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Cytotoxic activity of broussochalcone a against colon and liver cancer cells by promoting destruction complex-independent β-catenin degradation

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ABSTRACT

Aberrant activation of β-catenin-response transcription (CRT) is a well-recognized characteristic of colorectal and liver cancers and thus a potential therapeutic target for these malignancies. *Broussonetia papyrifera* (paper mulberry) has been used as a herbal medicine to treat various diseases. Using a sensitive cell-based screening system, we identified broussochalcone A (BCA), a prenylated chalcone isolated from *Broussonetia papyrifera*, as an antagonist of CRT. BCA accelerated the turnover of intracellular β-catenin that was accompanied by its N-terminal phosphorylation at Ser33/37/Thr41 residues, marking it for ubiquitin-dependent proteasomal degradation. Pharmacological inhibition of glycogen synthase kinase-3β could not abrogate BCA-mediated degradation of β-catenin. BCA decreased the intracellular β-catenin levels in colon and liver cancer cells with mutations in β-catenin, adenomatous polyposis coli, and Axin. BCA repressed the expressions of cyclin D1, c-Myc, and Axin2, which are β-catenin/T-cell factor-dependent genes, and thus decreased the viability of colon and liver cancer cell. Moreover, apoptosis was elicited by BCA, as indicated by the increase in the population of Annexin V-FITC positive cells and caspase-3/7 activities in colon and liver cancer cells. These findings indicate that BCA exerts its cytotoxic effects by promoting phosphorylation/ubiquitin-dependent degradation of β-catenin and may potentially serve as a chemopreventive agent for colonrectal and liver cancers.

1. Introduction

β-Catenin is a key component of Wnt/β-catenin signaling that controls cell proliferation, differentiation, and development (Wodarz and Nusse, 1998; Huelsken and Birchmeier, 2001; Miller, 2002). Intracellular β-catenin levels are tightly regulated by a proteasomal degradation pathway. Generally, β-catenin is phosphorylated in a destruction complex comprising adenomatous polyposis coli (APC), Axin, casein kinase 1 (CK1), and glycogen synthase kinase-3β (GSK-3β) (Hart et al., 1998; Ikeda et al., 1998) and is then recognized by the F-box β-transducin repeat-containing protein (β-TrCP) E3 ubiquitin ligase, thereby leading to its ubiquitin-dependent degradation (Aberle et al., 1997; Hart et al., 1999). Upon association of Wnt ligands with the Frizzled (Fz) and low-density lipoprotein receptor-related protein 5/6 (LRP5/6) co-receptors, β-catenin phosphorylation and degradation are inhibited via negative regulation of GSK-3β activity (Lee et al., 2003).

Abnormal activation of the Wnt/β-catenin pathway is associated with the development and progression of certain human cancer. Oncogenic mutations in β-catenin or other components of the destruction complex (APC or Axin) are frequently observed in colon cancer, hepatocellular carcinoma, and prostate cancer (Morin, 1999; Fearnhead et al., 2001; Miyoshi et al., 1998). In addition, Alterations in RNF43, a negative regulator of Wnt/β-catenin signaling, are found in over 18% of endometrial cancers and translocation of RSPO, which augments the Wnt/β-catenin pathway, are noted in 4–18% of patients with gastric, ovarian, and endometrial cancers (Kirshnamurthy and Kurzrock, 2018). These mutations result in the aberrant accumulation of β-catenin in the cytoplasm and its subsequent translocation into the nucleus, where it forms complexes with the T-cell factor/lymphocyte enhancer factor (TCF/LEF) family transcription factors that upregulate the expressions of Wnt/β-catenin dependent genes, such as *c-myc*, *cyclin D1*, metalloproteinase-7 (*MMP-7*), and peroxisome proliferators-

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activated receptor-δ (*PPAR-δ*), which play essential roles in tumorigenesis and metastasis (He et al., 1998, 1999; Tetsu and McCormick et al., 1999; Takahashi et al., 2002). Thus, promoting pathogenic β-catenin degradation may be a plausible strategy for developing anticancer therapeutics.

