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ORIGINAL RESEARCH

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CRMP4 suppresses cervical cancer cell proliferation and EMT through the Wnt/β-catenin pathway

Meiqin Liu[1], Shile Gao[1], Xingjun Xu[1], Huan Ma[1], Dongmei Wang[1], Xueqin Cai[1], Yang Wang[2,*]

[1]Tumor Four Departments, The 901st Hospital of the Joint Logistics Support Force of the People's Liberation Army of China, 230031 Hefei, Anhui, China
[2]Department of Obstetrics and Gynecology, Nanjing Drum Tower Hospital Group Suqian Hospital (The Affiliated Suqian Hospital of Xuzhou Medical University), 233800 Suqian, Jiangsu, China

***Correspondence**
y_wang0514@163.com
(Yang Wang)

Abstract

Cervical cancer is the 4th leading cause of tumor-related deaths among women, primarily due to high-risk Human Papillomavirus (HPV). The role of Collapsin Response Mediator Protein 4 (CRMP4) in cervical cancer remains poorly understood, despite its variable expression across various cancers. This study aimed to investigate the mechanisms by which CRMP4 regulates cervical cancer cell growth as well as Epithelial-Mesenchymal Transition (EMT). Based on transcriptome data from The Cancer Genome Atlas (TCGA), CRMP4 is significantly downregulated in cervical cancer tissues compared to normal tissues. Immunoblot assays revealed lower CRMP4 expression in cervical cancer cell lines. Cell Counting Kit-8 (CCK-8), colony formation and flowcytometry demonstrated that CRMP4 overexpression inhibited cell growth as well as stimulated apoptosis in HeLa as well as SiHa cells. In addition, CRMP4 overexpression increased Epithelial cadherin (E-cadherin) levels and decreased Neural cadherin (N-cadherin) and alpha-Smooth Muscle Actin (α-SMA) levels, indicating EMT suppression. CRMP4 overexpression downregulated the Wnt/β-catenin axis by reducing expressions of β-catenin, Wnt family member 3A (Wnt3a), c-Myc and cyclin D1. In summary, CRMP4 inhibits cervical cancer cell proliferation as well as EMT by mediating the Wnt/β-catenin axis. CRMP4 may therefore be a potential therapeutic target of cervical cancer.

Keywords

Cervical cancer; Collapsin response mediator protein 4 (CRMP4); Proliferation; Epithelial-mesenchymal transition (EMT); Wnt/β-catenin

1. Introduction

Cervical cancer remains the 4th leading cause of tumor-related deaths among women [1]. According to 2012 estimates, there were approximately 527,600 new cases as well as 265,700 deaths [2]. There are only a minority of women exposed to this virus who eventually develop cervical cancer, suggesting other biological and environmental factors also contribute to its development [3]. To progress from HPV infection to cervical cancer, additional genetic or environmental factors (the second hit) required. Besides contributing directly, persistent HPV infection also causes chronic inflammation, which contributes to cervical cancer formation [4]. Inflammation promotes cell proliferation and survival, enhances angiogenesis and alters hormones and chemotherapy agents responses [5]. The inherent complexity of these mechanisms represents substantial challenges in battling cervical cancer, especially with regard to drug resistance.

Recent oncological research showed Collapsin Response Mediator Protein 4 (CRMP4) due to its variable expression across various types of human cancers [6]. Functionally, CRMP4 plays a key role in neuronal development, where it is essential in assembling cytoskeletal proteins and the outgrowing neuronal axons. In studies, CRMP4 is found to interact with actin filaments and affect cytoskeletal architecture, influencing cellular dynamics such as migration and invasion [7]. Interestingly, CRMP4 modulates several cancer-related pathways. CRMP4 modulates the activity of macrophages and myeloid-derived suppressor cells, which are crucial for tumor immune evasion [8]. Cancer types such as prostate and pancreatic cancer respond differently to therapies due to CRMP4's alteration of cellular motility and immune responses [9, 10]. CRMP4's role in other cancer types has been explored, however its function and regulatory mechanisms in cervical cancer remain poorly understood.

