Submitted: 22 January, 2024 Accepted: 18 March, 2024 Published: 15 April, 2024 DOI:10.22514/ejgo.2024.037

ORIGINAL RESEARCH

European Journal of Gynaecological Oncology

Targeting CENPN to inhibit breast cancer progression: insights into cell growth and aerobic glycolysis modulation

Zhe Ye[1], Qiuyan Lin[2], Hualong Lin[2], Yiwei Huang[1,*]

[1]Department of Tumor Radiotherapy, The Third Affiliated Hospital of Wenzhou Medical University, 325200 Rui'an, Zhejiang, China
[2]Department of Oncology, The Third Affiliated Hospital of Wenzhou Medical University, 325200 Rui'an, Zhejiang, China

***Correspondence**
yw_huang0711@163.com
(Yiwei Huang)

Abstract

Breast cancer represents a significant health challenge for women globally. Although centromere protein N (CENPN) is reported to be upregulated in breast cancer, its detailed role remains unclear. This study used *in silico* analysis to investigate the expression profile and survival impact of CENPN in breast cancer. The CENPN level was evaluated using real-time quantitative polymerase chain reaction and western blot assays. The influence of CENPN on cell proliferation and aerobic glycolysis was investigated through cell counting kit-8, 5-ethynyl-2′-deoxyuridine incorporation assays, measurements of the extracellular acidification rate (ECAR), glucose uptake, lactate production, and additional western blot analyses. Moreover, *in vivo* studies on xenografted mice were conducted, utilizing immunohistochemistry and western blot techniques to evaluate the functions of CENPN. These findings revealed an upregulation of CENPN, correlating with decreased survival rates in breast cancer patients. Functionally, CENPN was shown to stimulate cell growth in both cell culture and animal models. Through gain- and loss-of-function experiments, CENPN was found to increase the ECAR, glucose uptake, lactate production, and the protein levels of glucose transporter 1 (GLUT1) and hexokinase 2 (HK2). Furthermore, CENPN was observed to increase the levels of phosphorylated protein kinase B (AKT) (p-AKT) relative to total AKT and hypoxia-inducible factor 1α (HIF-1α) in both cellular and animal models. The HIF-1α overexpression mitigated the suppressive effects of sh-CENPN on cell proliferation and aerobic glycolysis in breast cells. In conclusion, CENPN is upregulated and is related to a lower survival probability in breast cancer. It facilitates cell growth and aerobic glycolysis by upregulating AKT/HIF-1α signaling pathways. These insights provide novel perspectives for the diagnosis and treatment of breast cancer.

Keywords

Breast cancer; CENPN; Growth; Aerobic glycolysis; PI3K/AKT; HIF-1α

1. Introduction

Breast cancer is still a major global public health concern for women. Recent statistics indicate that there were an estimated 43,170 deaths and 297,790 new cases of breast cancer among women in the United States in 2023 [1], positioning breast cancer as the most prevalent among female cancers, accounting for 31% of cases, and the second leading cause of cancer-related deaths in women, representing 15% of such mortalities [1]. Additionally, both breast cancer death and incidence rates are rising in developing countries [2]. Various treatment modalities, such as breast-conserving surgery, mastectomy, hormonal therapy, chemotherapy and radiotherapy, are currently utilized in clinical settings for managing breast cancer [3, 4], and improvements in these treatments, coupled with earlier detection efforts, have significantly improved the 5-year relative survival rate for breast cancer patients [3, 5]. However, the challenges associated with late-stage diagnosis in breast cancer patients persist, largely due to gaps in early screening programs. While stage I breast cancer patients have a 5-year relative survival rate of 100%, this rate drastically drops to 28% for those diagnosed with stage IV breast cancer [3]. Thus, it is imperative to look into the molecular pathways underlying breast cancer in more detail and to find new biomarkers for the disease's early diagnosis and therapy.

Aerobic glycolysis, known as the Warburg effect, describes the phenomenon whereby cancer cells exhibiting rapid growth preferentially convert glucose to lactate in the existence of adequate oxygen [6–8]. This metabolic pathway is primarily regulated by glucose transporter 1 (GLUT1) and the three rate-limiting enzymes: phosphoglycerate kinase 1 (PGK1), lactate dehydrogenase A (LDHA), and hexokinase 2 (HK2)

[9–11]. Extensive research has demonstrated that aerobic glycolysis contributes to various aspects of cancer pathophysiology, including proliferation, apoptosis resistance, metastasis, invasion, angiogenesis, maintenance of stem cell-like characteristics, immune system evasion, and drug resistance [12–14]. Thus, targeting the mechanisms of aerobic glycolysis presents a strategic approach for developing therapeutic interventions against breast cancer.

Centromere protein N (CENPN), located on chromosome 16q23.2, is broadly distributed in the chromosomal DNA of eukaryotic cells [15]. The CENPN N-terminal and C-terminal regions are known to interact with CENP-A nucleosomes and CENP-L, respectively, facilitating the formation of a network associated with mitophagy [16]. Several studies have highlighted CENPN's association with cancer progression. Zheng *et al.* [17] demonstrated that CENPN promotes lung adenocarcinoma cell proliferation, invasion and stemness while decreasing apoptosis through the phosphatidylinositol 3 kinase (PI3K)/protein kinase B (AKT) signaling pathway. Xu *et al.* [18] reported that the downregulation of CENPN inhibits glucose metabolism in esophageal cancer cells and induces G1 arrest by affecting the PI3K/AKT pathway. Additionally, Qi *et al.* [19] found that silencing CENPN suppresses nasopharyngeal carcinoma cell proliferation and cell cycle progression and reduces glucose metabolism, leading to decreased apoptosis *via* the AKT signaling pathway in both cell culture and animal models. Moreover, increased CENPN expression has been associated with higher mortality and recurrence risk in breast cancer patients with a history of smoking [20]. Importantly, CENPN level is predicted to be aggrandized in breast cancer through *in silico* analysis and confirmed by real-time quantitative polymerase chain reaction (RT-qPCR) assays, demonstrating its significance in the prognosis of breast cancer patients [21]. However, the comprehensive role of CENPN in breast cancer requires further elucidation.

Herein, we designed this current study to elucidate the function and underlying mechanism of CENPN in breast cancer to provide new insights that could be potentially used as a referential basis for improving the diagnosis and therapeutic strategies for breast cancer.

2. Materials and methods

2.1 CENPN's expression profile in breast cancer

The GEPIA platform (http://gepia2.cancer-pku.cn) was utilized to assess the expression profile of *CENPN* on samples of para-carcinoma tissues and breast invasive carcinoma (BRCA) and to determine the influence of *CENPN* expression levels on the survival probability of breast cancer patients. The association between *CENPN* and *HIF-1*α was investigated with the Tumor Immune Estimation Resource (TIMER, https://cistrome.shinyapps.io/timer/).