Broussochalcone A (BCA), a prenylated chalcone, is originally isolated from the cortex of *Broussonetia papyrifera*, which has been used as a traditional medicine for diuresis, hemostasis, and relief from edema and cough (Matsumoto et al., 1985). BCA inhibits neutrophil respiratory bursts (Wang et al., 1997) and platelet aggregation (Lin et al., 1996). In addition, it suppresses nitric oxide production in lipopolysaccharide-stimulated Raw 264.7 cells (Cheng et al., 2001) and exhibits cytotoxic effects in HepG2 hepatoma cells (Park et al., 2018). Here, we identified BCA as an antagonist of the Wnt/β-catenin pathway using cell-based small-molecule screening. BCA exhibited cytotoxic activity against colon and liver cancer cell by promoting the degradation of intracellular β-catenin.

2. Materials and methods

2.1. Cell culture, reporter assay, and chemicals used

The HEK293, HCT116, SW480, and SNU475 cell lines were obtained from the American Type Culture Collection (Manassas, VA) and maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS), 120 μg/ml penicillin, and 200 μg/ml streptomycin. HEK293-FL (firefly luciferase) reporter (TOPFlash), control (FOPFlash), and HEK293-secreted alkaline phosphatase (SEAP) reporter cells were established as previously described (Park et al., 2006; Ryu et al., 2008). Wnt3a-conditioned medium (Wnt3a-CM) was prepared as previously described (Park et al., 2006). Luciferase assay was performed using the dual luciferase assay kit (Promega, Madison, WI), and the SEAP assay was performed using the Phospha-Light assay kit (Applied Biosystems, Foster City, CA), according to the manufacturers' instructions. 6-Bromoindirubin-3′-oxime (BIO) and MG-132 were purchased from Sigma-Aldrich (St. Louis, MO). SKL2001 was obtained from Merck-Millipore (Burlington, MA). Broussochalcone A (BCA; purity, 95.8%) was kindly donated by the National Development Institute of Korean Medicine (Gyeongsna, Korea).

2.2. Screening for small-molecule inhibitors of the Wnt/β-catenin pathway

HEK293-FL reporter cells (15×10^3 cells/well) were plated onto 96-well plates and incubated at 37 °C in 5% CO_2 atmosphere for 24 h. Post incubation, the cells were again incubated with 480 natural compounds, which were kindly donated by the National Development Institute of Korean Medicine (Gyeongsna, Korea), at a final concentration of 20 μM for 15 h and the plates were assayed for FL activity and cell viability simultaneously. We used CGK062 (Merck-Millipore) as a positive control in initial screening.

2.3. Cell viability assay

Cells were plated onto 96-well plates and treated with BCA for 48 h. The cell viability of each treated sample was measured in triplicates using the CellTiter-Glo assay kit (Promega), according to the manufacturer's instructions.

2.4. Western blotting

Cytosolic and nucleic fractions from each treated sample were prepared as previously described (Dignam et al., 1983). Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 4%–12% gradient gel (Invitrogen, Carlsbad, CA, USA) and transferred onto nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA, USA). The membranes were blocked with 5% nonfat milk and probed with anti-β-catenin (BD Transduction Laboratories, Palo Alto, CA, USA), anti-axin2 (Cell Signaling Technology, Danvers, MA, USA), anti-cyclin D1 (Santa Cruz Biotechnology, Dallas, TX, USA), anti-c-myc (Santa Cruz Biotechnology, Dallas, TX, USA), anti-poly(ADP) ribose polymerase (anti-PARP; Cell Signaling Technology), anti-caspase-3 (Cell Signaling Technology), anti-p21 (Santa Cruz Biotechnology), anti-firefly luciferase (Abcam, Cambridge, UK), anti-active-β-catenin (Sigma-Aldrich), and anti-actin (Cell Signaling Technology) antibodies. The membranes were then incubated with horseradish peroxidase-conjugated anti-mouse (Santa Cruz Biotechnology) or anti-rabbit (Santa Cruz Biotechnology) IgGs and