This study aims to investigate the mechanisms by which CRMP4 regulates cervical cancer cell proliferation and EMT.

2. Materials and methods

2.1 Bioinformatics

Transcriptome data were obtained from The Cancer Genome Atlas (TCGA) database. CRMP4 transcripts per million (TPM) in cervical cancer were downloaded from the Gene Expression Profiling Interactive Analysis (GEPIA) website,

which analyzed 306 tumor tissues and 13 normal tissues (URL: http://gepia.cancer-pku.cn/).

2.2 Cell culture and transfection

Normal cervical cell line HaCaT, and 4 types of cervical cancer cell lines HeLa, SiHa, CaSki and C33A cells were purchased from American Type Culture Collection (ATCC). Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) (14190-144, Gbico, Carlsbad, CA, USA), supplemented with 10% Fetal Bovine Serum (FBS) (10099-141, Gbico, Carlsbad, CA, USA) and incubated at 37 °C, 5% CO_2. We transfected cells with Ad-vector or ad-CRMP4 plasmids (49340, Addgene, Watertown, MA, USA) for 24 h using Lipofectamine 3000 (L3000015, Thermo Fisher Scientific, Waltham, MA, USA).

2.3 Immunoblot assay

Samples were separated by 10% Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and sequentially transferred to Polyvinylidene Fluoride (PVDF) membranes. We blocked membranes with 5% dry milk (Bio-Rad Cat# 1706404) and incubated them with primary antibodies including CRMP4 (Abcam Cat# ab129082, 1:1000), α-SMA (Abcam Cat# ab7817, 1:1000), Epithelial Cadherin (Abcam Cat# ab231303, 1:2000), Neural Cadherin (Abcam Cat# ab76011, 1:2000), anti-c-Myc (Abcam Cat# ab32072, 1:2000), anti-Wnt3a (Abcam Cat# ab219412, 1:1000), anti-cyclin D1 (Abcam Cat# ab16663, 1:2000) and Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) (Abcam Cat# ab8245, 1:5000). Membranes were then incubated with secondary antibodies at a 1:5000 dilution and visualized using Enhanced Chemiluminescence (ECL) kit (GE Healthcare, Cat# RPN2232, Chicago, IL, USA).

2.4 Cell growth assays

For CCK-8, 10^5 cells were plated into 96-well plates and maintained for 24 h at 37 °C. Cells were exposed to CCK-8 (C0038, Beyotime, Beijing, China) for 1.5 h at 37 °C. Optical Density (OD450) value was then determined (Bio-Rad, USA).

For colony formation assay, 1000 cells were plated into 24-well plates and maintained for 14 d at 37 °C. Cells were then incubated in 0.2% crystal violet and photographed with a fluorescence microscope (Carl Zeiss AG, Oberkochen, BW, Germany).

2.5 Flow Cytometry (FCM) assay

Cells were resuspended in 1$\times$ binding buffer (BD Biosciences, Cat# 556454, lot 839204, Franklin Lakes, NJ, USA) at a concentration of 1 $\times$ 10^6 cells/mL. 5 μL of Annexin V-Fluorescein Isothiocyanate (FITC) (BD Biosciences, Cat# 556547, lot 849302, Franklin Lakes, NJ, USA) and 5 μL of Propidium Iodide (PI) (50 μg/mL, BD Biosciences, Cat# 556463, lot 849304, Franklin Lakes, NJ, USA) were added to each 100 μL of cell suspension. We used FlowJo software (Tree Star Inc., version 10.6.1, Franklin Lakes, NJ, USA) to perform compensation and gating.

2.6 Statistics

Data analysis was performed using GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA). Data were presented as mean $\pm$ standard deviation (SD). A p-value $<$ 0.05 indicates statistically significant differences. Asterisks denote significance levels: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

3. Results

3.1 CRMP4 exhibits low expression in cervical cancer

To discover CRMP4's role in cervical cancer progression, we first detected its expression. TCGA database revealed abnormally low CRMP4 expression in cervical cancer tissues (Fig. 1A). A CRMP4 immunoblot was performed in 4 cervical cancer cell lines to detect CRMP4 expression. CRMP4 was downregulated in cancer cells (Fig. 1B). Therefore, CRMP4 exhibits low expression in cervical cancer.