2.2 Tissue specimen

Thirty pairs of breast cancer tissues and adjacent non-cancerous tissues were yielded from patients diagnosed with breast cancer at the Third Affiliated Hospital of Wenzhou Medical University. Immediately after excision, the specimens were snap-frozen in liquid nitrogen and stored at −80 °C until further analysis. The breast cancer samples were confirmed *via* pathological examinations and with no evidence of other cancer types. Furthermore, none of the participants had undergone chemoradiotherapy prior to the surgery.

2.3 RT-qPCR

Total RNA was harvested from both breast cancer tissues and adjacent non-cancerous tissues by TRIzol reagent (cat. no.: 15596026, Invitrogen, Carlsbad, CA, USA). Then, the RNA was reverse-transcribed into cDNA with the Bio-Rad Script™ cDNA Synthesis Kit (cat. no.: 1708890, Bio-Rad Laboratories, Hercules, CA, USA) in keeping with the manufacturer's protocol. qPCR was performed using the Bio-Rad CFX Manager software (3.1, Bio-Rad Laboratories, Hercules, CA, USA) and 2×SYBR Green PCR Mastermix (cat. no.: SR1110, Solarbio, Beijing, China). The specific primer sequences used were: *CENPN* forward, 5′-CACAAAGCCAAACCAGTACAAAC-3′; *CENPN* reverse, 5′-GATACCGACTTCTCAGGTCCATT-3′; β-*actin* forward, 5′-CTCCATCGTCCACCGCAAATGCTTCT-3′; and β-*actin* reverse, 5′-GCTCCAACCGACTGCTGTCACCTTC-3′.

2.4 Culture and transfection of cells

MCF7 (cat. no.: CL-0149) and MDA-MB-231 (cat. no.: CL-0150B) cell lines were purchased from Procell (Wuhan, China). The MCF7 cells were cultivated in Minimum Essential Medium (MEM, supplemented with Non-Essential Amino Acids (NEAA), cat. no.: PM150410, Procell) enriched with 10% fetal bovine serum (FBS, cat. no.: 164210, Procell, Wuhan, China), 0.01 mg/mL insulin (97% purity, cat. no.: I8830, Solarbio, Beijing, China), and 1% penicillin-streptomycin (P/S, cat. no.: PB180120, Procell, Wuhan, China) at 37 °C in 5% CO_2. The MDA-MB-231 cells were grown in DMEM medium (cat. no.: PM150210, Procell, Wuhan, China) supplemented with 10% FBS and 1% P/S solution under the same conditions.

To knock down CENPN expression, short hairpin RNA (shRNA) targeting CENPN (sh-CENPN) and a negative control (sh-NC) were purchased from GenePharma (Shanghai, China) and introduced into both MCF7 and MDA-MB-231 cells. Then, the sequences encoding CENPN or HIF-1α were cloned into pcDNA vector plasmids and transfected into MDA-MB-231 and MCF7 cells to elevate the expressions of CENPN or HIF-1α, as previously described [22]. All transfections were performed using Lipofectamine 3000 (cat. no.: L3000001, Invitrogen, Carlsbad, CA, USA). Cells were harvested 48 hours post-transfection for subsequent analyses.

2.5 Cell counting kit-8 (CCK-8) detection

Cells were seeded at a density of 5×10^3 cells per well into 96-well plates. As previously described [23], 10 μL of CCK-8 reagent (cat. no.: CA1210, Solarbio, Beijing, China) was mixed to each well and hatched at 37 °C for 2 hours. Subsequently, absorbance was detected by a microplate reader

(Multiskan SkyHigh, Thermo Fisher Scientific, Waltham, MA, USA) at 450 nm.

2.6 The 5-ethynyl-2′-deoxyuridine (EdU) incorporation experiment

The EdU incorporation assay was carried out with the BeyoClick™ EdU Cell Proliferation Kit with Alexa Fluor 647 (cat. no.: C0081S, Beyotime, Shanghai, China) following the manufacturer's instructions. Cell nuclei were counterstained with Hoechst 33342 (5 μg/mL, cat. no.: C1022, Beyotime, Shanghai, China). Fluorescence images were obtained using a fluorescence microscope (IX71, Olympus, Tokyo, Japan).

2.7 Extracellular acidification rate (ECAR) detection

For ECAR, 1×10^4 cells were seeded in Seahorse XF 96 cell culture microplates. Using the Seahorse XF Glycolysis Stress Test Kit (cat. no.: 103020-100, Seahorse Bioscience, Chicopee, MA, USA), the ECAR was measured on a Extracellular Flux analyzer (XF96, Seahorse Bioscience, Chicopee, MA, USA) with the Seahorse XF-96 Wave software (2.6, Seahorse Bioscience, Chicopee, MA, USA). Glucose, oligomycin, and 2-deoxy-D-glucose (2-DG) were sequentially appended to each well after 20, 40 and 60 minutes, respectively. ECAR readings were taken every six minutes.

2.8 Detection of glucose uptake and lactate generation

Glucose uptake and lactate production in the culture supernatants, collected 48 hours post-transfection, were quantified with a glucose assay kit (cat. no.: CBA086, Sigma, St. Louis, MO, USA) and a lactate assay kit (cat. no.: K607, Biovision, Mountain View, CA, USA) following the provided instructions.

2.9 Experiment on animals

For the *in vivo* investigations, four-week-old BALB/c nude mice (Cyagen, Jiangsu, China) were kept under specific pathogen-free (SPF) room with a controlled temperature and 12-hour light-dark cycle. The mice were stochastically apportioned into sh-NC and sh-CENPN groups, with six mice per group. The sh-CENPN group was injected subcutaneously with 1×10^7 MCF7 cells transduced with sh-CENPN [24], while the sh-NC group received an equal number of MCF7 cells transduced with sh-NC. Tumor volume was examined weekly for four weeks by the formula volume = $1/2 \times$ length $\times$ width^2. At the end of five weeks, mice were euthanized with 100 mg/kg sodium pentobarbital intraperitoneally. Tumors were collected, weighed, immobilized in 4% formaldehyde for immunohistochemistry (IHC) assays, and kept at −80 °C for western blot analysis.