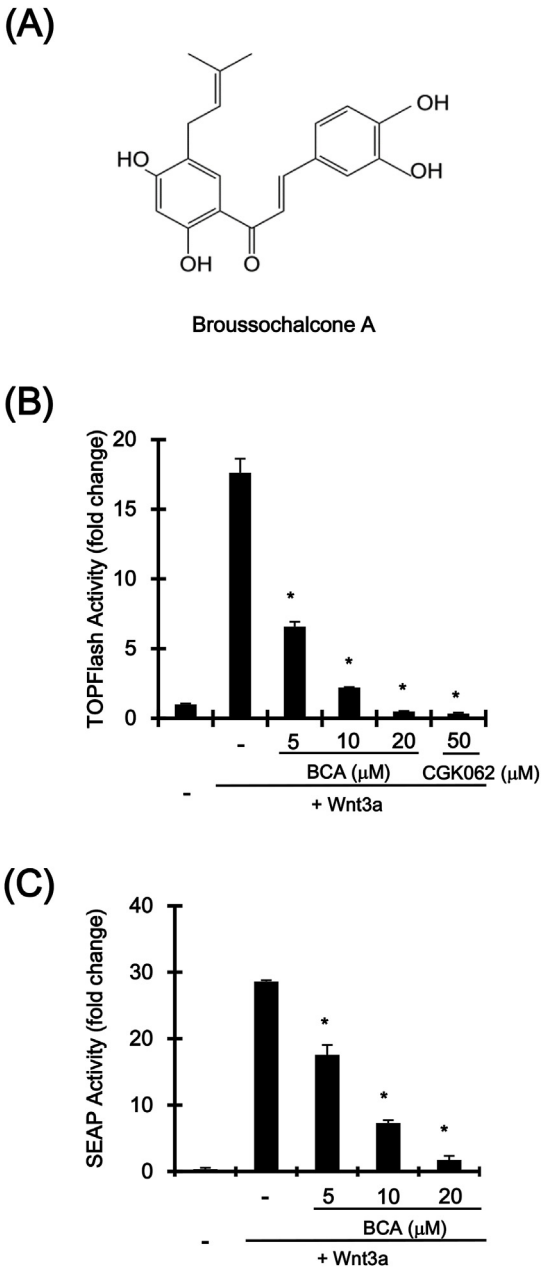

Fig. 1. Identification of BCA as an inhibitor of the Wnt/β-catenin pathway. (A) Chemical structure of BCA. (B) and (C) Dose-dependent inhibition of the Wnt/β-catenin pathway. HEK293-FL and HEK293-SEAP reporter cells were incubated with the indicated concentrations of BCA in the presence of Wnt3a-CM. After 15 h, the firefly luciferase activity (B) and SEAP activity (C) were measured. CGK062 (an inhibitor of the Wnt/β-catenin pathway) was used as a positive control. The results represent the mean ± S.D. of three independent experiments. *$P < 0.01$ compared with the Wnt3a-CM-treated control group.

