

ORIGINAL RESEARCH

Overexpression of URG11 can inhibit the proliferation and motility of breast cancer cells

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Abstract

Breast cancer (BC) is the most common type of cancer among women worldwide, and identifying the key molecules that regulate cell metastasis is essential to improve the patients' prognosis and developing potential therapeutic strategies. Upregulator gene 11 (URG11) plays a significant role in cancer cell motility. Despite its relevance, its role and underlying mechanism in BC remains underexplored. This study aimed to investigate the significance and mechanism of action of URG11 in BC. The results indicated a low expression of URG11 in BC and revealed that its overexpression could suppress BC cell growth and motility. Through mechanistic studies, we discovered that URG11 overexpression inhibited the Wnt/ β -Catenin pathway in BC cells. In summary, URG11 overexpression could inhibit the growth and motility of BC cells by suppressing the Wnt/ β -Catenin pathway, suggesting URG11 as a potential target for BC management.

Keywords

Breast cancer (BC); Upregulator gene 11 (URG11); Invasion; Migration; Wnt/ β -catenin

1. Introduction

Breast cancer (BC) is the most common type of tumor among women globally [1]. Recent statistics have indicated a rapid increase in its incidence [2], with BC accounting for 30% of all new cancer diagnoses in women. Advances in treatment have notably enhanced patient prognoses [3]. Despite these advancements, 25–50% of patients diagnosed at an early stage will eventually progress to metastatic BC [3]. Targeted therapy for breast cancer focuses on minimizing damage to healthy cells and enhancing treatment efficacy by specifically targeting molecules or signaling pathways in cancer cells [4, 5]. The common targeted therapy drugs include trastuzumab for human epidermal growth factor receptor 2 (HER2), tamoxifen and aromatase inhibitors for estrogen receptors, and palbociclib for Cyclin Dependent Kinase 4 (CDK)4/6 [6, 7]. Presently, treatment selection is tailored based on the molecular characteristics of the tumor and may be administered as monotherapy or in combination with other therapeutic modalities [6, 7]. Given that tumor metastasis presents a significant challenge in BC management, identifying the key molecules involved in metastasis is essential for improving patient outcomes and devising novel therapeutic approaches [8].

The Upregulator gene 11 (URG11) encodes a protein of approximately 70 kDa in size [9–11]. These domains have been reported to be involved in cell adhesion, motility and cell/matrix interactions [9, 10]. URG11 is found to be overexpressed in various cancers, including breast, prostate, and gastric cancers, where it has been shown to facilitate the growth of cancer cells. Furthermore, URG11 is involved in regulating

cell migration as well as metastasis through its impact on cell adhesion and interactions with the extracellular matrix [9–12]. Specifically, in prostate cancer, URG11 overexpression has been linked to increased β -catenin expression [9]. Similarly, in gastric cancer, URG11 contributes to tumor growth and metastasis, at least in part, by activating the β -catenin pathway [12]. Additionally, URG11 has been implicated in mediating hypoxia-induced Epithelial-Mesenchymal Transition (EMT) through the inhibition of E-cadherin and activation of the β -catenin/T cell specific transcription factor (TCF) axis [13]. This evidence supports the oncogenic role of URG11 in the development of prostate cancer cells, suggesting its viability as a novel therapeutic target for prostate cancer [10]. Overall, these findings underscore the multifaceted role of URG11 in promoting tumor development and progression. Despite its significance, the expression and mechanistic role of URG11 in breast cancer remain underexplored.

This study aims to elucidate the potential role of URG11 in BC and reveal its mechanism. Our findings demonstrate that URG11 plays an inhibitory role in the growth and motility of breast cancer cells through the modulation of the Wnt/ β -Catenin pathway, representing a viable target for therapeutic intervention in BC.

2. Materials and methods

2.1 Cell culture and transfection

The MCF10A, MDA-MB-231, MCF-7 and SK-BR-3 cell lines were purchased from American Type Culture Collection (ATCC). They were cultured in Dulbecco's Modified

Eagle Medium (DMEM) (Gibco, 11960077, CA, USA), supplemented with 10% Fetal Bovine Serum (FBS) (Gibco, 10099-133, CA, USA), and maintained in an incubator at 37 °C and 5% CO₂. Then, they were transfected with either Ad-vector or Ad-URG11 plasmids (Addgene, USA) using Lipofectamine 3000 (Thermo, L3000075, Waltham, MA, USA) and incubated for 24 h.