2.10 IHC

The fixed tumor tissues were dehydrated in ethanol and then embedded in paraffin (cat. no.: YA0011, Solarbio, Beijing, China). Sections of 5 μm thickness were prepared, and antigen retrieval was performed with sodium citrate buffer (pH 6.0, cat. no.: P0081, Beyotime, Shanghai, China) for 15 minutes at 94 °C. The slices were blocked with 1% bovine serum albumin (BSA, cat. no.: SW3015, Solarbio, Beijing, China) for 60 minutes, followed by overnight incubation at 4 °C with primary antibodies targeted CENPN (1:100, cat. no.: PA5-100721, Thermo Fisher Scientific) and Ki-67 (1:1000, cat. no.: ab15580, Abcam, Cambridge, UK). Next, the sections were administrated with HRP-conjugated anti-rabbit IgG antibody (cat. no.: ab288151, Abcam) at 37 °C for 30 minutes and counterstained with hematoxylin. The slides were assessed by a light microscope (CX31P, Olympus, Tokyo, Japan).

2.11 Western blot

The experiment was executed as previously described [25, 26]. Briefly, MCF7 and MDA-MB-231 cells, along with tumor tissues, were lysed using RIPA buffer (cat. no.: R0010, Solarbio, Beijing, China) to yield total proteins. Protein concentration was determined using a BCA kit (cat. no.: P0012S, Beyotime, Shanghai, China) following the manufacturer's instructions. Protein samples (20 μg) were electrophoresed on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then shifted onto polyvinylidene difluoride (PVDF) membranes (cat. no.: IPVH00010, EMD Millipore, Billerica, MA, USA). The membranes were managed with primary antibodies targeting CENPN (1:2000, cat. no.: PA5-100721, Thermo Fisher Scientific), GLUT1 (1:2500, cat. no.: ab14683, Abcam), HK2 (1:20000, cat. no.: ab227198, Abcam), AKT (1:10000, cat. no.: ab227385, Abcam), phosphorylated AKT (Ser473) (1:5000, cat. no.: ab81283, Abcam), HIF-1α (1:1000, cat. no.: ab216842, Abcam), and β-actin (1:1000, cat. no.: ab8227, Abcam). Goat anti-rabbit IgG H&L (HRP) (cat. no.: ab6721, 1:10000, Abcam) was used as the secondary antibody. Bands were visualized using the ECL Western Blotting Detection Kit (cat. no.: SW2030, Solarbio, Beijing, China), and band intensity was analyzed using ImageJ software (National Institutes of Health, USA).

2.12 Statistical analysis

Statistical analysis was conducted with SPSS 26.0 software (IBM, Armonk, NY, USA). Data are displayed as mean $\pm$ standard deviation (SD). The Student's *t*-test was used for comparisons between two groups, while one-way analysis of variance (ANOVA) followed by a *post hoc* Bonferroni test was applied for comparisons among more than two groups. A p-value < 0.05 was considered statistically significant.

3. Results

3.1 CENPN is upregulated in breast cancer

To inquire the role of CENPN in breast cancer, its level was first analyzed in breast cancer tissues using *in silico* data. The GEPIA database revealed significantly higher levels of *CENPN* in breast cancer tissues relative to adjacent non-tumor tissues (Fig. 1A). Besides, elevated *CENPN* expressions were associated with poorer survival outcomes in breast cancer patients, as indicated by GEPIA data (Fig. 1B). These obser-

vations were consistent with findings from tumor samples of breast cancer patients (Fig. 1C,D), confirming that increased *CENPN* expression correlates with adverse survival rates in this population.

3.2 CENPN promoted breast cancer cell proliferation

The elevated expression of CENPN suggested its potential involvement in breast cancer progression. To investigate this further, knock-in and out studies were conducted in MCF7 and MDA-MB-231 cells. Notably, cells transfected with a CENPN overexpression plasmid exhibited a significant elevation in CENPN protein levels relative to those transfected with a control plasmid (Fig. 2A). Similarly, transfection with sh-CENPN leaded to a substantial decrease in CENPN protein levels relative to the control group (Fig. 2A). The overexpression of CENPN significantly enhanced cell viability and the number of Edu+ cells, whereas CENPN knockdown led to a noticeable reduction in these indicators in both cell lines (Fig. 2B,C). These results elucidate that CENPN plays a role in promoting the proliferation of breast cancer cells.

3.3 CENPN enhanced cell aerobic glycolysis in breast cancer cells

The influence of CENPN on aerobic glycolysis was examined in cells, and the outcomes displayed that ECAR was significantly augmented in cells overexpressing CENPN, while it significantly decreased in cells with reduced CENPN ex-