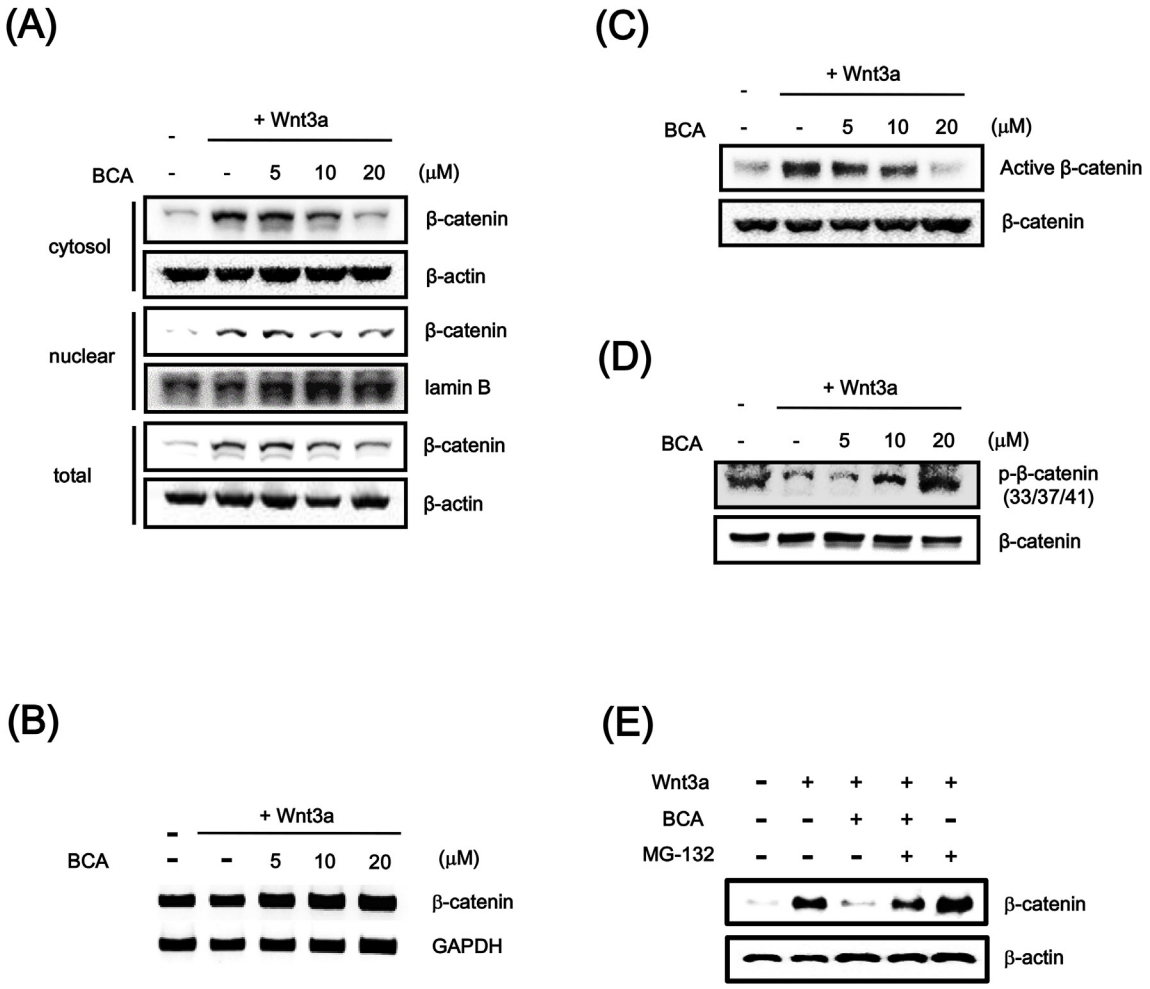

Fig. 2. BCA promotes the degradation of β-catenin through a proteasomal degradation pathway. (A) Proteins were prepared from HEK293-FL reporter cells treated with either vehicle (DMSO) or the indicated concentrations of BCA in the presence of Wnt3a-CM for 15 h and then immunoblotted with an anti-β-catenin antibody. The blot was re-probed with anti-actin or lamin B antibodies to confirm equal loading. B) Semi-quantitative RT-PCRs for β-catenin and GAPDH were performed with total RNA prepared from HEK293-FL reporter cells treated with either vehicle (DMSO) or the indicated concentrations of BCA in the presence of Wnt3a-CM for 15 h. (C) and (D) Proteins were prepared from HEK293-FL reporter cells treated with either vehicle (DMSO) or indicated concentrations of BCA in the presence of Wnt3a-CM for 15 h and then subjected to western blotting with anti-active-β-catenin (C) and anit-phospho-Ser33/37/Thr41-β-catenin (D) antibodies. The blot was re-probed with anti-β-catenin antibody actin to confirm equal loading, (E) Cytosolic proteins prepared from HEK293-FL reporter cells incubated with vehicle (DMSO) or BCA (20 μM) in the presence of Wnt3a-CM and then exposed to MG-132 (10 μM) for 8 h, were immunoblotted using an anti- β-catenin antibody. All results indicated are representative of three independent experiments.

visualized using the enhanced chemiluminescence system (Santa Cruz Biotechnology) and detected using ImageQuant LAS 4000 (GE Healthcare Life Sciences, Chicago, IL).

2.5. RNA extraction and semi-quantitative RT-PCR

Total RNA was isolated using TRIzol Reagent (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions. cDNA synthesis and amplification as well as polymerase chain reaction (PCR) were performed as previously described (Park et al., 2006). Amplified cDNA was electrophoresed on a 2% (w/v) agarose gel and stained with ethidium bromide for visualization of bands.

2.6. Apoptosis analysis

After treatment with BCA for 48 h, the cells were washed with cold phosphate-buffered saline and resuspended in staining buffer containing annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) provided in the apoptosis detection kit (ApoScanTM annexin V-FITC apoptosis detection kit). Apoptosis was measured using a Cellometer Vision image cytometer (Nexcelom Bioscience).

2.7. Caspase-3/7 assay

The activities of caspase-3 and -7 were measured using the Caspase-Glo 3/7 Assay (Promega, Madison, WI, USA), according to the manufacturer's instructions.

2.8. Statistical analysis

The student's t-test was used to compare means between control and experimental groups. All experiments were performed three times. Statistical significance was set at $P < 0.05$ or $P < 0.01$. Results are presented as the mean ± SD.