3.2 CRMP4 overexpression inhibits cervical cancer cell proliferation

Detecting CRMP4's role in cervical cancer was made possible by low CRMP4 expression in human cervical cancer cells. A CRMP4 overexpression adenovirus was infected into HeLa as well as SiHa cells. CRMP4 expression was significantly increased following transfection with CRMP4 (Fig. 2A). CRMP4 overexpression decreased OD450 values in HeLa as well as SiHa cells, suggesting cell growth inhibition (Fig. 2B). Similarly, CRMP4 overexpression decreased colony numbers in HeLa as well as SiHa cells (Fig. 2C). Moreover, its overexpression further stimulated apoptosis in HeLa as well as SiHa cells (Fig. 2D). Therefore, CRMP4 suppressed cervical cancer cell growth.

3.3 CRMP4 overexpression blocks cell EMT in cervical cancer cells

Subsequently, we detected CRMP4 effects on cervical cancer cell EMT process. CRMP4 overexpression increased E-cadherin expression, and decreased N-cadherin and α-SMA expressions in HeLa as well as SiHa cells, suggesting EMT suppression (Fig. 3). Therefore, CRMP4 overexpression inhibited cervical cancer cell EMT process.

3.4 Overexpressed CRMP4 downregulates the Wnt/β-catenin pathway

We then investigated the mechanism underlying CRMP4's effect on cervical cancer progression. CRMP4 effects on the Wnt/β-catenin axis have been reported, which could mediate cell growth and motility. CRMP4 was then investigated in cervical cancer cells to see if it mediates this axis. CRMP4 overexpression decreased expressions of β-catenin, Wnt3a, c-Myc and cyclin D1, in HeLa as well as SiHa cells (Fig. 4). Therefore, CRMP4 suppresses the Wnt/β-catenin axis in cervical cancer cells.

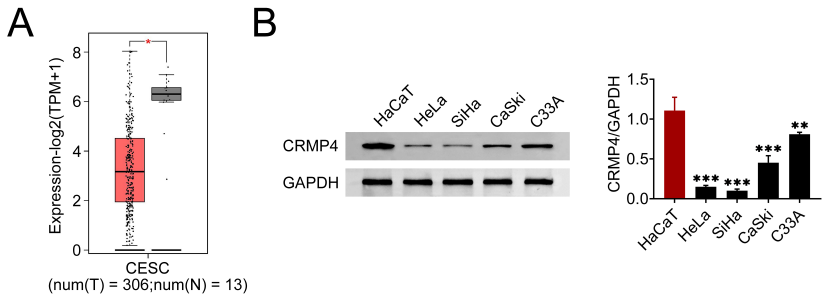

FIGURE 1. CRMP4 exhibits low expression in cervical cancer. (A) TCGA database revealed CRMP4 transcripts per million (TPM) in 13 normal tissues and 306 tumor tissues of cervical cancer patients. (B) Immunoblot assays showed CRMP4 expression levels in the indicated cell lines. CRMP4 relative expression was quantified. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, *vs.* HaCaT: Human Adult Low Calcium High Temperature keratinocytes; TPM: Transcripts Per Million; CESC: Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma; CRMP4: Collapsin Response Mediator Protein 4; GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase.

