Submitted: 11 March, 2024 Accepted: 16 April, 2024 Published: 15 August, 2024 DOI:10.22514/ejgo.2024.073

ORIGINAL RESEARCH

European Journal of Gynaecological Oncology

Beta-Caryophyllene can inhibit the proliferation and autophagy of HPV16-infected immortalized cervical epithelial cells

Meng Fei[1,*], Wen Fan[2]

[1]Department of Gynecology and Obstetrics, The second people's Hospital of Jingzhou, 434000 Jingzhou, Hubei, China
[2]Department of General Practice, The first people's Hospital of Jingzhou, 434000 Jingzhou, Hubei, China

***Correspondence**
FM_meng037@163.com
(Meng Fei)

Abstract

Cervical cancer arises due to the progressive dysplasia of cervical epithelial cells, characterized pathologically as cervical intraepithelial neoplasia (CIN). Given the urgency for effective treatments against CIN, exploring novel pharmaceutical candidates is of paramount importance. Beta-Caryophyllene (BCP), derived from various plants, has shown promise in impeding the progression of various malignancies. However, its potential efficacy in CIN treatment remains unexplored. In this study, we investigate the impact of BCP on CIN progression and elucidate its mechanism of action. An immortalized cervical epithelial cell line (H8) infected with HPV16 was used as a model for CIN. The results revealed that BCP significantly inhibited the proliferation of H8 cells and induced apoptosis. Moreover, BCP demonstrated the ability to suppress autophagy in H8 cells. Mechanistically, our findings indicate that BCP exerts its effects by modulating the Wnt/β-catenin signaling pathway. Specifically, BCP was found to block this pathway, thereby impeding cell growth and autophagy in H8 cells. These results suggest that BCP holds promise as a therapeutic agent for CIN, potentially through its regulatory effects on the Wnt/β-catenin pathway.

Keywords

Cervical intraepithelial neoplasia (CIN); Beta-Caryophyllene (BCP); HPV16; Autophagy; Wnt/β-catenin pathway

1. Introduction

Cervical cancer ranks as the second leading cause of tumor-related mortality among women globally, with an estimated 530,000 new cases and 275,000 deaths annually. It occurs from the progressive dysplasia of the cervical epithelium, clinically termed cervical intraepithelial neoplasia (CIN) [1–3]. Human papillomavirus (HPV) infection is widely recognized as a primary sexual vector contributing to CIN and cervical cancer [4, 5]. Since persistent HPV infection significantly elevates the risk of CIN progressing to invasive cervical cancer, there is an urgent need to discover novel pharmaceutical interventions to combat CIN [6].

Natural products represent a vast repository of bioactive agents, offering a rich source for drug discovery research since the turn of the millennium. These naturally occurring chemical products have emerged as a novel paradigm for disease prevention [7]. Beta-Caryophyllene (BCP), a sesquiterpene found in various plants, including certain essential oils and cannabis, has received attention for its reported inhibitory effects on the growth of diverse cancer cell types, such as bladder and lung cancers [8, 9]. In anti-cancer research, BCP has demonstrated anti-proliferative properties in human melanoma and breast cancer cell lines [10]. Moreover, synergistic interactions between BCP and certain plant molecules have been shown to induce apoptosis in epidermoid skin cancer cells [11]. In regard to other diseases, BCP has been shown to possess neuroprotective effects by activating autophagy, thereby shielding neurons from damage due to brain ischemia-reperfusion injury [12]. Additionally, BCP displays anti-proliferative activity in multiple myeloma cells through modulation of the Wnt/β-catenin signaling pathway [13]. The transfection of primary human cervical epithelial (HCE) cells with full-length HPV type 16 DNA has led to the establishment of a cell line capable of sustained growth, morphologically resembling cervical intraepithelial neoplasia [5]. This *in vitro* model, involving human cervical epithelium infected with HPV genes, serves as a fundamental tool for investigating the physiological mechanisms underlying cervical epithelial biology and cervical diseases [5]. Notably, the E6/papillomavirus E6-associated protein (E6AP) complex has been implicated in enhancing the Wnt signaling pathway, potentially contributing to HPV-induced carcinogenesis [5]. However, despite the extensive research on BCP's pharmacological properties, its role in CIN progression and the underlying mechanisms remain unclear.

This study investigates the impact of BCP on the progression of CIN. The findings showed that BCP effectively restrained

the growth and autophagy of HPV16-infected immortalized cervical epithelial cells by modulating the Wnt/β-catenin pathway, supporting the potential of BCP as a promising therapeutic candidate for CIN.