3. Results

3.1. BCA suppresses the Wnt/β-catenin pathway

HEK293 cells harbor an intact Wnt/β-catenin signaling and allow

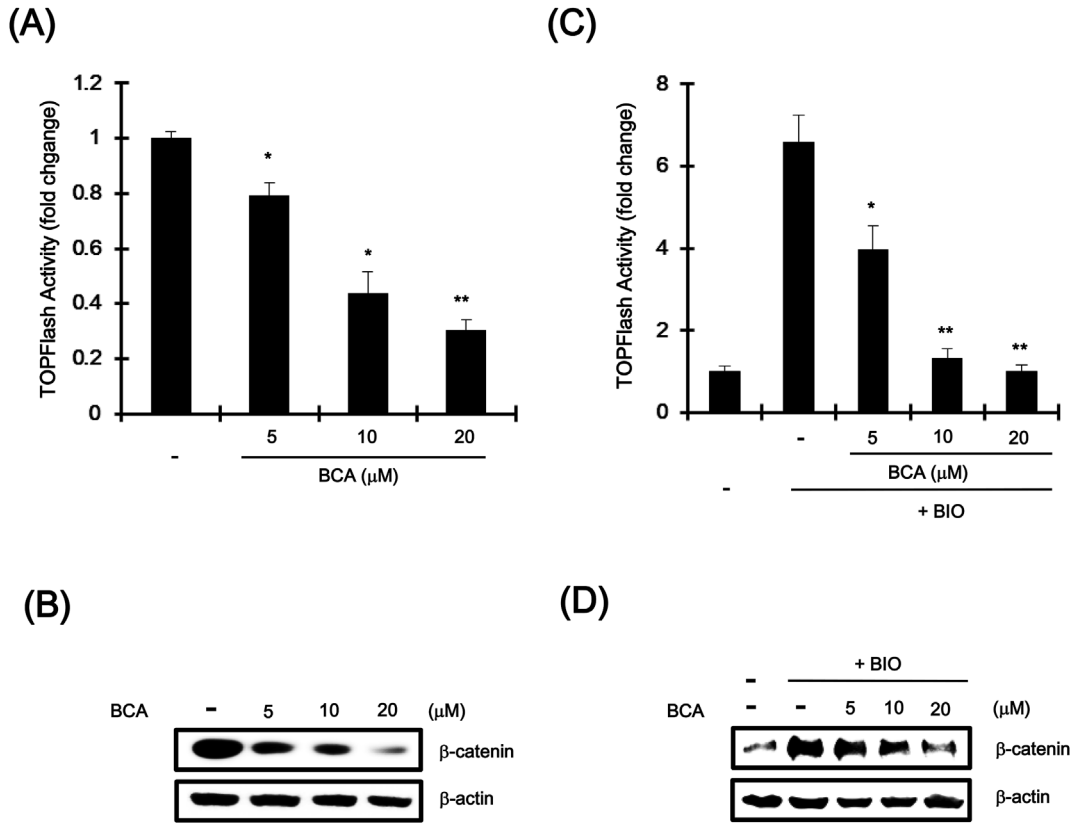

Fig. 3. BCA promotes β-catenin degradation via a CK1/GSK-3β independent mechanism. (**A**) HCT116 cells were co-transfected with TOPFlash and pCMV-RL plasmids and incubated with BCA for 15 h. Luciferase activities were measured 39 h after transfection. TOPFlash activity has been normalized to *Renilla* luciferase activity. The results represent the mean ± S.D. of three independent experiments. $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with the vehicle control group. (**B**) Cytosolic proteins were prepared from HCT116 cells treated with the vehicle (DMSO) or BCA for 15 h and immunoblotted with an anti-β-catenin antibody. (C) HEK293-FL reporter cells were incubated with BCA (5, 10, and 20 μM) in the presence of BIO (0.75 μM). After 15 h, the firefly luciferase activity was determined. The results represent the mean ± S.D. of three independent experiments. $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with the BIO-treated control group. (D) Cytosolic proteins were prepared from HEK293-FL reporter cells treated with either vehicle (DMSO) or BCA (5, 10, and 20 μM) in the presence of BIO (0.75 μM) for 15 h and immunoblotted with anti- β-catenin antibody. In (B) and (D), the blot was re-probed with an anti-actin antibody to confirm equal loading, and the results indicated are representative of three independent experiments.