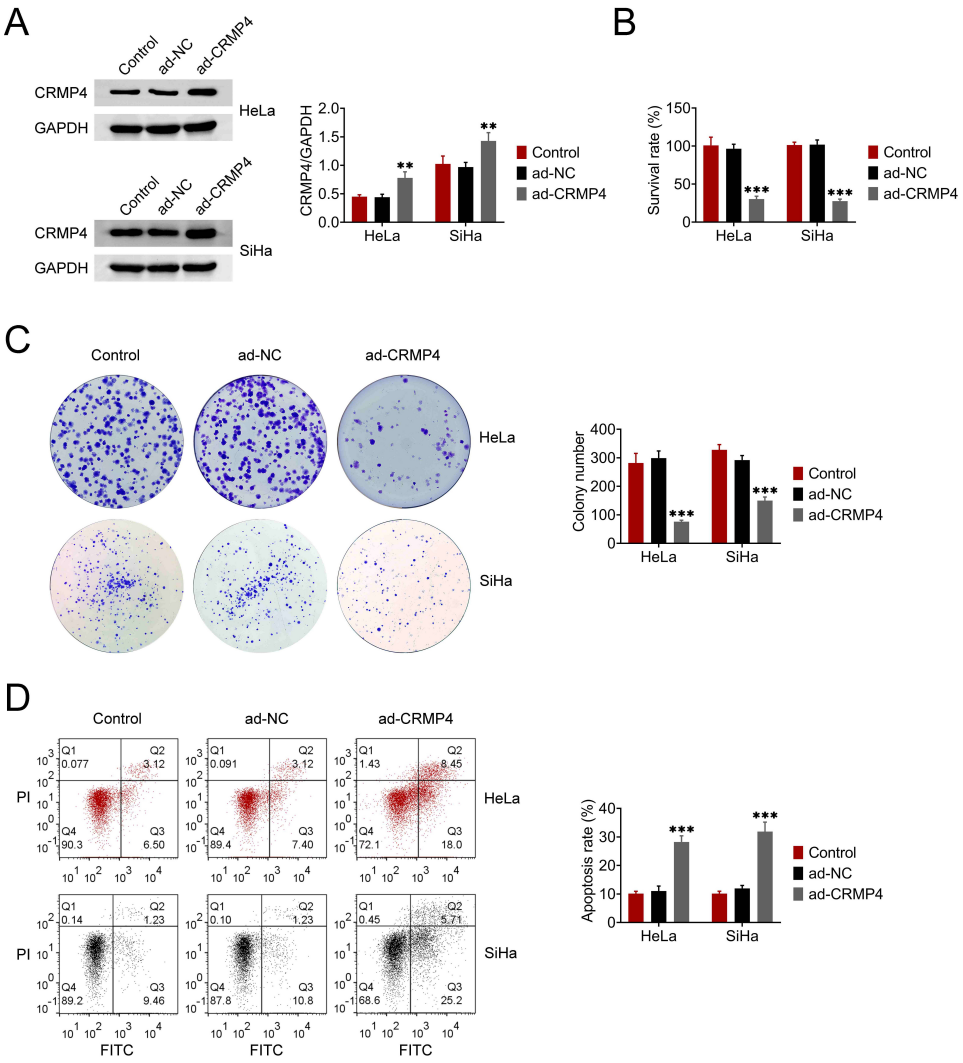

FIGURE 2. CRMP4 overexpression inhibits cervical cancer cell proliferation. (A) Immunoblot assays indicated CRMP4 expression in HeLa as well as SiHa cells upon infection with ad-NC and ad-CRMP4 for 24 h. CRMP4 relative expression was quantified. (B) CCK-8 assays showed HeLa as well as SiHa cell growth upon infection with ad-NC and ad-CRMP4 for 24 h. OD450 value was measured. (C) Colony formation assays indicated HeLa as well as SiHa cell growth upon infection with ad-NC and ad-CRMP4 for 24 h. Colony numbers were counted. (D) FCM assays revealed HeLa as well as SiHa cell apoptosis upon infection with ad-NC and ad-CRMP4 for 24 h. Percentage of apoptosis cells was measured. **$p < 0.01$, ***$p < 0.001$, ad-CRMP4 *vs.* ad-NC. NC: negative control; CRMP4: Collapsin Response Mediator Protein 4; GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase; FITC: Fluorescein Isothiocyanate.