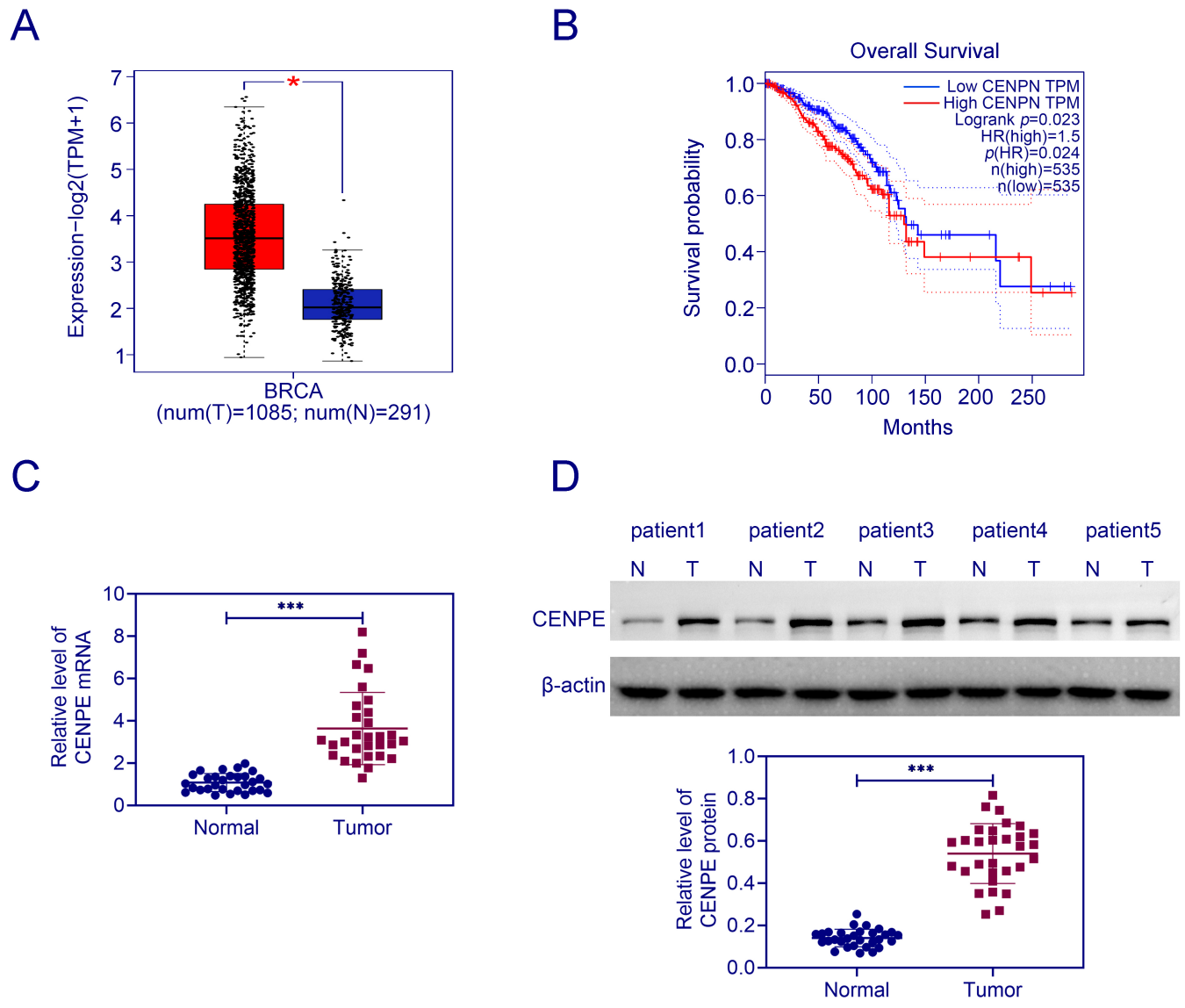

FIGURE 1. ***CENPN* is highly expressed in breast cancer.** (A) *CENPN* expression profile examined using the GEPIA datasets. (B) The survival of breast cancer patients predicated based on *CENPN* expression. (C) The relative mRNA expression of *CENPN* investigated using RT-qPCR. The outcomes were expressed using *β-actin* normalization. (D) The relative protein expression of CENPN was confirmed using western blot. The outcomes were expressed using β-actin normalization. *$p < 0.05$ and ***$p < 0.001$. CENPN: centromere protein N; mRNA: messenger RNA; RT-qPCR: real-time quantitative polymerase chain reaction.

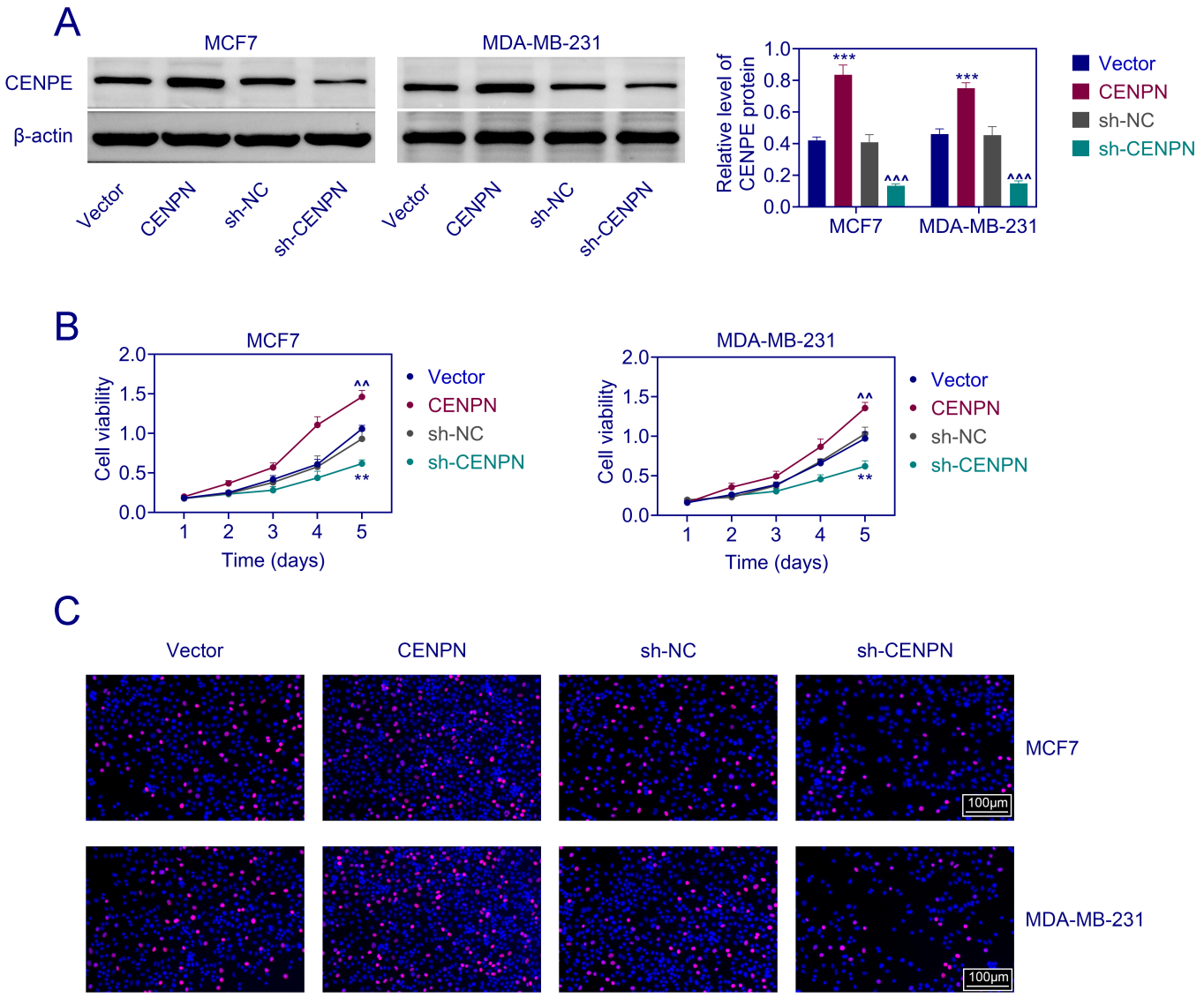

FIGURE 2. CENPN enhances cell proliferation of breast cancer. (A) Western blotting assessing the relative expression of the CENPN protein following transfection of MCF7 and MDA-MB-231 cells with plasmids expressing CENPN overexpression and sh-CENPN, as well as the corresponding negative controls. (B) CCK-8 assays for determining the cell viability. (C) Assessment of cell proliferation by Edu assays. $^{**}p < 0.01$ and $^{***}p < 0.001$ *vs.* vector; $\hat{}\hat{}p < 0.01$ and $\hat{}\hat{}\hat{}p < 0.001$ *vs.* sh-NC. CENPN: centromere protein N; CCK-8: cell counting kit-8; EdU: 5-ethynyl-2′-deoxyuridine; sh-CENPN: short hairpin RNA against centromere protein N; sh-NC: short hairpin RNA against negative control.

pression compared to the controls (Fig. 3A). Furthermore, the upregulation of CENPN substantially increased glucose uptake and lactate production, whereas CENPN knockdown significantly reduced these levels in both cell lines relative to the controls (Fig. 3B). Additionally, the levels of GLUT1 and HK2 were notably higher in cells overexpressing CENPN and markedly lower in cells with sh-CENPN relative to the control groups (Fig. 3C,D). These findings demonstrate that CENPN promotes aerobic glycolysis in breast cancer cells.