2.2 Immunoblot assay

The samples were lysed using Radio-Immunoprecipitation Assay (RIPA) buffer (P0013D, Beyotime, Beijing, China), separated by 10% Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, USA). To blocking, the membranes were incubated with 5% dry milk, then probed with primary antibodies, including URG11 (1:500, ab184772, Abcam), Matrix Metalloproteinase 2 (MMP2) (1:2000, ab92536, Abcam), MMP9 (1:2000, ab76003, Abcam), anti-C-myc (1:500, ab32072, Abcam), anti-C-myc (duplicate mention should be removed), anti-cyclin D1 (1:500, ab16663, Abcam) and β -catenin (1:500, ab32572, Abcam). β -actin (1:3000, ab8226, Abcam) was used as the loading control. Next, the membranes were treated with appropriate horseradish peroxidase-conjugated secondary antibodies, and the protein bands were detected using an enhanced chemiluminescence (ECL) kit (P0018FC, Beyotime, Beijing, China).

2.3 Cell growth assays

For the cell counting kit 8 (CCK-8) assay, the cells were seeded into 96-well plates and incubated for 24 h at 37 °C, followed by treatment with the CCK-8 solution (C0038, Beyotime, Beijing, China) and incubation for an additional 1.5 h at 37 °C. The Optical density (OD450) value was measured using a microplate reader (iMark, Bio-Rad, Hercules, CA, USA).

In the colony formation assay, the BC cells were seeded into 24-well plates and cultured for 14 days at 37 °C, following which the cells were stained with 0.2% crystal violet and images were captured using a fluorescence microscope (LSM710, Zeiss, Oberkochen, Germany).

2.4 Transwell assay

Cells were seeded into the upper chamber of a transwell apparatus filled with complete medium to facilitate either migration (without matrigel) or invasion assays (with 10% matrigel, 356237, BD, Franklin Lake, NJ, USA). After 24 h, cells were fixed and stained with 0.2% crystal violet.

2.5 Statistical analysis

Statistical analysis was conducted using GraphPad Prism (version 6.0, GraphPad Prism PLC., San Diego, CA, USA). Data are presented as mean \pm standard deviation (SD). *p* less than 0.05 was considered statistically significant.

3. Results

3.1 Low expression of URG11 in BC tissues and cells

To investigate URG11's involvement in BC progression, its expression was first examined in human BC tissues and various cell lines. Utilizing the Gene Expression Profiling Interactive Analysis (GEPIA) database, we observed notably lower expression levels of URG11 in BC tissues compared to normal counterparts (Fig. 1A). Further analysis in a non-tumorigenic breast cell line (MCF10A) and three BC cell lines (MCF-7, MDA-MB-231 and SK-BR-3) also confirmed the reduced expression of URG11 in the BC cell lines (Fig. 1B), thus confirming that URG11 is lowly expressed in BC.

3.2 URG11 overexpression suppressed BC cell growth

Given the observed reduction of URG11 in BC cells, we explored its functional role by overexpressing URG11 in MDA-MB-231 and MCF-7 cell lines. Transfection with a URG11 overexpression plasmid significantly enhanced its expression levels in these cells (Fig. 2A). Subsequent analysis demonstrated that URG11 overexpression led to a decrease in OD at 450 nm in both MDA-MB-231 as well as MCF-7 cells, indicating suppressed cell growth (Fig. 2B). Additionally, URG11 overexpression resulted in a reduced number of colonies in both cell lines (Fig. 2C). URG11 could act as a suppressor of BC cell growth.