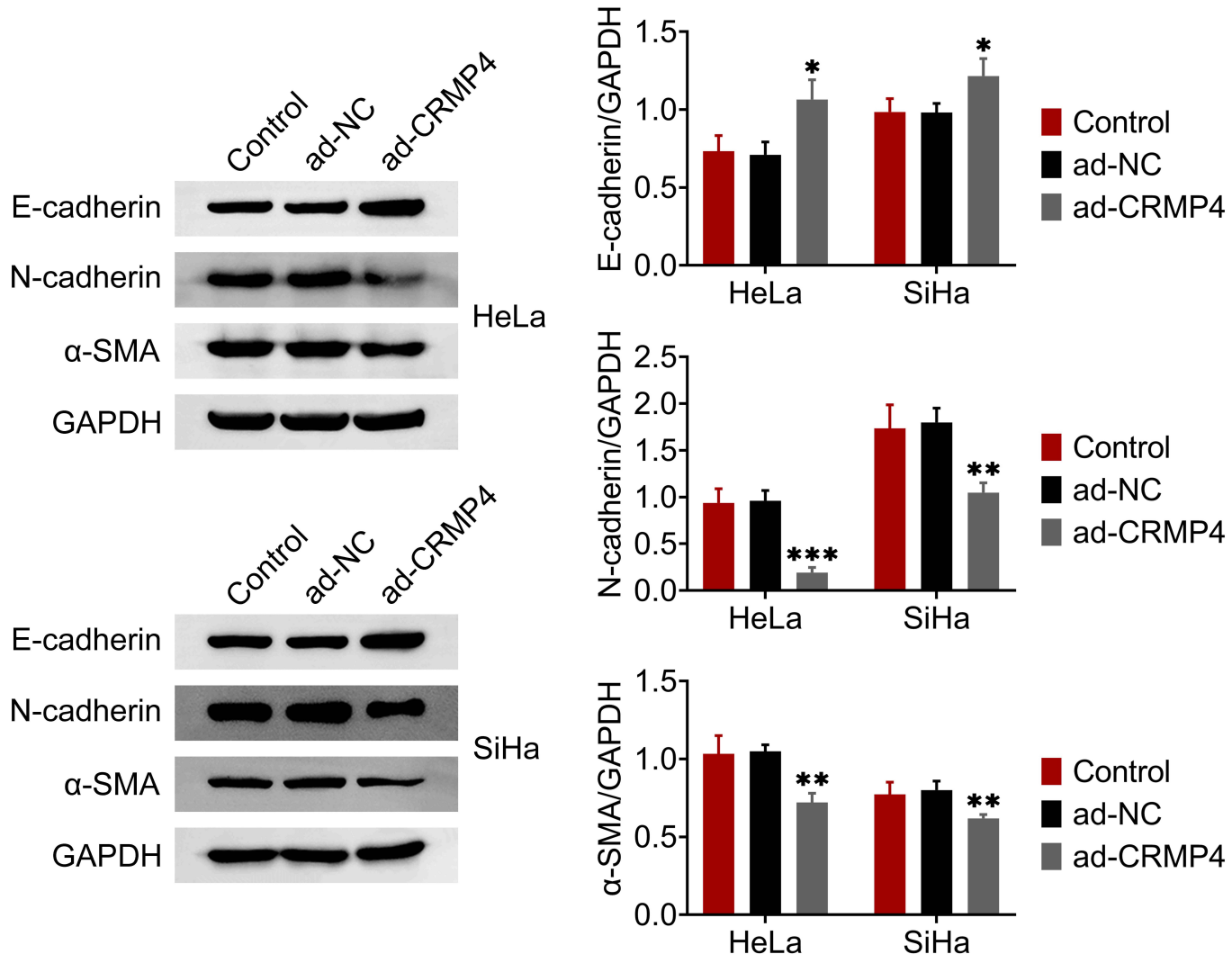

FIGURE 3. CRMP4 overexpression blocks cell EMT in cervical cancer cells. Immunoblot assays showed E-cadherin and N-cadherin, and α-SMA expressions in HeLa as well as SiHa cells upon infection with ad-NC and ad-CRMP4 for 24 h. Relative protein expression levels were calculated. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, ad-CRMP4 *vs.* ad-NC. NC: negative control; E-cadherin: Epithelial Cadherin; N-cadherin: Neural Cadherin; α-SMA: Alpha-Smooth Muscle Actin; CRMP4: Collapsin Response Mediator Protein 4; GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase.