3.4 CENPN accelerated breast cancer cell growth and aerobic glycolysis through the AKT/HIF-1α signaling

Herein, we investigated the potential mechanism through which CENPN may participate in the development of breast cancer. Given the extensive reports that the AKT signaling pathway regulates aerobic glycolysis in breast cancer, the activity of AKT was assessed using Western blot. The ratio of phosphorylated AKT to total AKT was signally aggrandized in cells transfected with CENPN overexpression plasmids and notably decreased in cells treated with sh-CENPN relative to the controls (Fig. 4A). Additionally, as HIF-1α is a well-known downstream target of the AKT signaling pathway, its expression level was also evaluated. The findings indicated a notable increase in HIF-1α protein levels in cells overexpressing CENPN and a decrease in cells with CENPN knockdown relative to the controls (Fig. 4B). Moreover, a significant positive correlation between CENPN and HIF-1α expression was identified using data from the TIMER database (Fig. 4C). The overexpression of HIF-1α significantly reversed the diminishment in cell viability caused

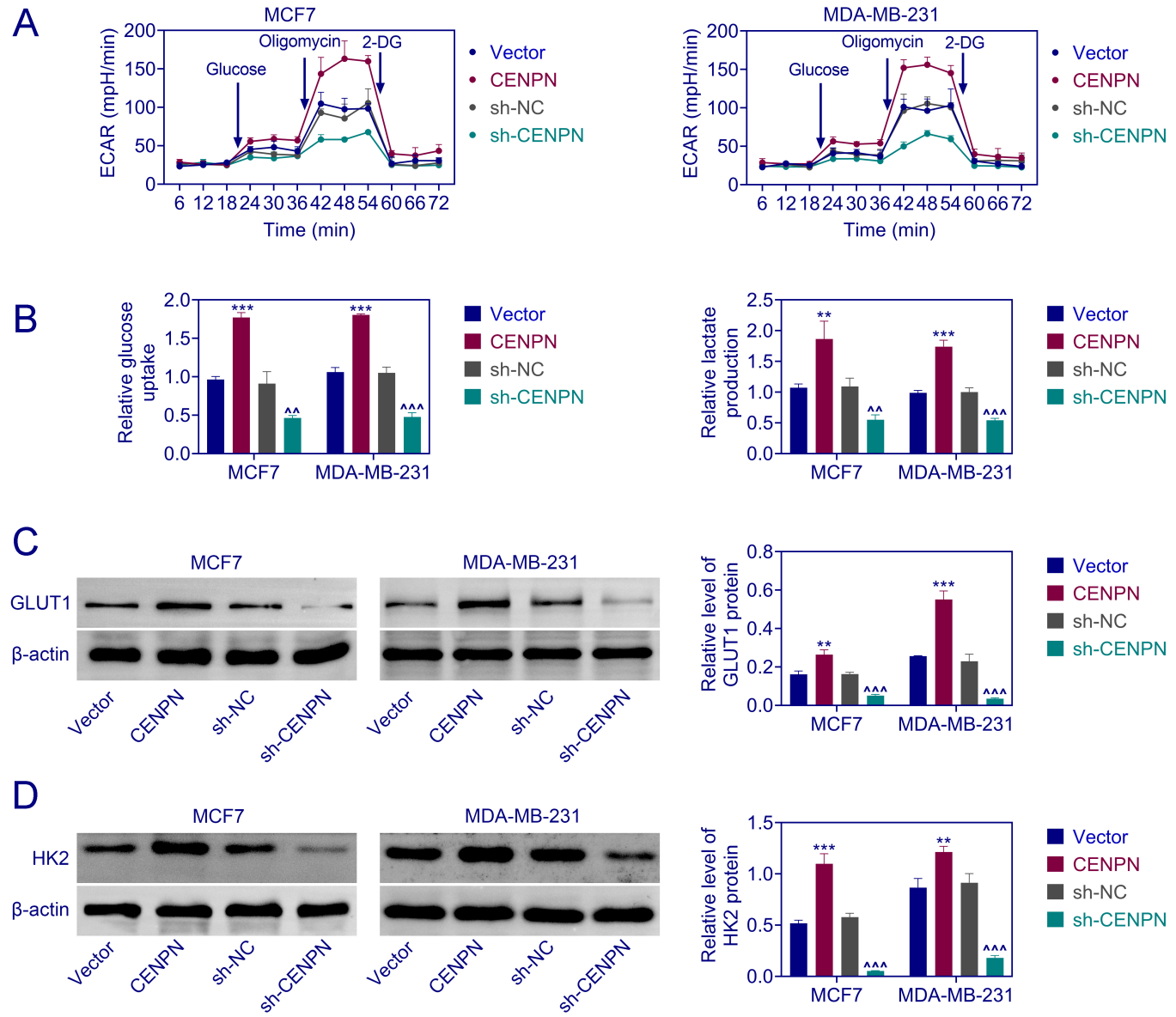

FIGURE 3. CENPN accelerated cell aerobic glycolysis of breast cancer. (A) Determination of the ECAR in MCF7 and MDA-MB-231 cells with an XF96 extracellular flux analyzer. (B) Measurement of the relative levels of glucose uptake and lactate production in two cells. (C,D) The relative protein expression of GLUT1 and HK2 evaluated using western blot. Outcomes are expressed using β-actin normalization. $^{**}p < 0.01$ and $^{***}p < 0.001$ *vs.* vector; $\wedge\wedge p < 0.01$ and $\wedge\wedge\wedge p < 0.001$ *vs.* sh-NC. CENPN: centromere protein N; ECAR: extracellular acidification rate; GLUT1: glucose transporter 1; HK2: hexokinase 2; sh-CENPN: short hairpin RNA against centromere protein N; sh-NC: short hairpin RNA against negative control.

by sh-CENPN in the two cell lines (Fig. 4D). Furthermore, the decrease in glucose uptake and lactate production, along with the downregulation of GLUT1 and HK2 protein levels in cells treated with sh-CENPN, were substantially reversed by the upregulation of HIF-1α (Fig. 4E,F). Overall, these findings suggest that CENPN supports cell proliferation and aerobic glycolysis in breast cancer through the AKT/HIF-1α pathway.