the inducible activation of β-catenin response transcription (CRT). Thus, we used HEK293-FL reporter cells stably transfected with human Frizzled-1 (hFz-1) expression and synthetic β-catenin/TCF-dependent FL reporter (TOPFlash) plasmids to identify the natural compounds that downregulate CRT. Using a microplate reader, FL activity and cell viability were measured in HEK293-FL reporter cells incubated with Wnt3a-CM. The effects of the screened compounds on CRT were evaluated by normalizing the FL activity with the cell viability values in each assay well. Of all the screened natural compounds, BCA was identified as a CRT suppressor (Fig. 1A) that induced concentration-dependent decreases in the CRT and FL expression stimulated by incubation in Wnt3a-CM, without detectable cytotoxicity (Fig. 1B and Supplemental Fig. S1). In the HEK293-SEAP reporter cells stably harboring the synthetic β-catenin/TCF-dependent SEAP reporter and hFz-1 expression plasmids, BCA consistently abrogated Wnt3a-induced SEAP activity in a dose-dependent manner (Fig. 1C). SKL2001, an activator of the Wnt/β-catenin pathway (Gwak et al., 2012a), reversed the inhibitory effect by BCA treatment (Supplemental Fig. S2). These results indicate that BCA is a specific antagonist of the Wnt/β-catenin pathway.

3.2. BCA promotes proteasomal degradation of β-catenin

In Wnt/β-catenin signaling, CRT is primarily mediated by β-catenin levels (Latres et al., 1999). To investigate the effects of BCA on intracellular β-catenin levels, we performed Western blot analysis using an anti-β-catenin antibody. Consistent with the effects of BCA on CRT, the incubation of HEK293-FL reporter cells with BCA resulted in decreased cytoplasmic and nuclear β-catenin levels that were elevated by Wnt3a-CM exposure (Fig. 2A and Supplemental Fig. S3). In contrast, β-catenin mRNA levels were not altered in samples exposed to different BCA concentrations in Wnt3a-CM (Fig. 2B). We next examined whether BCA induces the phosphorylation of β-catenin at N-terminal residues (Ser33/37/Thr41), which is essential for its degradation. As expected, the level of non-phospho-β-catenin (active β-catenin) was decreased by treatment of BCA (Fig. 2C). Notably, BCA induced β-catenin phosphorylation at Ser33/37/Thr41 residues (Fig. 2D). Intracellular β-catenin levels are controlled via a proteasomal degradation pathway (Aberle et al., 1997). To assess the involvement of the proteasome in BCA-induced β-catenin downregulation, we used MG-132, a proteasome inhibitor. As shown in Fig. 2E, the intracellular β-catenin levels were consistently reduced after BCA treatment, which was reversed after the addition of MG-132. In addition, BCA treatment resulted in an increase in β-catenin ubiquitination (Supplemental Fig. S4). Taken together, these results indicate that BCA attenuates Wnt/β-catenin signaling by promoting phosphorylation and ubiquitin-dependent proteasomal degradation of β-catenin without affecting β-catenin gene expression.