2. Materials and methods

2.1 Cell culture and treatment

The human HPV16-infected immortalized cervical epithelial cell line H8 was purchased from American Type Culture Collection (ATCC) and maintained in Dulbecco's Modified Eagle Medium (DMEM) complete medium (Gibco, 11995065, Logan, UT, USA) at 37 °C with 5% Carbon dioxide (CO_2). For experimental treatments, BCP (CRM40483, Sigma, St. Louis, MO, USA) was administered to the H8 cells at concentrations of 0, 20, 40 and 80 μM for a duration of 24 hours.

2.2 Cell viability assays

H8 cells were seeded into 96-well plates and incubated at 37 °C. After the specified treatments for 24 hours, the cells were exposed to Cell counting kit-8 (CCK-8, C0038, Beyotime, Beijing, China) reagent at 37 °C for 4 hours. The relative cell viability was evaluated using a spectrophotometer set to measure absorbance at 450 nm wavelength (Bio-Rad, 1681, Hercules, CA, USA) over 3 days.

2.3 Edu assay

Edu-treated H8 cells were fixed and blocked with 10% goat serum in Phosphate Buffer Triton X 100 (PBST) (0.1% Triton X-100 in Phosphate Buffer (PBS)) for 1 hour. Then, the cells were stained with 4,6-diamino-2-phenyl indole (DAPI) for 3 minutes, and after PBS washing, the sections were examined using a microscope (Leica, DM 5000B, Witzler, HE, Germany).

2.4 Cell apoptosis assay

H8 cells were rinsed with PBS and then fixed by incubation in 70% ethanol at −20 °C for 2 hours. After fixation, the cells were stained with propidium iodide (PI) and Annexin V at 4 °C and then analyzed using a FACSCalibur flow cytometer (BD Biosciences, Inc., BD-FACSCanto-II, Franklin Lake, NJ, USA).

2.5 Immunofluorescent staining

H8 cells were blocked with 4% paraformaldehyde (PFA) in 5% bovine serum albumin (BSA) and subsequently incubated with primary LC3B antibody (anti-LC3, 1:1000, ab192890, Abcam, Cambridge, UK). After three rinses, Alexa Fluor 488 secondary antibodies (Invitrogen, A30006, Carlsbad, CA, USA) were applied, and photographs were taken using a fluorescent microscope.

2.6 Immunoblot assay

The cell or tissue samples were lysed and subjected to 10% Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to separate the total proteins, which were then transferred onto Polyvinylidene fluoride (PVDF) membranes (Millipore, USA). Subsequently, the membranes were blocked with 5% dry milk. Primary antibodies, including anti-Bax (1:500, ab32503, Abcam, Cambridge, UK), anti-B-cell lymphoma-2 (BCL-2) (1:500, ab182858, Abcam, Cambridge, UK), anti-cleaved-Caspase-3 (1:1000, ab32042, Abcam, Cambridge, UK), LC3 (1:2000, ab192890, Abcam, Cambridge, UK), P62 (1:500, ab109012, Abcam, Cambridge, UK), anti-Aptera MMTV integration site family member 3A (WNT-3a) (1:500, ab81614, Abcam, Cambridge, UK), anti-β-catenin (1:500, ab32572, Abcam, Cambridge, UK), anti-p-β-catenin (1:500, ab305261, Abcam, Cambridge, UK), anti-Glycogen synthase kinase (GSK)-3β (1:500, ab93926, Abcam, Cambridge, UK), anti-p-GSK-3β (1:500, ab75814, Abcam, Cambridge, UK), and anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:3000, ab8245, Abcam, Cambridge, UK), were applied. After washing, the membranes were incubated with Horseradish peroxidase (HRP)-labelled secondary antibodies (A0208, Beyotime, Beijing, China) for 45 minutes. Lastly, each blot was visualized using the Enhanced chemiluminescence (ECL) kit (P0018A, Beyotime, Beijing, China).

2.7 Statistical analysis

Data analysis was conducted using GraphPad 8.0 (Graphpad plc., San Diego, CA, USA) and is presented as mean ± standard deviation (SD). A p-value less than 0.05 was considered statistically significant.

3. Results

3.1 BCP restrained the growth of H8 cells

To investigate the effects of BCP on CIN progression *in vitro*, we utilized the HPV16-infected immortalized cervical epithelial cell line H8 as the model. BCP was administered to H8 cells at concentrations of 20, 40 and 80 μM for 24 hours. The molecular formula of BCP is shown in Fig. 1A. CCK-8 assays revealed significant suppression of H8 cell growth after BCP treatment, as indicated by decreased Optical density (OD)450 values (Fig. 1B). Similarly, Edu assays demonstrated a reduction in the percentage of Edu-positive H8 cells after BCP treatment (Fig. 1C,D). Overall, these results indicate that BCP effectively restrains the growth of H8 cells.

