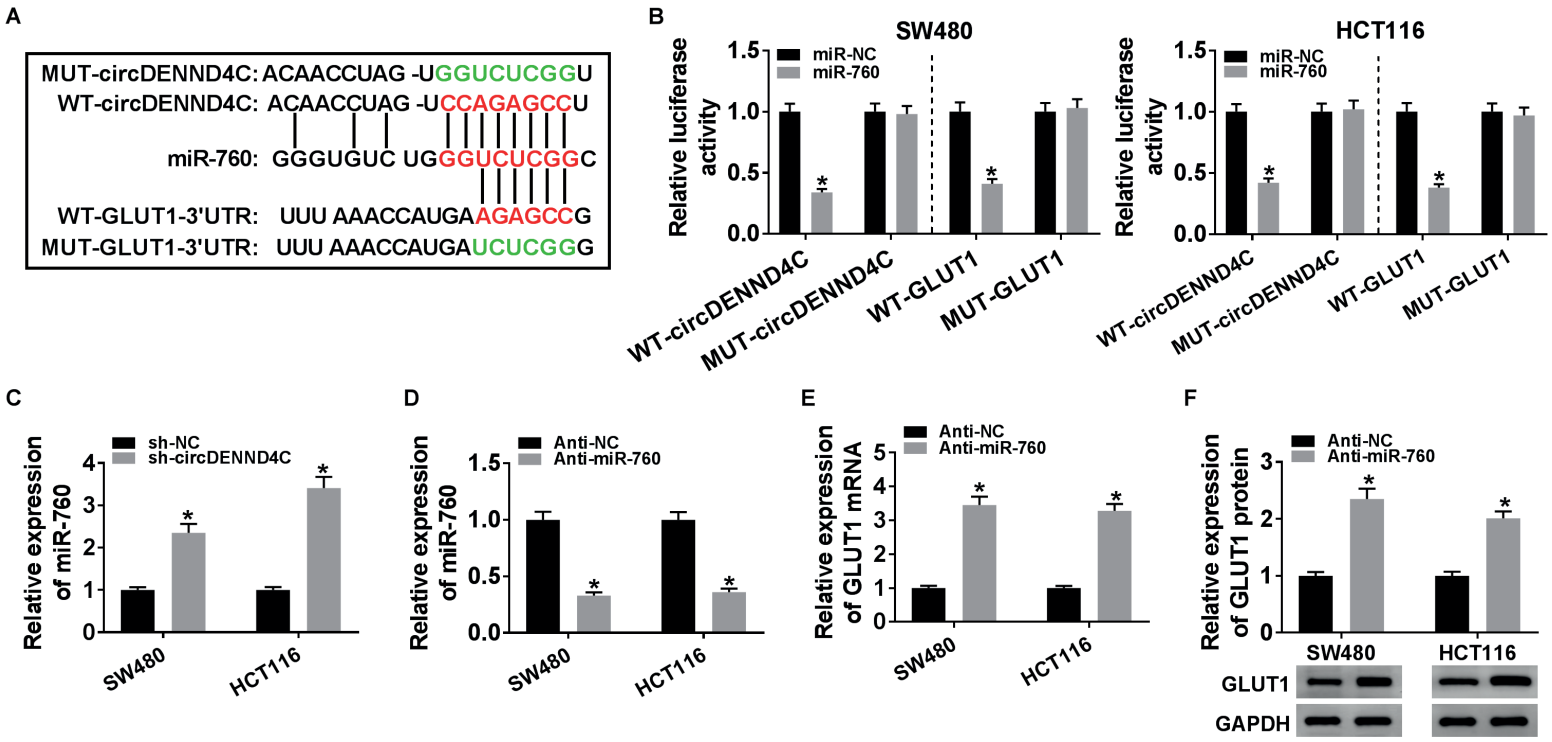

Figure 5. MiR-760 acted as a target of circDENND4C and miR-760 could bind to GLUT1. **A,** The potential binding sites between WT-circDENND4C and miR-760, WT-GLUT1-3'UTR and miR-760 were predicted by the starBase 3.0 and TargetScan. **B,** Dual-Luciferase reporter assay was performed to detect the luciferase activity of WT-circDENND4C or MUT-circDENND4C, WT-GLUT1 or MUT-GLUT1 reporter plasmid in SW480 cells and HCT116 cells transfected with miR-NC or miR-760. **C,** QRT-PCR was used to detect the level of miR-760 in SW480 cells and HCT116 cells transfected with sh-circDENND4C. **D,** The transfection efficiency of Anti-miR-760 in SW480 cells and HCT116 cells was detected by qRT-PCR. **E,** and **F,** The expression of GLUT1 in SW480 cells and HCT116 cells transfected with Anti-miR-760 was detected by qRT-PCR and Western blot. *$p < 0.05$.

CircDENND4C Regulated GLUT1 by Sponging MiR-760

To further elucidate the functional mechanism of circDENND4C, miR-760, and GLUT1, the level of GLUT1 was detected by Western blot. The results showed that GLUT1 was downregulated in W480 cells and HCT116 cells transfected with sh-circDENND4C, while in W480 cells and HCT116 cells transfected with sh-circDENND4C + Anti-miR-760, the effect of sh-circDENND4C on the level of GLUT1 was reversed (Figure 6A). Moreover, CCK-8 assay and transwell assay were performed, and the results indicated that the proliferation and migration of W480 cells and HCT116 cells transfected with sh-circDENND4C + Anti-miR-760 were increased compared with cells transfected with sh-circDENND4C (Figure 6B and C). In addition, Western blot showed that the knockdown of miR-760 also reversed the effects of circDENND4C silence on the protein levels of Cyclin D1 and MMP-9 (Figure 6C and D). Finally, the transfection of Anti-miR-760 also abolished the inducting effects of sh-circDENND4C on the glucose uptake and lactate production (Figure 6E and F). These data suggested that miR-760 inhibition reversed the effects of circDENND4C knockdown in W480 cells and HCT116 cells, and circDENND4C regulated the functions of GLUT1 by targeting miR-760.

Silence of CircDENND4C Decreased the Growth of Colorectal Cancer Cells In Vivo