3.3. BCA promotes β-catenin downregulation via a CK1/GSK-3β-independent mechanism

CK1 and GSK-3β catalyze β-catenin phosphorylation at Ser45 and

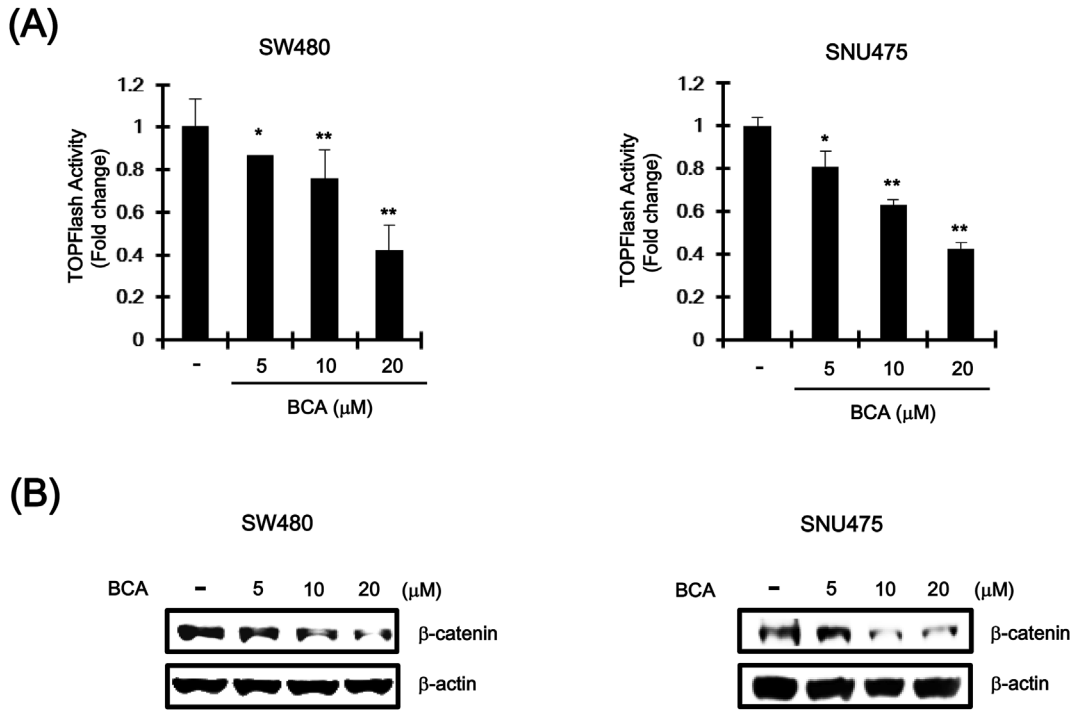

Fig. 4. Axin and APC are not involved in BCA-mediated β-catenin degradation. (A) SW480 and SNU475 cells were co-transfected with TOPFlash and pCMV-RL plasmids and incubated with broussochalcone A for 15 h. Luciferase activities were measured 39 h after transfection. TOPFlash activity has been normalized to *Renilla* luciferase activity. The results represent the mean ± S.D. of three independent experiments. $*P < 0.05$ and $**P < 0.01$, compared with the vehicle control group. (B) Cytosolic proteins were prepared from SW480 and SNU475 cells treated with the vehicle (DMSO) or BCA for 15 h and immunoblotted with an anti-β-catenin antibody. The blots were re-probed with anti-actin antibody as a loading control, and the results indicated are representative of three independent experiments.

Ser33/37/Thr41, respectively. Therefore, we investigated the roles of CK1 and GSK-3β in BCA-induced β-catenin degradation. HCT116 colon cancer cells having a Ser45 (CK1 phosphorylation site) deletion mutation in β-catenin (Ilyas et al., 1997), were transfected with TOPFlash and then treated the transfected cells with BCA. As shown in Fig. 3A, CRT was decreased in response to BCA. Consistently, incubation of HCT116 cells with BCA resulted in the reduction of intracellular β-catenin (Fig. 3B), indicating that CK1 activity is not required for BCA-mediated β-catenin downregulation. Next, we examined the effects of GSK-3β activity on BCA-promoted β-catenin degradation. Consistent with previous findings (Meijer et al., 2003), the incubation of HEK293-FL reporter cells with BIO, a GSK-3β inhibitor, resulted in increased TOPFlash reporter activity. Fig. 3C indicates that BCA suppressed this activity in a dose-dependent manner. Western blot analysis consistently revealed decreased cytosolic β-catenin levels in samples exposed to BIO and subsequently treated with BCA (Fig. 3D), indicating that BCA reduces β-catenin levels via a CK1- and GSK-3β − independent mechanism.

3.4. BCA promotes β-catenin degradation via an Axin/APC-independent mechanism

Next, we evaluated the effects of Axin and APC, the key components of the β-catenin destruction complex, on BCA-mediated β-catenin downregulation. For this, we first transfected SW480 colon cancer and SNU475 hepatoma cells having inactivating mutations of APC and Axin, respectively (Ilyas et al., 1997), with the TOPFlash plasmid. We treated the transfected cells with increasing concentrations of BCA and then observed consistent repression of CRT in a dose-dependent manner (Fig. 4A). In parallel, Western blot analysis revealed a concentration-dependent decrease in the intracellular β-catenin levels in SW480 and SNU475 cells treated with BCA (Fig. 4B). Moreover, when we over-expressed wild-type APC and wild-type Axin in SW480 and SNU475 cells, respectively, BCA was still able to decrease the level of β-catenin in these cells (Supplemental Fig. S5). These results indicate that Axin and APC are not required for BCA-promoted β-catenin degradation.