4. Discussion

This study fills some gaps in previous research by exploring the pathogenesis of cervical cancer and its possible association with CRMP4. Our findings demonstrated that as a regulatory protein, CRMP4 affects cervical cancer cell growth as well as EMT by modulating the Wnt/β-catenin pathway, providing a new perspective on cervical cancer molecular mechanisms [11]. Cervical cancer development is associated with multiple factors. Cervical cancer is currently treated with surgery and chemotherapy, but their effectiveness is limited due to late diagnosis and low five-year survival rate [12]. New targets could enable more personalized treatment options for cervical cancer patients, improving survival rates and quality of life. Furthermore, a deeper understanding of the mechanisms underlying cervical cancer is crucial for advancing this field.

In this study, CRMP4 mediates numerous cellular mechanisms, particularly in cancer biology. CRMP4 influences cytoskeletal dynamics, impacting cellular processes such as motility, and differentiation [13]. Our research highlights its capability to modulate the Wnt/β-catenin axis, thereby affecting cancer cell growth and EMT. CRMP4 not only contributes to cells' structural integrity but also acts as a significant mediator in signaling pathways that govern cancer progression, making it a potential target.

CRMP4 has therapeutic potential in various cancers [9, 10, 13]. CRMP4 inhibits prostate cancer cell migration and invasion by modulating actin cytoskeleton [9]. It appears that CRMP4 suppresses tumor metastasis in pancreatic cancer by promoting its expression [9, 10]. Based on these studies and our findings, CRMP4 may serve as a potential therapeutic target in cervical cancer by inhibiting key tumor-progressing processes.

Wnt/β-catenin is vital in regulating cell fate, growth and migration in various cancers [14, 15]. Cancer cells' survival and invasiveness are associated with aberrant activation of this pathway [16, 17]. Our findings demonstrated that CRMP4 overexpression significantly inhibits the Wnt/β-catenin axis