3.2 BCP stimulated the apoptosis of H8 cells

Expanding our investigation into BCP's impact on cell growth inhibition, we examined its influence on apoptosis in H8 cells to comprehensively understand its therapeutic potential. Flow cytometry (FCM) assays revealed that BCP treatment induced apoptosis in H8 cells, as indicated by an increased percentage of apoptotic cells (Fig. 2A). Immunoblot analysis demonstrated that BCP treatment resulted in elevated expression levels of Bax and cleaved caspase-3, followed by decreased expression of BCL-2 in H8 cells, suggesting the suppression of cell apoptosis (Fig. 2B), indicating that BCP promotes apoptosis in H8 cells.

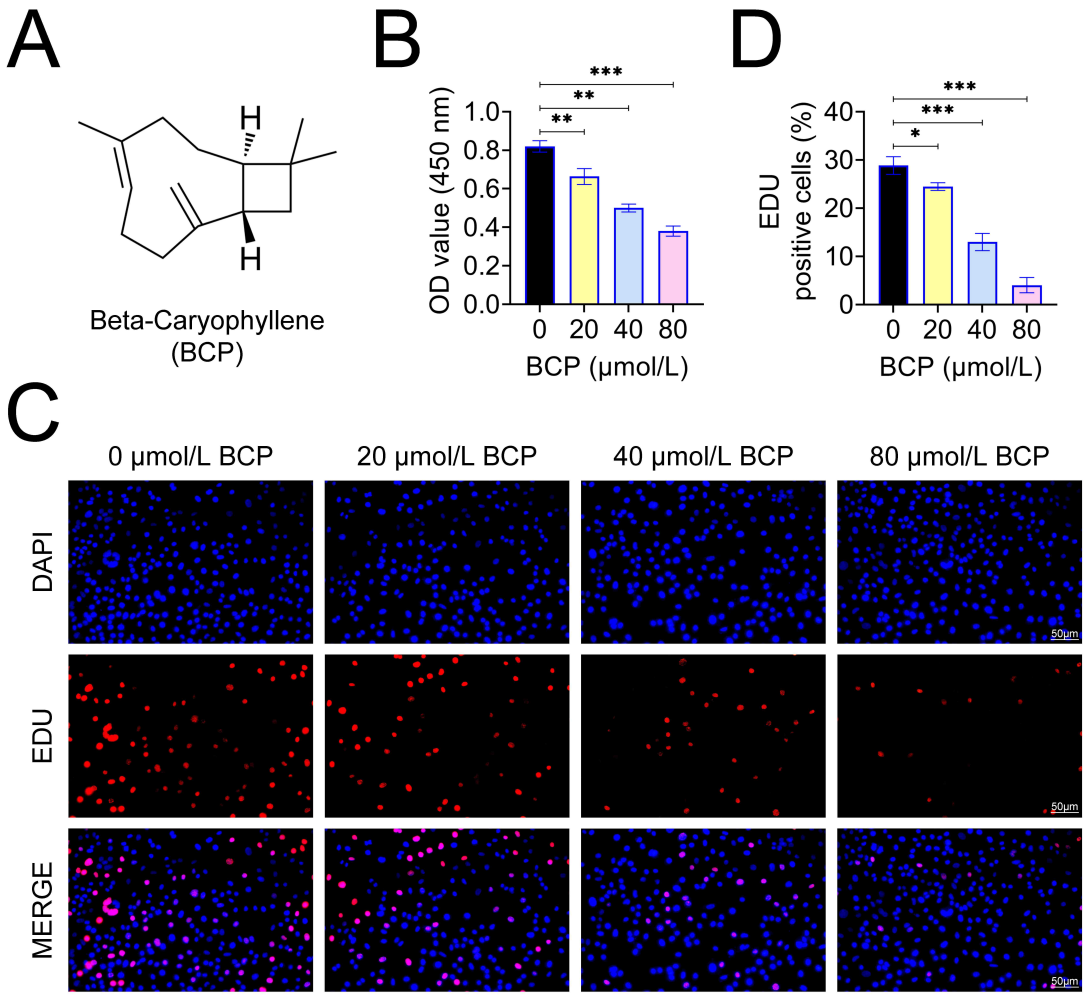

FIGURE 1. BCP inhibits the growth of H8 cells. (A) Molecular structure of BCP. (B) CCK-8 assays showing the growth inhibition of H8 cells following treatment with BCP at concentrations of 0, 20, 40 and 80 μM for 24 h. OD450 values were measured. (C) Edu assays illustrating the growth inhibition of H8 cells following treatment with BCP at concentrations of 0, 20, 40 and 80 μM for 24 h. Scale bar, 50 μm. (D) Quantification of panel C, showing the percentage of Edu-positive cells. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. BCP: Beta-Caryophyllene; OD: Optical density; EDU: 5-Ethynyl-2′- deoxyuridine; DAPI: 4′,6-Diamidino-2′-phenylindole.