To further investigate the functional effects of circDENND4C *in vivo*, W480 cells transfected with sh-circDENND4C were inoculated subcutaneously into the nude mice to establish the xenograft tumor. The tumor volume was measured every 5 d and the results indicated that the tumor volume was markedly reduced in xenograft tumor (Figure 7A). Then, the xenograft tumor weight appeared with an evident reduction detected in d 20 (Figure 7B). Finally, qRT-PCR was used to detect the expression of circDENND4C, miR-760, and GLUT1. The results showed that circDENND4C and GLUT1 were downregulated while miR-760 was upregulated in xenograft tumor (Figure 7C), and Western blot further indicated that the protein level of GLUT1 was reduced in xenograft tumor (Figure 7D).

Discussion

Colorectal cancer is the most common malignant tumor with high mortality and easy recurrence worldwide[21]. CircRNA might be a potential biomarker for the diagnosis and treatment of tumors in the future. Liang et al[22] indicated that circDENND4C was a hypoxia-inducible factor 1 alpha (HIF1α)-associated circRNA and it could promote the proliferation of breast cancer cells in a hypoxic environment. In our study, the functions of circDENND4C in colorectal cancer were firstly explored, and our results indicated that circDENND4C was upregulated in colorectal cancer tissues and cells. Notably, circDENND4C knockdown inhibited cell proliferation, migration, and glycolysis. Cyclin D1 was an effective marker to identify tumor and reactive the tumor proliferation[23]. MMP-9 acted as a crucial marker for migration and invasion in breast cancer[24-26]. Meanwhile, the expression level of GLUT1 was also increased in colorectal cancer tissues and cells, which was consist of the previous research[27]. GLUT1 was reported to be associated with the process of glycolysis, and silence of GLUT1 suppressed the proliferation, migration, and glycolysis of colorectal cancer tissues[18]. Our study also indicated that the overexpression of GLUT1 reversed the effects of circDENND4C knockdown on cell proliferation, migration, and glycolysis. But the molecular mechanism between circDENND4C and GLUT1 was unclear.