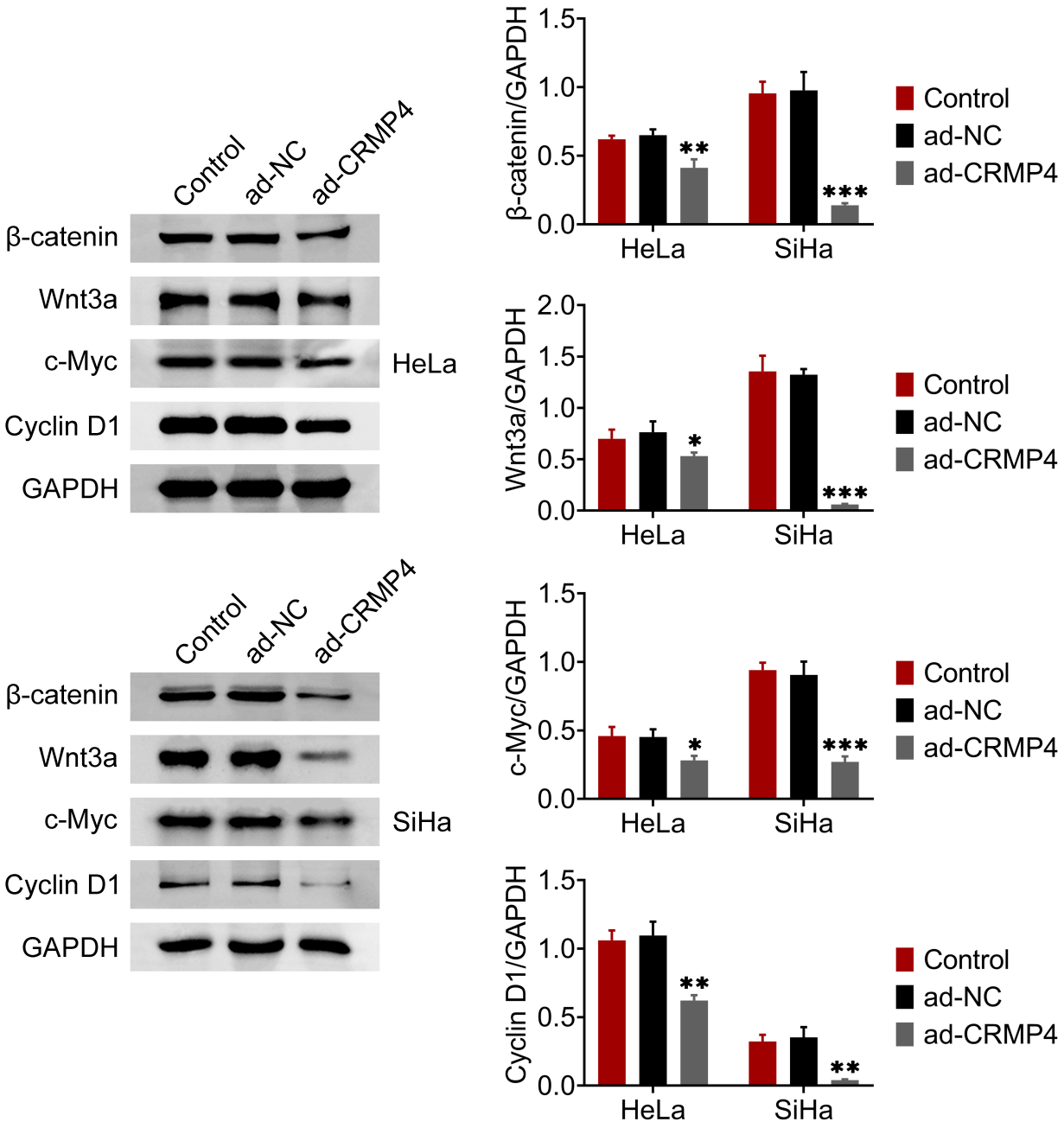

FIGURE 4. Overexpressed CRMP4 downregulates the Wnt/β-catenin pathway. Immunoblot assays revealed β-catenin, Wnt3a, c-Myc and cyclin D1 expressions in HeLa as well as SiHa cells upon infection with ad-NC and ad-CRMP4 for 24 h. Relative protein expression levels were calculated. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ ad-CRMP4 *vs.* ad-NC. NC: negative control; Wnt3a: Wingless-Type MMTV Integration Site Family, Member 3A; CRMP4: Collapsin Response Mediator Protein 4; GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase.

in cervical cancer cells, suppressing cell proliferation and EMT. This study highlights CRMP4's dual function in cervical cancer pathogenesis. In addition to affecting cell proliferation, CRMP4 also regulates critical processes of cell phenotype transformation.

In spite of this study's contribution to the understanding of CRMP4 as a potential therapeutic target, we recognize that cervical cancer treatment and prevention are still facing many challenges. CRMP4 has complex regulatory mechanisms, and its role may differ across cancer types [9, 18]. To develop CRMP4-based therapeutic strategies, further research is needed to assess its specific functions and mechanisms of action in cervical cancer's unique environment.

5. Conclusions

This study provides significant insight into CRMP4's role-CRMP4 in cancer progression. In particular, it emphasizes particularly highlighting its function in modulating key signaling pathways like Wnt/β-catenin, which influences cell proliferation and EMT. In this study, CRMP4 is demonstrated to have therapeutic potential, offering potential for developing novel cancer treatments.

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

MQL, YW—designed the study and carried them out; prepared the manuscript for publication and reviewed the draft of the manuscript. MQL, SLG, XJX, HM, DMW, XQC—supervised the data collection; analyzed the data. MQL, SLG, XJX, HM—interpreted the data. 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.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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