3.3 BCP suppressed the autophagy of H8 cells

Given the observed inhibitory effects of BCP on cell growth and its pro-apoptotic role in H8 cells, we explored how BCP influences the autophagy process in these cells. Immunoblot assays were conducted to detect the expression of autophagy markers, including LC3 and P62, and the findings revealed that BCP treatment increased the expression of P62 and decreased the ratio of LC3II/LC3I in H8 cells, suggesting suppressed autophagy (Fig. 3A). Similarly, immunostaining assays demonstrated that BCP treatment reduced the expression of LC3B in H8 cells, as evidenced by decreased staining intensity (Fig. 3B). These findings indicate that BCP can suppress the autophagy process in H8 cells.

3.4 BCP blocked the Wnt/β-catenin pathway in H8 cells

Investigations into the impact of BCP on the Wnt/β-catenin signaling pathway revealed mixed outcomes. While some indicators suggested potential inhibition by BCP, the general consensus remains unclear. Given the complexity of the pathway's regulation and its interaction with BCP, further examination is warranted. Herein, our immunoblot assay results showed that BCP treatment suppressed the phosphorylation of β-catenin and GSK-3β in H8 cells, alongside a decrease in the expression of WNT-3a, indicating inhibition of the Wnt/β-catenin pathway (Fig. 4). Thus, we hypothesize that BCP could block the Wnt/β-catenin pathway in H8 cells, thereby suppressing cell growth and autophagy.

4. Discussion

CIN is a precancerous lesion of the cervix, mainly caused by HPV infection [5]. CIN is classified into three grades: CIN1, CIN2 and CIN3, with CIN3 representing the most severe manifestation. Treatment modalities include physical interventions such as cryotherapy, laser therapy and loop electrosurgical excision procedures, and immunomodulatory agents [14, 15]. Given the associated side effects and recurrence risks of phys-

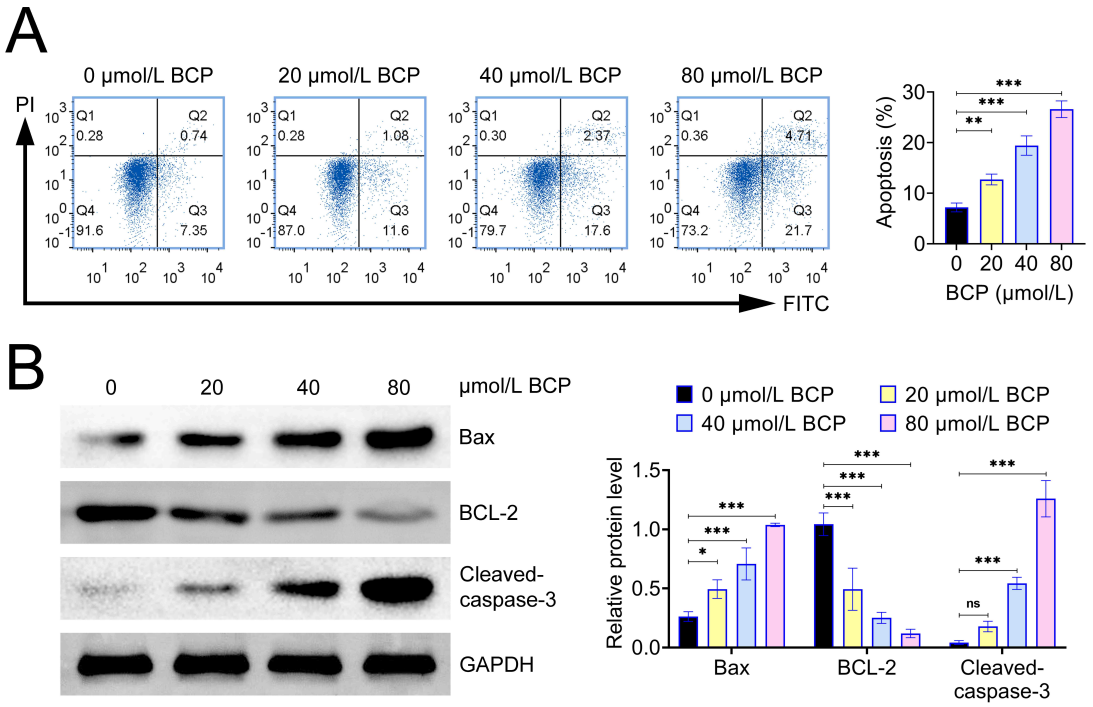

FIGURE 2. BCP induces apoptosis in H8 cells. (A) FCM assays showing the degree of apoptosis in H8 cells after treatment with BCP at concentrations of 0, 20, 40 and 80 μM for 24 h. The percentage of apoptotic cells was quantified. (B) Immunoblot assays illustrating the expression levels of Bax, BCL-2 and cleaved caspase-3 in H8 cells following treatment with BCP at concentrations of 0, 20, 40 and 80 μM for 24 h. The relative expression of these proteins was quantified. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. BCP: Beta-Caryophyllene; PI: propidium iodide; FITC: Fluorescein Isothiocyanate; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