3.3 URG11 overexpression restrained the migration and invasion of BC cells

Here, we investigated URG11's impact on cell motility. Utilizing transwell assays, we observed that URG11 overexpression significantly reduced MDA-MB-231 and MCF-7 cells' motility, as evidenced by decreased numbers of migrating and invading cells (Fig. 3A). Immunoblot assays further supported these findings, showing that URG11 overexpression led to reduced expression of MMP2 and MMP9, the key enzymes involved in cell motility (Fig. 3B). Thus, these results indicate that URG11 overexpression suppresses the motility capabilities of BC cells.

3.4 URG11 overexpression suppressed the Wnt/ β -catenin axis in BC cells

Next, we explored the mechanism by which URG11 influences BC cell behavior, particularly its impact on the Wnt/ β -catenin axis, which is known to regulate both cell growth and motility by examining the effects of URG11 overexpression on this pathway in BC cells. Our results showed that URG11 overexpression led to reduced expression levels of key components of the Wnt/ β -catenin pathway (Fig. 4). Based on these findings, we propose that URG11 negatively regulates the Wnt/ β -catenin axis in BC cells.

4. Discussion

BC remains one of the leading causes of cancer-related morbidity worldwide, with its incidence and mortality rates on an upward trend [2, 14]. As a disease influenced by a combination

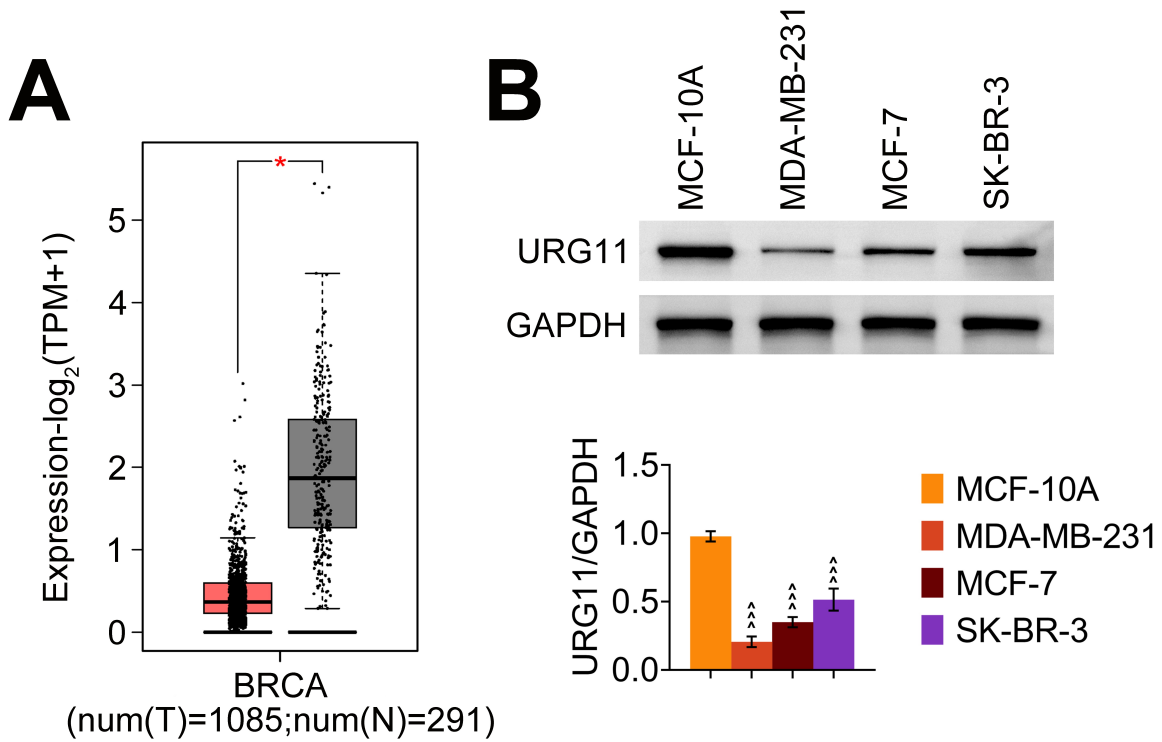


FIGURE 1. Reduced expression of URG11 in BC tissues and cells. (A) Analysis of URG11 expression levels using the GEPIA database, comparing transcripts per million (TPM) in 1085 BC tissues to 291 normal breast tissues. $*p < 0.05$, vs. Normal (N). (B) Western blot analysis demonstrating URG11 protein levels in normal breast epithelial cell line MCF10A and three BC cell lines: MDA-MB-231, MCF-7 and SK-BR-3. $^{\wedge\wedge\wedge}p < 0.001$, vs. MCF10A. TPM: Transcripts per million; BRCA: Breast cancer; URG11: up-regulatedgene11; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