3.5 Knockdown of CENPN suppressed tumor growth in vivo

To validate the function of CENPN in breast cancer *in vivo*, further experiments were performed using BALB/c nude mice. The knockdown of CENPN was found to significantly reduce tumor volume and weight (Fig. 5A). Moreover, the injection of MCF7 cells with sh-CENPN notably decreased the expression of CENPN and Ki-67 in the tumor tissues (Fig. 5B). Additionally, the suppression of CENPN significantly reduced the ratio of phosphorylated AKT to total AKT and the expression levels of HIF-1α (Fig. 5C). These findings indicate that the knockdown of CENPN inhibits tumor growth and downregulates AKT/HIF-1α signaling *in vivo*.

4. Discussion

Identifying biomarkers for cancer is important for diagnosis, treatment and improving the chances of early detection, which has been shown to significantly increase the 5-year relative

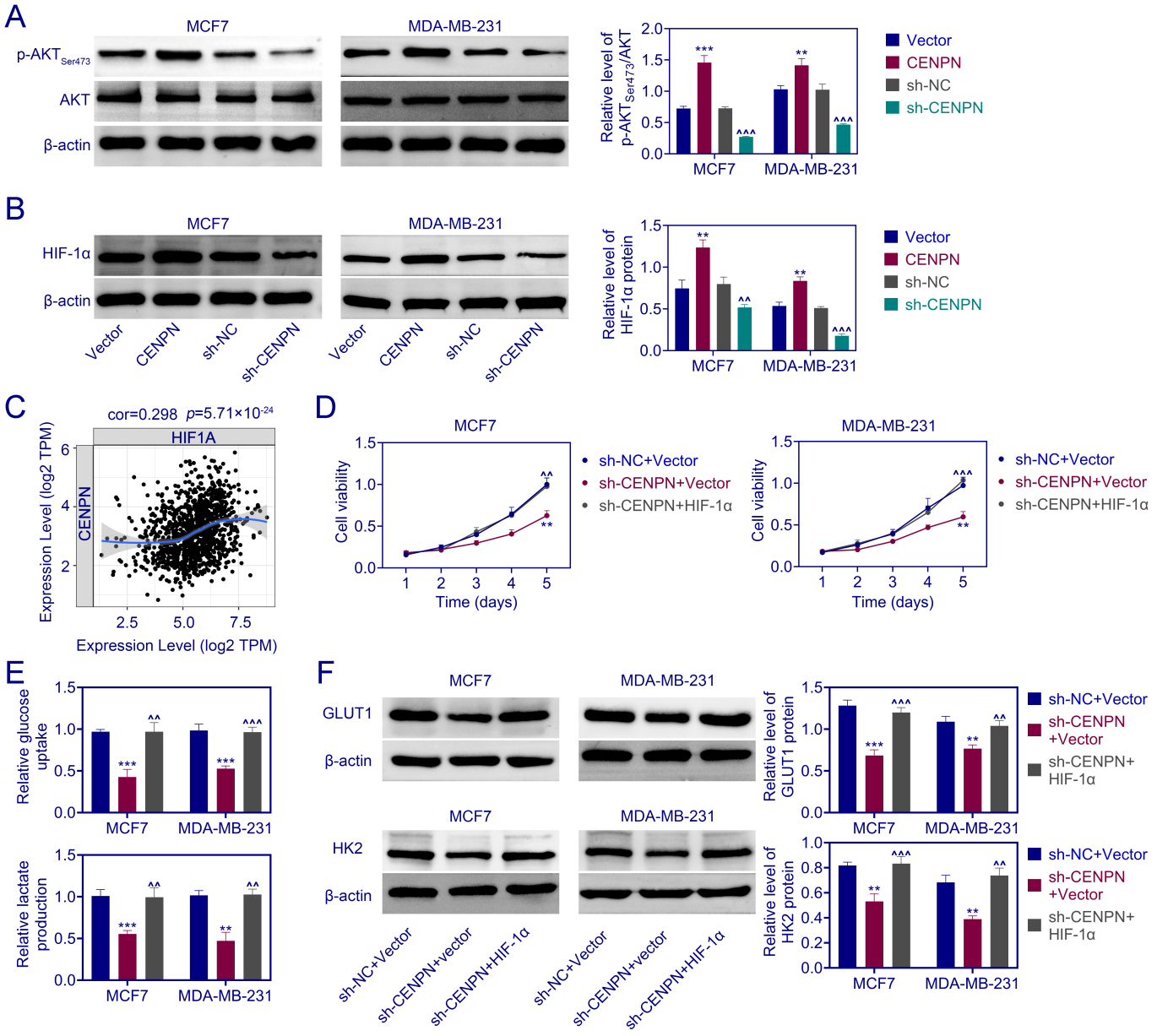

FIGURE 4. CENPN enhanced breast cancer cell growth and aerobic glycolysis *via* AKT/HIF-1α signaling. (A) The relative protein expression of p-AKT and AKT assessed *via* western blot after MCF7 and MDA-MB-231 cells were transfected with CENPN overexpression plasmids and sh-CENPN, normalized to β-actin. $^{**}p < 0.01$ and $^{***}p < 0.001$ *vs.* vector; $^{\wedge\wedge\wedge}p < 0.001$ *vs.* sh-NC. (B) Western blotting to determine the relative protein expression of HIF-1α following transfection of MCF7 and MDA-MB-231 cells with plasmids expressing CENPN overexpression and sh-CENPN. The results are expressed following β-actin normalization. $^{**}p < 0.01$ *vs.* vector; $^{\wedge\wedge}p < 0.01$ and $^{\wedge\wedge\wedge}p < 0.001$ *vs.* sh-NC. (C) The relationship between CENPN and HIF-1α predicted based on the data from the TIMER database. (D) Detection of the cell viability *via* CCK-8 assays. $^{**}p < 0.01$ *vs.* sh-NC + vector; $^{\wedge\wedge}p < 0.01$ and $^{\wedge\wedge\wedge}p < 0.001$ *vs.* sh-CENPN + vector. (E) The relative levels of glucose uptake and lactate production measured in MCF7 and MDA-MB-231 cells. $^{**}p < 0.01$ and $^{***}p < 0.001$ *vs.* sh-NC + vector; $^{\wedge\wedge}p < 0.01$ and $^{\wedge\wedge\wedge}p < 0.001$ *vs.* sh-CENPN + vector. (F) The relative protein expression of GLUT1 and HK2 was determined using western blot. The outcomes are expressed using β-actin normalization. $^{**}p < 0.01$ and $^{***}p < 0.001$ *vs.* sh-NC + vector; $^{\wedge\wedge}p < 0.01$ and $^{\wedge\wedge\wedge}p < 0.001$ *vs.* sh-CENPN + vector. CENPN: centromere protein N; AKT: protein kinase B; p-AKT: phosphorylated protein kinase B; HIF-1α: hypoxia-inducible factor 1α; CCK-8: cell counting kit-8; GLUT1: glucose transporter 1; HK2: hexokinase 2; sh-CENPN: short hairpin RNA against centromere protein N; sh-NC: short hairpin RNA against negative control.