ical therapies, the development of new drugs targeting HPV infection and CIN is of great significance [14] as it could inhibit HPV viral replication, enhance immune response and regulate the cell cycle to prevent cervical cancer occurrence [16]. Herein, our data confirm that BCP effectively suppresses CIN progression at the cellular level, suggesting its promise as a therapeutic agent for CIN.

At the cellular level, the mechanism underlying CIN primarily involves HPV infection and dysregulated regulation of the host cell cycle [17]. Key components in this process are the early proteins E6 and E7 of HPV, wherein E6 facilitates the degradation of the tumor suppressor protein p53, while E7 disables the Rb protein, resulting in uncontrolled cell cycle advancement and unbridled cell proliferation [18]. Moreover, HPV infection induces alterations in host cell gene expression and aberrant immune responses, further promoting CIN development [18] and leading to the anomalous proliferation of cervical epithelial cells and the formation of precancerous lesions [18]. In this study, our findings confirm that BCP suppressed the proliferation of H8 cells, induced cell apoptosis, and restrained cell autophagy, thus impeding CIN progression.

CIN has been shown to be closely associated with increased cell proliferation, inhibition of apoptosis, and alterations in the autophagy process [19, 20]. HPV infection, particularly high-risk types, disrupts normal cell cycle regulation through its early proteins E6 and E7, resulting in uncontrolled proliferation of cervical epithelial cells [19, 20]. Simultaneously, E6-mediated degradation of the p53 protein inhibits apoptosis, allowing abnormal cells to persist and increasing cancer risk. Additionally, HPV infection may disrupt the normal autophagy process by influencing the expression of autophagy-related genes, thus promoting cell survival [19]. The imbalance in these cellular processes is an important factor in CIN progression to cervical cancer. Our data indicate that BCP affects these cellular processes, including the growth, apoptosis, and autophagy of H8 cells, a CIN cell model. However, further studies are needed to elucidate the precise mechanism.

CIN is closely associated with the Wnt/β-catenin signaling pathway [21, 22]. Normally, β-catenin levels are tightly regulated, but in CIN, this pathway is often abnormally activated [21]. The early proteins E6 and E7 of HPV can disrupt the pathway's normal regulation, leading to increased stability and nuclear accumulation of β-catenin, promoting unrestricted proliferation of cervical epithelial cells [21]. Moreover, gene mutations linked to CIN and cervical cancer may also contribute to pathway activation [22]. Thus, the Wnt/β-catenin pathway plays an important role in the occurrence and progression of CIN and has become a potential target for the prevention and treatment of cervical cancer. Interestingly, our data confirmed that BCP inhibited the growth and autophagy of H8 cells and stimulated cell apoptosis *via* the Wnt/β-catenin pathway; therefore, BCP suppressed CIN at the cellular level.

To elucidate the broader implications of our findings, it is essential to consider the role of BCP in modulating the Wnt/β-catenin signaling pathway, an important element in the proliferation and survival of cancer cells. Our study demonstrates that BCP not only inhibits the growth of HPV16-infected cervical epithelial cells but also induces apoptosis, suggesting