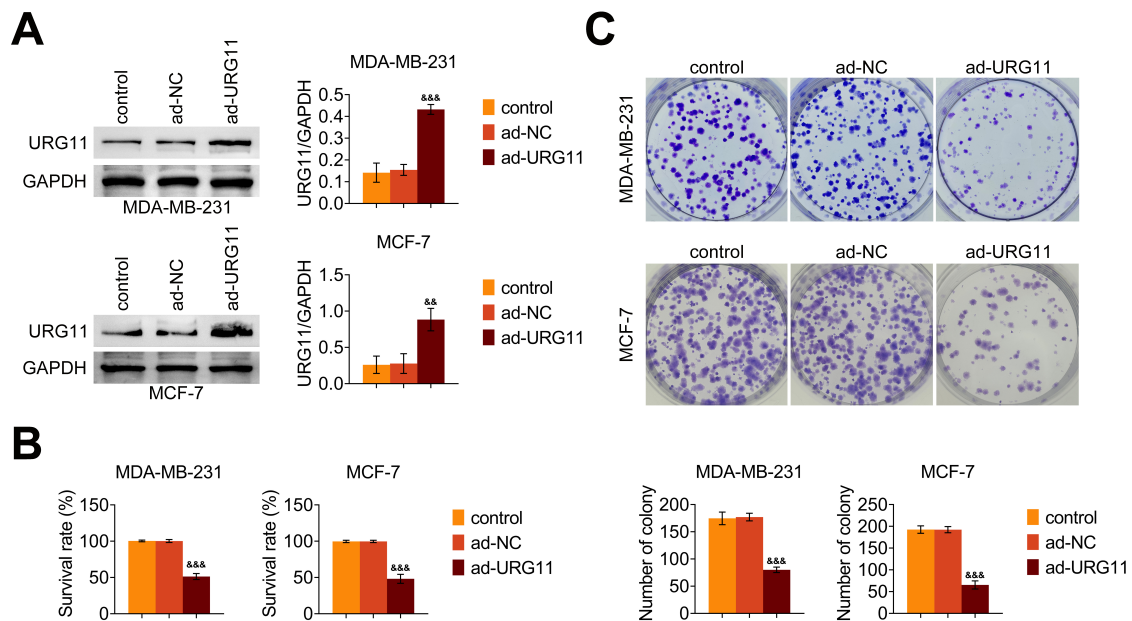


FIGURE 2. URG11 overexpression suppressed the growth of BC cells. (A) Immunoblot assays showing the expression of URG11 in MDA-MB-231 and MCF-7 cells upon transfection with NC or URG11 overexpression plasmids for 24 h. (B) CCK-8 assays showing the growth of MDA-MB-231 and MCF-7 cells upon the transfection with NC or URG11 overexpression plasmids for 24 h. (C) Colony formation assays showing the growth of MDA-MB-231 and MCF-7 cells upon the transfection with NC or URG11 overexpression plasmids for 24 h. Colonies were quantified to assess growth. $\&\&p < 0.01$, $\&\&\&p < 0.001$, ad-URG11 vs. ad-NC. NC: negative control; URG11: up-regulatedgene11; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