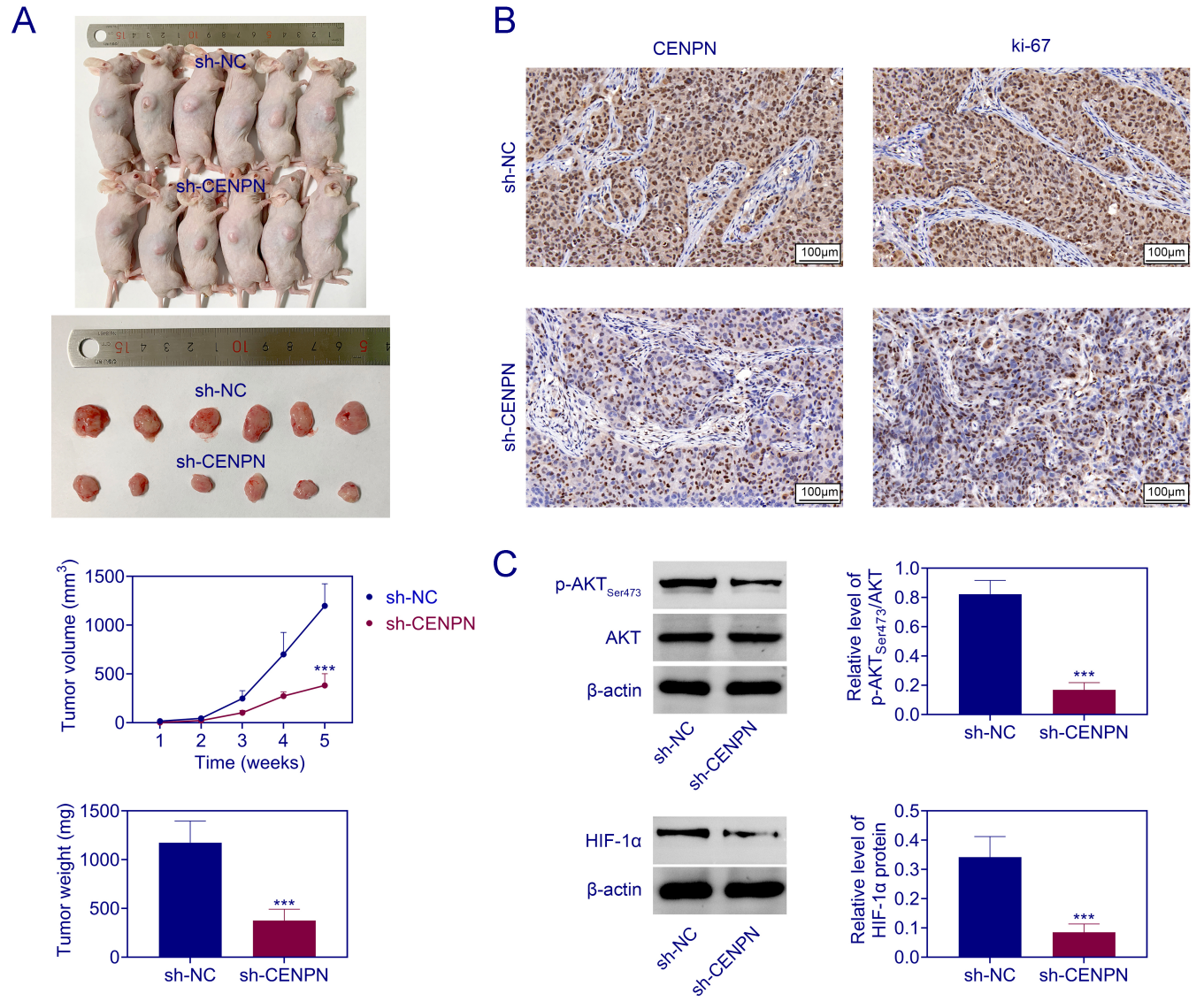

FIGURE 5. Knockdown of CENPN inhibited tumor growth *in vivo*. Mice were subcutaneously inoculated with 1×10^7 of MCF7 cells transfected with sh-NC and sh-CENPN. (A) Images of tumors from naked mice and assessment of tumor volume and weight. (B) The expression levels of CENPN and Ki-67 detected *via* IHC. (C) Western blot for assessing the relative protein expression of p-AKT, AKT and HIF-1α, normalized to β-actin. ***$p < 0.001$ *vs.* sh-NC. CENPN: centromere protein N; IHC: immunohistochemistry; AKT: protein kinase B; p-AKT: phosphorylated protein kinase B; HIF-1α: hypoxia-inducible factor 1α; sh-CENPN: short hairpin RNA against centromere protein N; sh-NC: short hairpin RNA against negative control.

survival rate for cancer patients [5]. Numerous biomarkers have been identified as being related to the development, progression and prognosis of breast cancer [27–31]. Here, the *CENPN* level was elevated in breast cancer tissues, correlating with decreased survival rates for patients. Functional assays revealed that CENPN enhanced cell viability, Edu+ positive cell counts, ECAR, glucose uptake, lactate production and the protein levels of GLUT1 and HK2. Additionally, CENPN aggrandized the ratio of phosphorylated AKT to total AKT and the expression of HIF-1α. HIF-1α overexpression mitigated the effects of sh-CENPN on cell viability, glucose uptake, lactate production and the expression levels of GLUT1 and HK2 in both cell types. Furthermore, the knockdown of CENPN decreased tumor weight and size, the expressions of CENPN and Ki-67, and the signaling activity of AKT and HIF-1α. Overall, our findings indicate that elevated CENPN levels are correlative with lower survival rates in breast cancer patients and facilitate cell growth and aerobic glycolysis by upregulating AKT and HIF-1α signaling pathways.