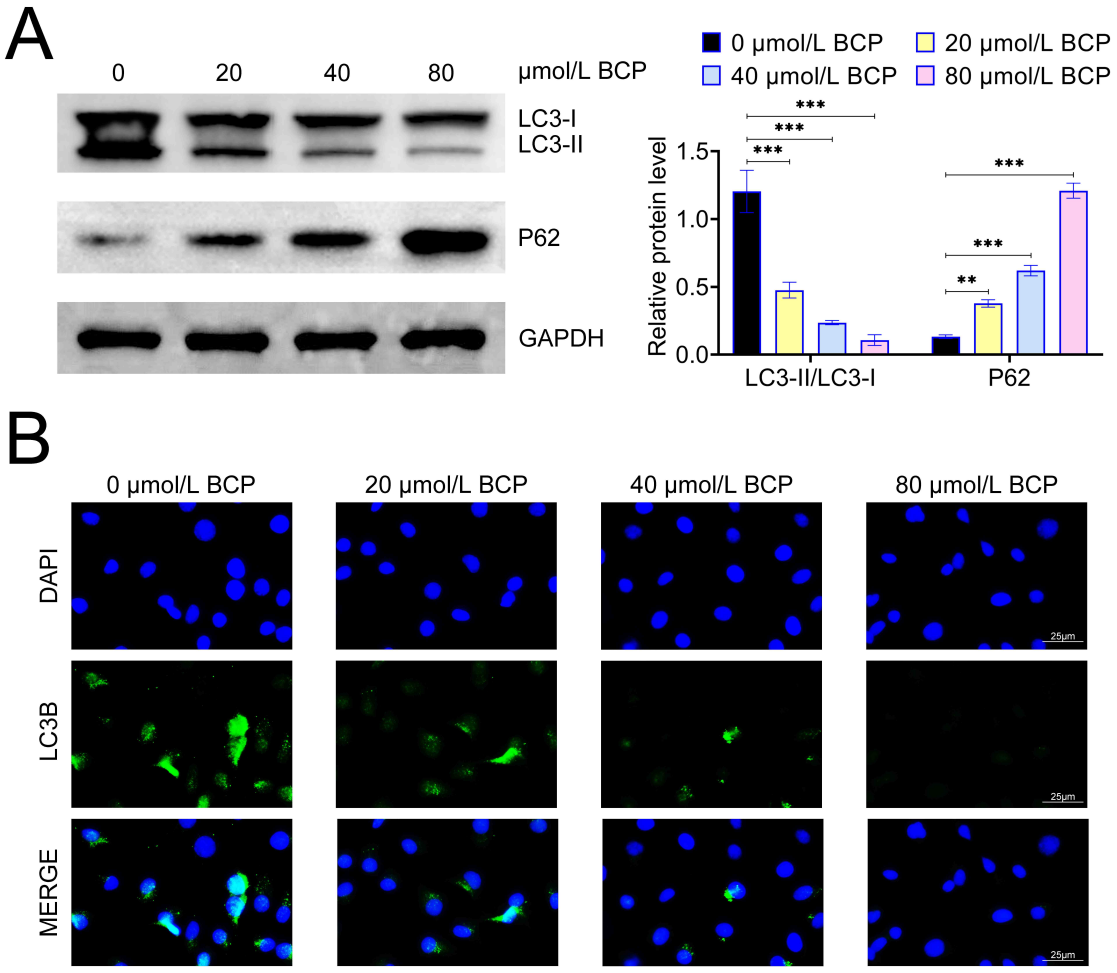

FIGURE 3. BCP suppresses autophagy in H8 cells. (A) Immunoblot assays for determining the expression levels of LC3 and P62 in H8 cells after treatment with BCP at concentrations of 0, 20, 40 and 80 μM for 24 h. The relative expression of P62 and the ratio of LC3II/LC3I were quantified. (B) Immunostaining assays showing the expression of LC3B in H8 cells following treatment with BCP at concentrations of 0, 20, 40 and 80 μM for 24 h. The red panel indicates LC3B. Scale bar, 25 μm. $^{**}p < 0.01$, $^{***}p < 0.001$. BCP: Beta-Caryophyllene; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; DAPI: 4′,6-Diamidino-2′-phenylindole.

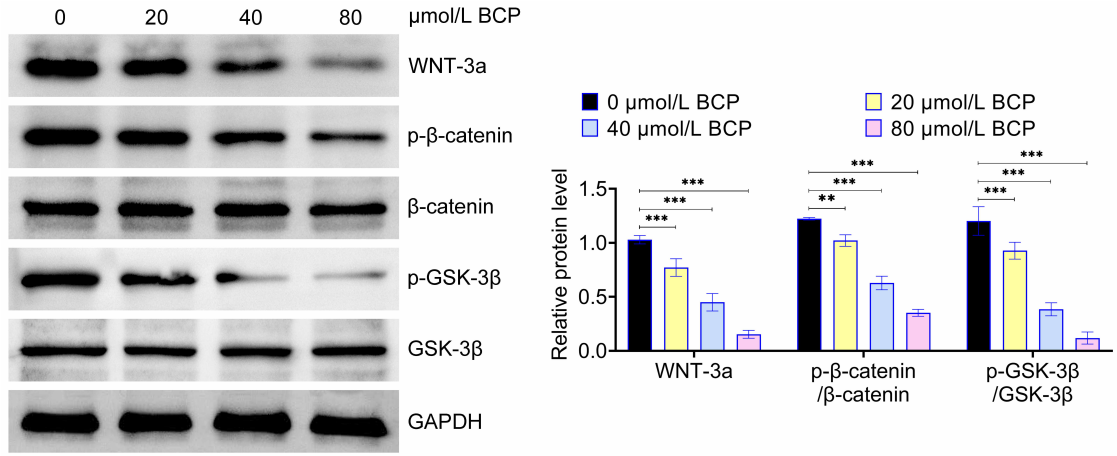

FIGURE 4. BCP inhibits the Wnt/β-catenin pathway in H8 cells. Immunoblot assays showing the expression levels of WNT-3a, β-catenin, and GSK-3β, as well as the phosphorylation levels of β-catenin and GSK-3β in H8 cells after treatment with BCP at concentrations of 0, 20, 40 and 80 μM for 24 h. The relative expression or phosphorylation of these proteins was quantified. $^{**}p < 0.01$, $^{***}p < 0.001$. BCP: Beta-Caryophyllene; Aptera MMTV integration site family member 3A; GSK-3β: Glycogen synthase kinase-3β; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