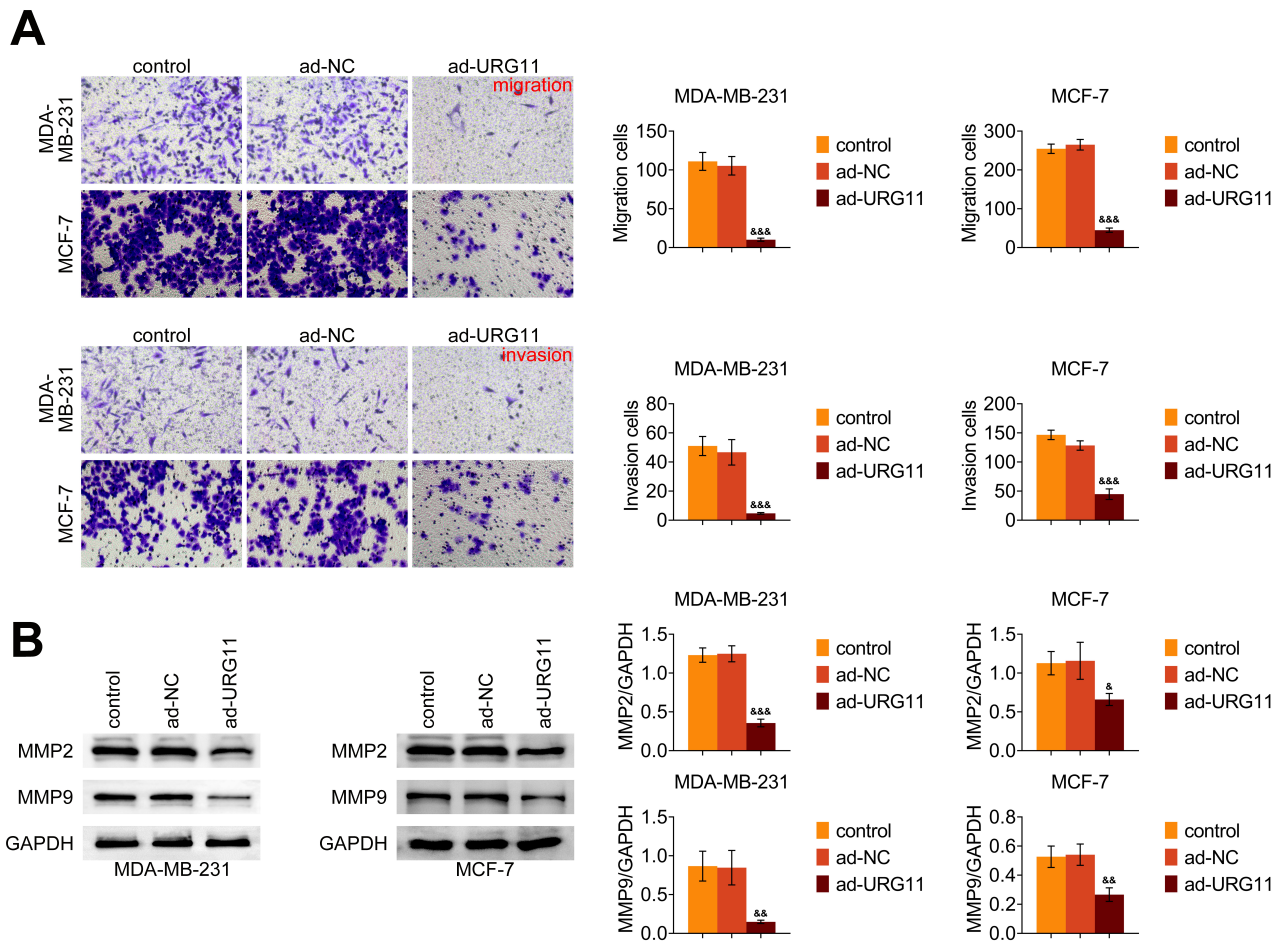


FIGURE 3. The overexpression of URG11 restrained the migration and invasion of BC cells. (A) Transwell assays illustrating the impact of URG11 overexpression on the migration (upper panel) and invasion (lower panel) of MDA-MB-231 and MCF-7 cells 24 h post-transfection with either a negative control (NC) or URG11 overexpression plasmids. The number of migrated and invaded cells was quantified. (B) Immunoblot analysis displaying the levels of MMP2 and MMP9 in MDA-MB-231 and MCF-7 cells 24 h post-transfection with NC or URG11 overexpression plasmids. Protein expression levels were quantified and compared. $&p < 0.05$, $&&p < 0.01$, $&&&p < 0.001$, ad-URG11 vs. ad-NC. NC: negative control; URG11: up-regulatedgene11; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