Notably, the online software starBase 3.0 and TargetScan were performed to predict the potential binding sites of circDENND4C and miR-760, GLUT1-3'UTR, and miR-760. The interaction of circRNA, miRNA, and mRNA provided a new perspective for a more detailed understanding of the pathogenesis of cancer and also provided a new reference for the discovery of innovative therapeutic strategies[28,29]. Kong et al[30] showed that miRNA-760 suppressed the biological progress of colorectal cancer cells through targeting Forkhead-box A1 transcription factor (FOXA1) and affecting epithelial-to-mesenchymal transition (EMT) process and the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) signaling pathway. In colorectal cancer cells, lncRNA KCNQ1OT1 was proved to enhance the methotrexate resistance by directly regulating miR-760 through the cyclic adenosine monophosphate (cAMP) signaling pathway[31]. Meanwhile, lncRNA SNHG6 acted as a ceRNA to accelerate the proliferation, invasion, and migration of colorectal cancer by regulating miR-760 and activating forkhead box C1 (FOXC1)[32]. Our study demonstrated that

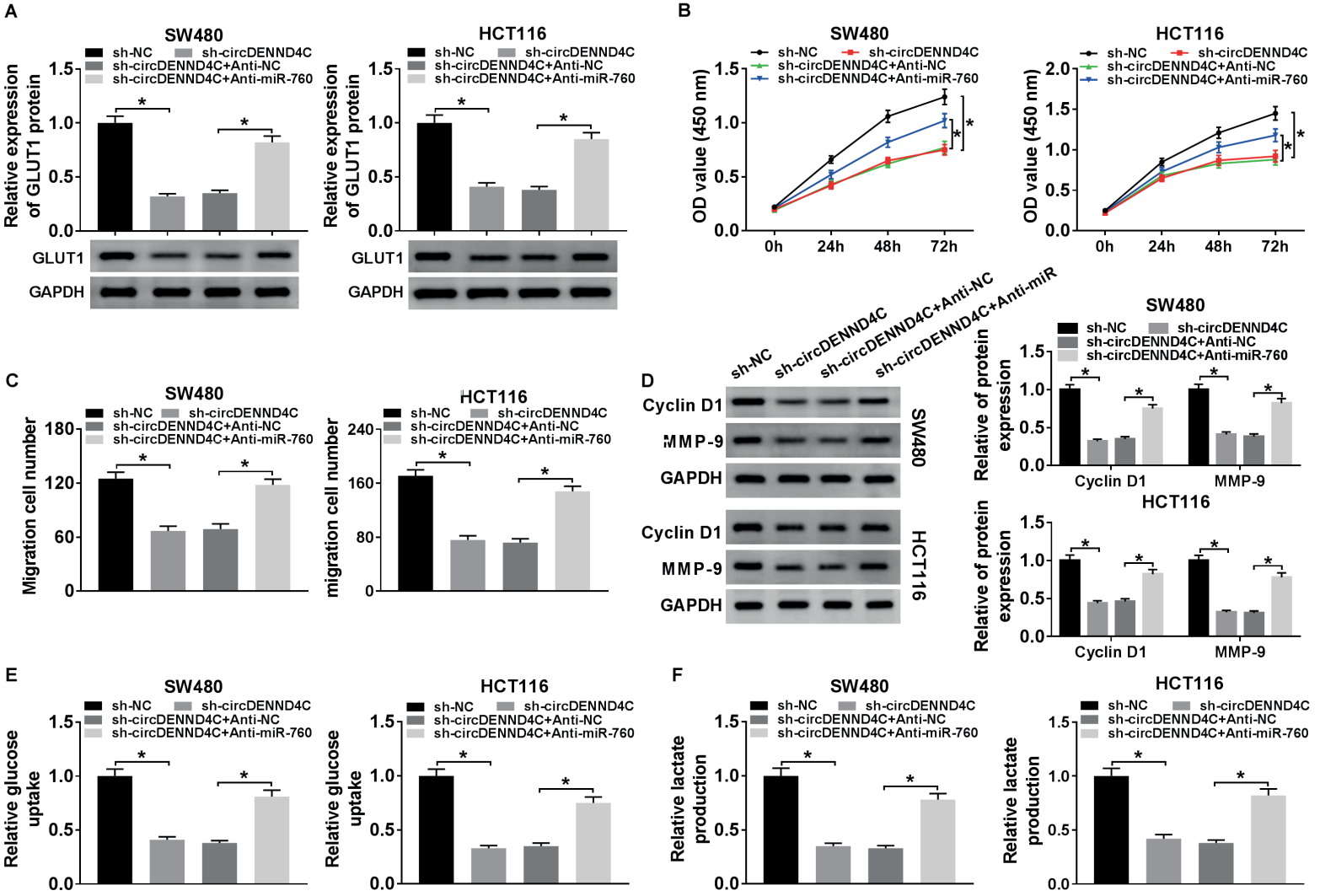

Figure 6. CircDENND4C regulated GLUT1 by sponging miR-760. **A,** Western blot was used to detect the expression of GLUT1 in SW480 cells and HCT116 cells transfected with sh-circDENND4C or sh-circDENND4C + Anti-miR-760. **B,** and **C,** The proliferation and migration of SW480 cells and HCT116 cells transfected with sh-circDENND4C or sh-circDENND4C + Anti-miR-760 were detected by CKK-8 assay and transwell assay. **D,** The protein levels of Cyclin D1 and MMP-9 in SW480 cells and HCT116 cells transfected with sh-circDENND4C or sh-circDENND4C + Anti-miR-760 were measured by Western blot. **E,** and **F,** The Assay Kits were used to measure the glucose uptake and lactate production of SW480 cells and HCT116 cells transfected with sh-circDENND4C or sh-circDENND4C + Anti-miR-760. *$p < 0.05$.