a potential therapeutic role in combating CIN and aligning with and expanding upon existing literature indicating that natural compounds can significantly impact cancer cell biology through various pathways [21]. Particularly, the inhibition of the Wnt/β-catenin pathway by BCP highlights its potential as a multifaceted therapeutic agent capable of targeting different aspects of cancer cell physiology [22]. Furthermore, these results contribute to a growing body of evidence supporting the use of plant-derived compounds in cancer treatment, challenging the conventional reliance on synthetic drugs [22]. In this context, our findings highlight the importance of exploring natural compounds as both alternative treatments and potential leads for developing more effective and less toxic cancer therapies.

We also investigated the potential mechanisms underlying BCP's inhibitory effects on H8 cell proliferation. Given the important role of the Wnt/β-catenin signaling pathway in regulating the cell cycle, we propose that BCP suppresses cell proliferation by inhibiting the activation of this pathway, consequently arresting cell cycle progression. Our data demonstrate that BCP treatment substantially decreases the phosphorylation levels of β-catenin, corroborating our hypothesis. This elucidates the potential molecular mechanism underlying BCP's inhibition of CIN cell proliferation and provides new insights for future therapeutic approaches.

Additionally, we investigated the impact of BCP on the autophagic process in H8 cells. Autophagy, an important intracellular degradation pathway, plays a multifaceted role in the survival and drug resistance of cancer cells. Herein, we observed that BCP treatment reduced the LC3-II/LC3-I ratio and increased P62 protein levels, indicating a suppression of autophagic flux. This finding suggests that BCP may influence the survival state of CIN cells by disrupting the normal progression of intracellular autophagic flux and offers novel molecular insights into the role of BCP in combating CIN.

5. Conclusions

In summary, our study provides compelling evidence that BCP exerts significant inhibitory effects on the proliferation and autophagy of HPV16-infected immortalized cervical epithelial cells, suggesting its potential as a therapeutic agent for CIN. In addition, we elucidated that BCP's actions are mediated, at least in part, by its modulation of the Wnt/β-catenin signaling 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

MF—designed the study and carried them out; prepared the manuscript for publication and reviewed the draft of the manuscript. MF and WF—supervised the data collection, analyzed the data, 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.

ACKNOWLEDGMENT

Not applicable.

FUNDING

This research received no external funding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Zang L, Feng R, Huang Y, Huang J, Hu Y. Relationship between vaginal microecology and human papillomavirus infection as well as cervical intraepithelial neoplasia in 2,147 women from Wenzhou, the southeast of China. Frontiers in Oncology. 2023; 13: 1306376.

[2] Gong L, Tang Y, Xie H, Zhang L, Sun Y. Predicting cervical intraepithelial neoplasia and determining the follow-up period in high-risk human papillomavirus patients. Frontiers in Oncology. 2023; 13: 1289030.

[3] Gupta S, Nagtode N, Chandra V, Gomase K. From diagnosis to treatment: exploring the latest management trends in cervical intraepithelial neoplasia. Cureus. 2023; 15: e50291.

[4] Shiravani Z, Nazari Z, Yazdani F, Najib FS, Jahromi MA, Momtahan M, *et al*. Evaluation of the therapeutic effect of quadrivalent human papillomavirus (HPV) vaccination on cervical intraepithelial neoplasia lesions. Indian Journal of Surgical Oncology. 2023; 14: 504–509.

[5] Sakai M, Ohara T, Suzuki H, Kadomoto T, Inayama Y, Shitanaka S, *et al*. Clinical impact of age-specific distribution of combination patterns of cytology and high-risk HPV status on cervical intraepithelial neoplasia grade 2 or more. Oncology Letters. 2023; 26: 384.

[6] Herzog C, Vavourakis CD, Barrett JE, Karbon G, Villunger A, Wang J, *et al*. HPV-induced host epigenetic reprogramming is lost upon progression to high-grade cervical intraepithelial neoplasia. International Journal of Cancer. 2023; 152: 2321–2330.

[7] Cui D, Chen Y, Ye B, Guo W, Wang D, He J. Natural products for the treatment of neurodegenerative diseases. Phytomedicine. 2023; 121: 155101.