of genetic, environmental and lifestyle factors, breast cancer presents a complex challenge in the field of oncology. The evolution of molecular biology and genomics has enriched our understanding of breast cancer's molecular underpinnings, opening avenues for the identification of novel therapeutic targets [2]. While conventional therapies such as surgery, chemotherapy and radiotherapy are foundational in breast cancer management, the advent of targeted therapies has marked a pivotal shift towards personalized treatment approaches as they are aimed at specific molecular aberrations, offering the promise of improved patient outcomes and survival rates [15]. Thus, the discovery and validation of new therapeutic targets are crucial for improving and enhancing the efficacy of BC treatment strategies [16]. In this context, our findings highlight that URG11 exhibits reduced expression in human BC tissues and cell lines. Moreover, we demonstrated that URG11 overexpressing impeded BC cell growth and motility.

URG11, which has been shown to be implicated in various cellular processes and the progression of multiple cancers, has

also been demonstrated to enhance growth and metastasis in gastric cancer by activating the β -catenin pathway, leading to increased expression of genes involved in cell growth as well as motility [9, 12, 17]. Furthermore, it activates the β -catenin promoter in hepatocellular carcinoma, promoting cell growth, and plays a significant role in hepatocarcinogenesis by transcriptionally upregulating β -catenin and influencing microRNA expression, including miR-148a [12]. Beyond its oncogenic roles, URG11 has been found to block apoptosis in osteosarcoma and prostate cancer, possibly *via* the Wnt/ β -catenin pathway [18]. Given its impact not only on cancer but also on inflammatory diseases, URG11 is considered a promising therapeutic target in various diseases, including both cancer and inflammation. Herein, our present findings support URG11's pivotal role in BC, where it influences cell growth and motility.

URG11, a protein with elevated expression levels in certain cancer types, is believed to participate in various biological processes [17, 19, 20]. In the context of breast cancer,