High expression of CENPN has been identified in various cancers, including esophageal squamous cell carcinoma [18, 32], stomach adenocarcinoma [33], nasopharyngeal carcinoma [19, 34], lung adenocarcinoma [17, 35], gliomas [36], hepatocellular carcinoma [37], and oral squamous cell carcinoma [38]. Similarly, increased levels of CENPN have also been reported in breast cancer [39]. Furthermore, the upregulation of CENPN has been correlative with the metastatic and aggressive characteristics of breast cancer, correlating with a poor prognosis for patients [39]. CENPN has also been identified as one of eight central genes serving as prognostic markers for

various subtypes of breast cancer [21]. Moreover, the overexpression of CENPN is interrelated in the survival and prognosis of different cancer types, including stomach adenocarcinoma [33], lung adenocarcinoma [17, 35], esophageal squamous cell carcinoma [32], lung adenocarcinoma [19], gliomas [36], hepatocellular carcinoma [37], anaplastic thyroid carcinoma [40], oral squamous cell carcinoma [38] and colorectal cancer [41]. In this context, the increased expression of *CENPN* suggests a negative prognosis for breast cancer patients, further supporting that CENPN as a marker of poor survival in breast cancer.

Dysregulated expression of CENPN has been implicated in the progression of breast cancer. Experimental results from *in vitro* gain- and loss-of-function studies demonstrated that CENPN increases cell viability, the number of Edu+ positive cells, ECAR, glucose uptake, lactate production, and the expression levels of GLUT1 and HK2 proteins. These findings suggest that CENPN supports the proliferation and aerobic glycolysis in breast cancer cells. Additionally, *in vivo* experiments using xenografted mice showed that silencing CENPN resulted in decreased tumor volume and weight, alongside reduced level of Ki-67, a proliferation marker, indicating CENPN downregulation restricts breast cancer cell growth. Oka *et al.* [38] reported that CENPN could enhance cell proliferation by modulating the cell cycle in human oral cancer. Zheng *et al.* [17] found that CENPN increased cell viability, invasiveness and stemness in PC9 lung cells. Aerobic glycolysis, a key feature of cancer cells, contributes to their growth and survival [14]. Xu *et al.* [18] reported that CENPN could circumvent G1 arrest and enhance glucose metabolism in esophageal squamous cell carcinoma. Furthermore, Qi *et al.* [19] demonstrated that CENPN promoted cell proliferation and cycling and inhibited apoptosis by fostering aerobic glycolysis in nasopharyngeal carcinoma. Collectively, these findings establish that CENPN serves a crucial role in enhancing cell growth and aerobic glycolysis in breast cancer.

The AKT signaling pathway is essential in regulating biological functions such as proliferation, apoptosis and invasion [42] and is known to be hyperactivated in various cancers, making it a pivotal target for cancer therapy [43, 44]. Specifically, AKT has been identified as a key therapeutic target for breast cancer, playing a significant role in aerobic glycolysis [45]. Zong *et al.* [42] have demonstrated that suppression of the PI3K/AKT pathway can inhibit aerobic glycolysis in breast cancer cells, highlighting the pathway's involvement in this metabolic process. Furthermore, the transduction of AKT signaling typically involves the activation of downstream effector molecules, among which HIF-1α is a well-recognized target [46]. HIF-1α is a key protein in the modulation of aerobic glycolysis, whose activation activates the expression of genes encoding glucose transporters and glycolytic enzymes [47]. Yang *et al.* [48] showed the repressive effect of coenzyme Q0 on aerobic glycolysis *via* suppressing the HIF-1α level in triple-negative breast cancer cells. Similarly, Liu *et al.* [49] have found that the long non-coding RNA LINC00365 diminishes HIF-1α-mediated aerobic glycolysis in breast cancer. Here, CENPN was observed to elevate the relative levels of p-AKT/AKT and HIF-1α both *in vitro* and *in vivo*. Furthermore, overexpression of HIF-1α neutralizes the suppression by sh-CENPN on cell proliferation and aerobic glycolysis. It has been elucidated that in nasopharyngeal carcinoma cells, CENPN can form a complex with AKT, where this CENPN/AKT complex leads to increased aerobic glycolysis, enhancing proliferation, apoptosis resistance, and cell cycling [19]. Additionally, CENPN promotes the progression of lung adenocarcinoma through activation of the PI3K/AKT axis [17] and influences G1 arrest and glucose metabolism in esophageal squamous cell carcinoma *via* the PI3K/AKT pathway [18]. Thus, it can be deduced that CENPN facilitates cell growth and aerobic glycolysis by upregulating AKT/HIF-1α signaling.

5. Conclusions

In conclusion, CENPN is elevated in breast cancer and correlates with poor prognosis. Functionally, it operates as an oncogene, contributing to the proliferation and aerobic glycolysis of breast cancer, as demonstrated through both gain- and loss-of-function experiments. Mechanistically, CENPN upregulates AKT and HIF-1α signaling pathways in cellular and animal models. Consequently, silencing CENPN impedes cell growth and aerobic glycolysis in breast cancer through attenuating AKT and HIF-1α signaling. Nonetheless, the absence of clinical data limits the exploration of the association between CENPN levels and the clinical features of metastasis and aggression in this study. While HIF-1α is identified as a downstream effector of AKT signaling, the direct linkage between AKT and HIF-1α requires further validation in MCF7 and/or MDA-MB-231 cell lines. Moreover, additional studies are warranted to investigate CENPN's role in other aspects of breast cancer progression, including migration, invasion, apoptosis and immune evasion. In summary, this research positions CENPN as a potential biomarker for the diagnosis and treatment of breast cancer.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article.

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

ZY—performed material preparation and the experiments; QYL and HLL—performed data collection and analysis; YWH—written the first draft of the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. All authors contributed to the study conception and design.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All procedures performed in studies involving human participants were in accordance with the standards upheld by the Ethics Committee of the Third Affiliated Hospital of Wenzhou

Medical University and with those of the 1964 Helsinki Declaration and its later amendments for ethical research involving human subjects (Approval No. YJ2023039).

All animal experiments were approved by the Ethics Committee of the Third Affiliated Hospital of Wenzhou Medical University for the use of animals and conducted in accordance with the National Institutes of Health Laboratory Animal Care and Use Guidelines (Approval No. YJ2023039).

Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

ACKNOWLEDGMENT

Not applicable.

FUNDING

This research received no external funding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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How to cite this article: Zhe Ye, Qiuyan Lin, Hualong Lin, Yiwei Huang. Targeting CENPN to inhibit breast cancer progression: insights into cell growth and aerobic glycolysis modulation. European Journal of Gynaecological Oncology. 2024; 45(2): 147-157. doi: 10.22514/ejgo.2024.037.