[8] Mannino F, Pallio G, Imbesi C, Scarfone A, Puzzolo D, Micali A, *et al*. Beta-Caryophyllene, a plant-derived CB2 receptor agonist, protects SH-SY5Y cells from cadmium-induced toxicity. International Journal of Molecular Sciences. 2023; 24: 15487.

[9] Agnes JP, Dos Santos B, das Neves RN, Luciano VMM, Benvenutti L, Goldoni FC, *et al*. Beta-Caryophyllene inhibits oxaliplatin-induced peripheral neuropathy in mice: role of cannabinoid type 2 receptors, oxidative stress and neuroinflammation. Antioxidants. 2023; 12: 1893.

[10] Di Sotto A, Gulli M, Minacori M, Mancinelli R, Garzoli S, Percaccio E, *et al*. Beta-Caryophyllene counteracts chemoresistance induced by cigarette smoke in triple-negative breast cancer MDA-MB-468 cells. Biomedicines. 2022; 10: 2257.

[11] Ghazwani M, Hani U, Alqarni MH, Alam A. Beta Caryophyllene-loaded nanostructured lipid carriers for topical management of skin disorders:

statistical optimization, *in vitro* and dermatokinetic evaluation. Gels. 2023; 9: 550.

[12] Chen F, Bai N, Yue F, Hao Y, Wang H, He Y, *et al*. Effects of oral Beta-Caryophyllene (BCP) treatment on perioperative neurocognitive disorders: attenuation of neuroinflammation associated with microglial activation and reinforcement of autophagy activity in aged mice. Brain Research. 2023; 1815: 148425.

[13] Mannino F, Pallio G, Corsaro R, Minutoli L, Altavilla D, Vermiglio G, *et al*. Beta-Caryophyllene exhibits anti-proliferative effects through apoptosis induction and cell cycle modulation in multiple myeloma cells. Cancers. 2021; 13: 5741.

[14] Choudhoury S, Latif T, Dhar S, Sarker K, Yasmin R, Begum A, *et al*. Analysis of socio demographic risk factors of cervical intraepithelial neoplasia at colposcopic clinic in Mymensingh medical college hospital. Mymensingh Medical Journal. 2024; 33: 62–67.

[15] Hibino Y, Okazawa-Sakai M, Yokoyama T, Fujimoto E, Okame S, Teramoto N, *et al*. Abnormal vaginal cytology after total laparoscopic hysterectomy in patients with cervical intraepithelial neoplasia. Acta Medica Okayama. 2023; 77: 627–634.

[16] Zhu P, Xiong J, Yuan D, Li X, Luo L, Huang J, *et al*. ZNF671 methylation test in cervical scrapings for cervical intraepithelial neoplasia grade 3 and cervical cancer detection. Cell Reports Medicine. 2023; 4: 101143.

[17] Santopietro R, Shabalova I, Petrovichev N, Kozachenko V, Zakharova T, Pajanidi J, *et al*. Cell cycle regulators p105, p107, Rb2/p130, E2F4, p21CIP1/WAF1, cyclin a in predicting cervical intraepithelial neoplasia, high-risk human papillomavirus infections and their outcome in women screened in three new independent states of the former Soviet Union. Cancer Epidemiology, Biomarkers & Prevention. 2006; 15: 1250–1256.

[18] Nuovo GJ, de Andrade CV, Wells SI, Brusadelli M, Nicol AF. New biomarkers of human papillomavirus infection in acute cervical intraepithelial neoplasia. Annals of Diagnostic Pathology. 2018; 36: 21–27.

[19] Leisching GR, Loos B, Botha MH, Engelbrecht AM. The role of mTOR during cisplatin treatment in an *in vitro* and *ex vivo* model of cervical cancer. Toxicology. 2015; 335: 72–78.

[20] Cheng HY, Zhang YN, Wu QL, Sun XM, Sun JR, Huang X. Expression of beclin 1, an autophagy-related protein, in human cervical carcinoma and its clinical significance. European Journal of Gynaecological Oncology. 2012; 33: 15–20.

[21] Li L, Yang WT, Zheng PS, Liu XF. SOX17 restrains proliferation and tumor formation by down-regulating activity of the Wnt/β-catenin signaling pathway *via* trans-suppressing β-catenin in cervical cancer. Cell Death & Disease. 2018; 9: 741.

[22] Chen M, Li L, Zheng PS. SALL4 promotes the tumorigenicity of cervical cancer cells through activation of the Wnt/β-catenin pathway *via* CTNNB1. Cancer Science. 2019; 110: 2794–2805.

How to cite this article: Meng Fei, Wen Fan. Beta-Caryophyllene can inhibit the proliferation and autophagy of HPV16-infected immortalized cervical epithelial cells. European Journal of Gynaecological Oncology. 2024; 45(4): 93-99. doi: 10.22514/ejgo.2024.073.