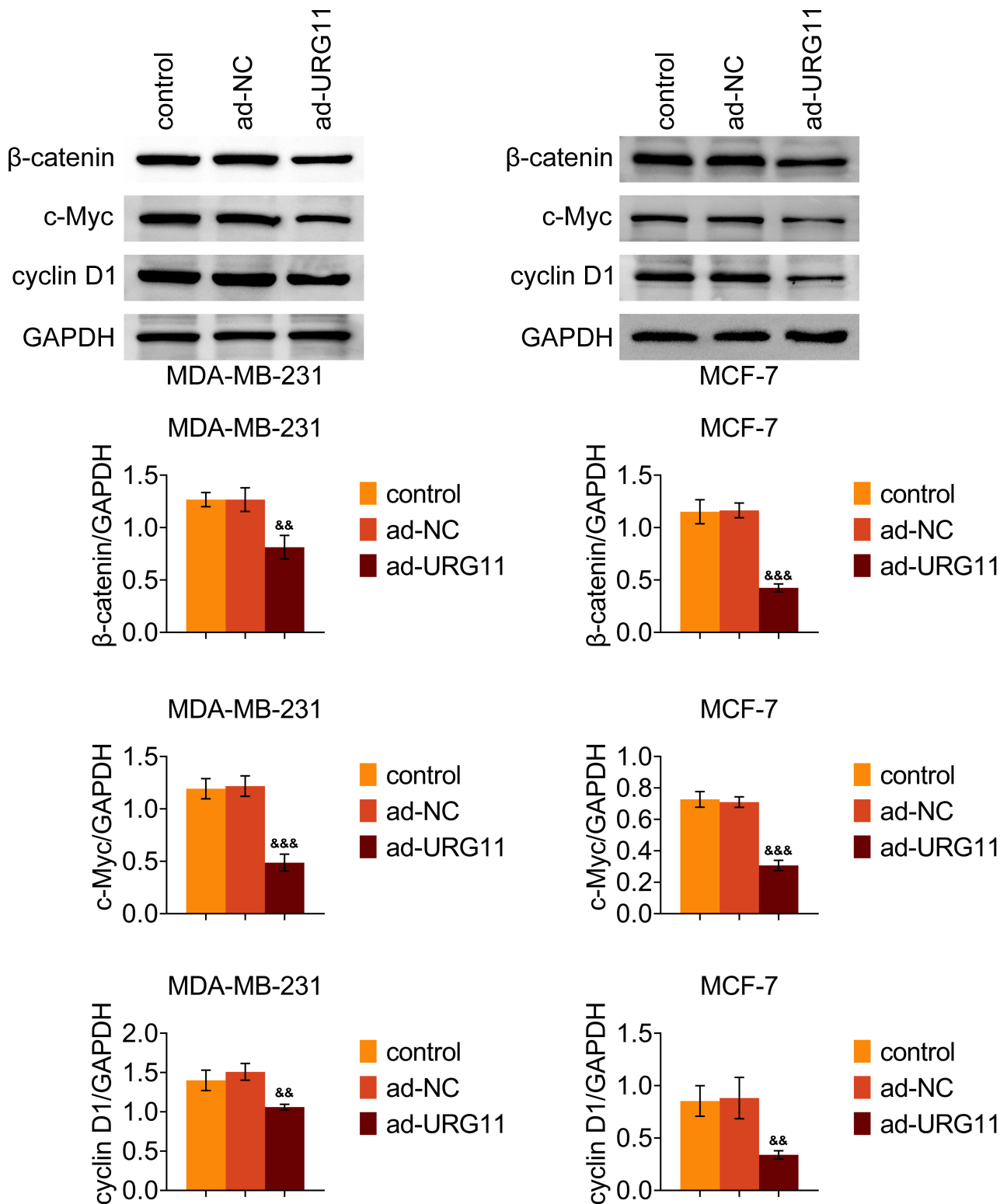


FIGURE 4. URG11 overexpression suppressed the Wnt/ β -Catenin pathway in BC cells. Immunoblot assays revealing the impact of URG11 overexpression on the expression levels of β -Catenin, c-Myc and Cyclin D1 in MDA-MB-231 and MCF-7 cells, 24 h post-transfection with either NC or URG11 overexpression plasmids. Quantitative analysis was conducted to assess the relative protein expression. $&&p < 0.01$, $&&&p < 0.001$, Ad-URG11 vs. Ad-NC. NC: negative control; URG11: up-regulated gene 11; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

URG11's expression might be linked to tumor development and progression, yet its specific role and underlying mechanisms require further exploration [20]. URG11 is thought to promote cancer cell growth by activating pathways such as Wnt/ β -catenin and nuclear factor kappa-B (NF- κ B) [10, 12]. Therefore, URG11 presents itself as a potential therapeutic target for breast cancer. Nonetheless, a detailed understanding of its exact mechanism warrants additional investigation.

The Wnt/ β -Catenin axis is vital in the development, progression, and stem cell characteristics of BC [21, 22]. Its abnormal activation is known to promote tumor formation, enhance tumor invasiveness and metastatic potential, and is linked to the maintenance of BC stem cells [23–26]. Consequently, targeting this pathway is viewed as a strategy for BC treatment, with inhibitors of this pathway emerging as potential therapeutic options [24]. In our study, we demonstrated that URG11 suppresses the growth and motility of BC cells by modulating the Wnt/ β -Catenin axis. However, the precise mechanisms through which URG11 exerts its effects require further investigation.

5. Conclusions

In conclusion, our findings suggest that URG11 can inhibit BC cell growth and motility through the regulation of the Wnt/ β -Catenin pathway.

AVAILABILITY OF DATA AND MATERIALS

The authors declare that all data supporting the findings of this study are available within the paper and any raw data can be obtained from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

JNQ, SHY—designed the study and carried them out; supervised the data collection, analyzed the data, interpreted the data; prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This article does not contain any studies with human participants or animals performed by any of the authors.

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: Jianing Qian, Shuhuan Yu. Overexpression of URG11 can inhibit the proliferation and motility of breast cancer cells. *European Journal of Gynaecological Oncology*. 2024; 45(3): 88-94. doi: 10.22514/ejgo.2024.052.