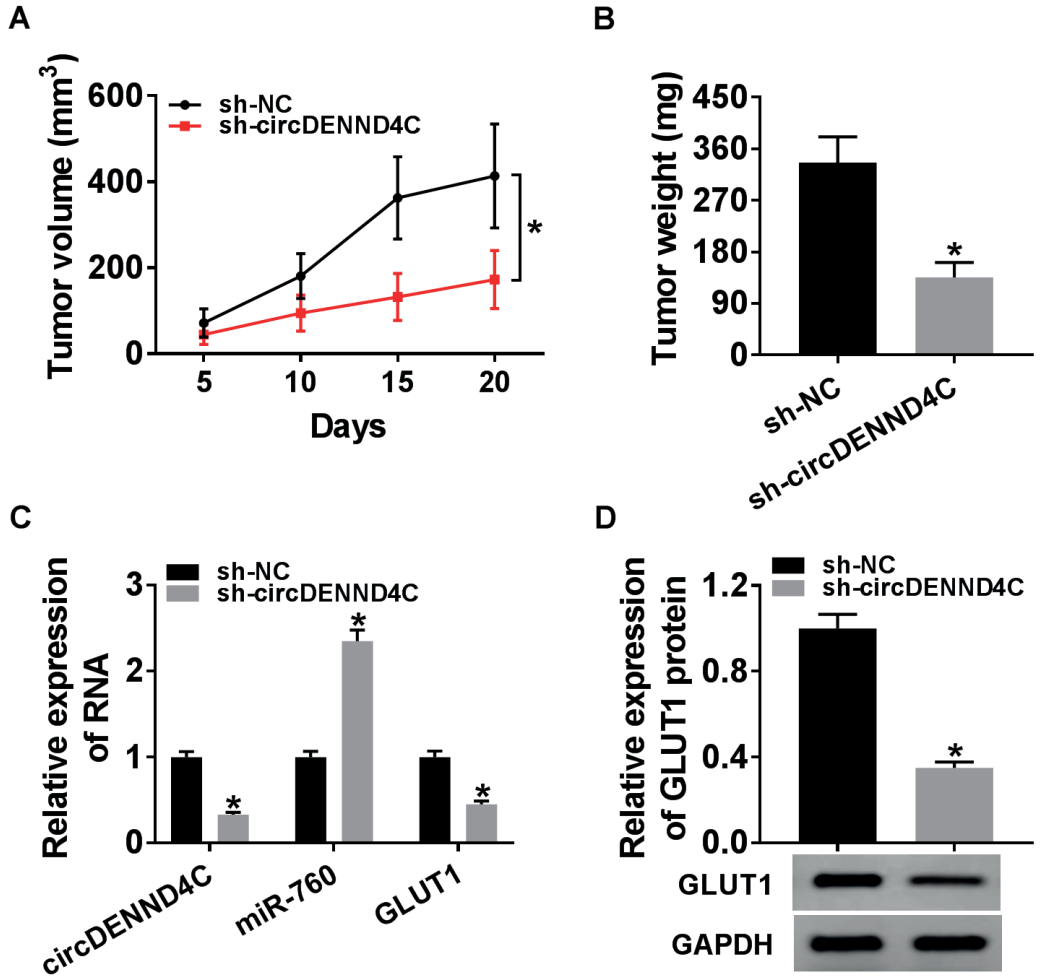

Figure 7. Silence of circDENND4C decreased the growth of colorectal cancer cells *in vivo*. SW480 cells transfected with sh-circDENND4C were inoculated subcutaneously into the nude mice to establish the xenograft tumor. **A,** The tumor volume was measured every 5 d after injection. **B,** Tumor weight was measured on d 20. **C,** QRT-PCR was used to detect the levels of circDENND4C, miR-760 and GLUT1 in xenograft tumor. **D,** Western blot was performed to detect the protein level of GLUT1 in xenograft tumor. *$p < 0.05$.

circDENND4C regulated the effects of GLUT1 on proliferation, migration, and glycolysis of colorectal cancer cells by sponging miR-760. Santasusagna et al[20] indicated that miR-328 regulated a metabolic shift through solute career family 2 member 1 (SLC2A1)/GLUT1 pathway in colon cancer cells. CircRNA_100290 acted as a ceRNA for miR-378a to mediate the progression of oral squamous cell carcinoma cells through GLUT1 and glycolysis[18]. Similarly, our results also demonstrated that circDENND4C affects proliferation and migration by regulating glycolysis of colorectal cancer cells.

Conclusions

These study results indicated that the levels of circDENND4C and GLUT1 were increased and circDENND4C or GLUT1 knockdown could reduce the proliferation, migration, and glycolysis of colorectal cancer cells. Furthermore, circDENND4C also promoted colorectal cancer cell growth *in vivo*. Further, the overexpression of GLUT1 reversed the effects of downregulation of circDENND4C. CircDENND4C could directly bind to miR-760, and miR-760 was upregulated in colorectal cancer cells transfected with sh-circDEN-

ND4C. GLUT1 also acted as a target of miR-760, and downregulation of miR-760 increased the level of GLUT1. Notably, we found that circDENND4C regulated the functions of GLUT1 by sponging miR-760. Collectively, our results revealed that circDENND4C knockdown inhibited colorectal cancer cell proliferation, migration, and glycolysis by regulating GLUT1 mediated by miR-760. These results provided a reference for finding new diagnostic targets and therapeutic strategies for colorectal cancer cells and provided a basis for a better understanding of the pathophysiology of colorectal cancer cells.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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