3.5. BCA represses the expression of β-catenin-dependent genes

Because BCA reduced the intracellular β-catenin levels in colon and liver cancer cells, we next examined whether BCA regulates the expression of β-catenin dependent genes in SW480, HCT116, and SNU475 cells using Western blot analysis. As shown in Fig. 5A, the addition of BCA to these colon and liver cancer cells produced a significant decrease in the expressions of cyclin D1, c-myc, and axin-2, which are established β-catenin-dependent genes. Previous studies have demonstrated that the specific disruption of β-catenin functions by antisense oligonucleotides or small interference RNAs inhibits cancer cell proliferation *in vitro* and tumor growth in a xenograft mouse model (Roh et al., 2001; Verma et al., 2003; Green et al., 2001). Given that BCA promoted β-catenin degradation, we evaluated its effects on the proliferation of colon and liver cancer cells. SW480, HCT116, and SNU475 cells were treated with different BCA concentrations, and cell viability was also measured. BCA efficiently decreased the viability of SW480, HCT116, and SNU475 cells in a concentration-dependent manner (IC_{50} values: 6.17, 8.59, and 6.41 μM, respectively; Fig. 5B).

3.6. BCA induces apoptosis in colon and liver cancer cells

To investigate the potential anti-proliferative mechanism of BCA, cells samples were exposed to the compound, and the number of apoptotic cells was the counted using cytometric analysis. The number of apoptotic cells was elevated in a dose-dependent manner in colon and liver cancer cells exposed to different BCA concentrations (Fig. 6A). Compared with control cells, the percentage of Annexin V-FITC/PI double-positive cells increased from 7.05% to 46.73% in SW480 cells, 6.99% to 49.69% in HCT116 cells, and 4.52% to 42.0% in SNU475 cells

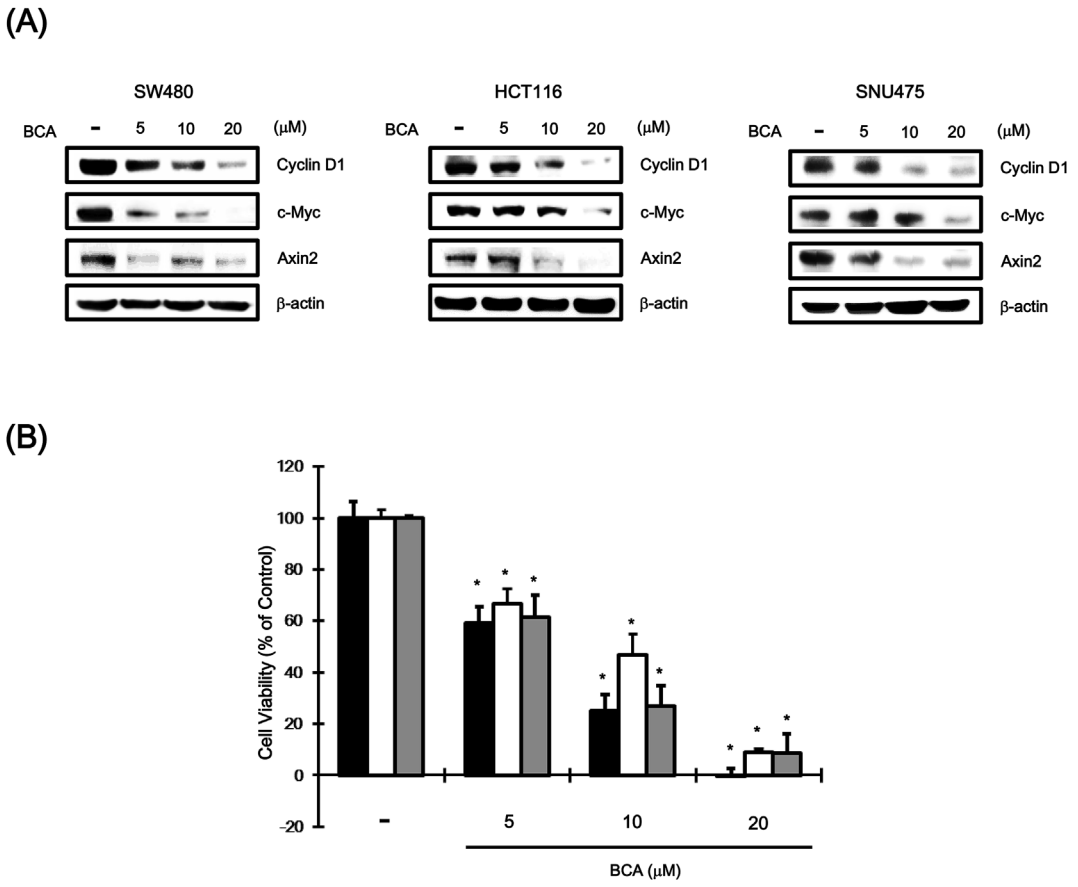

Fig. 5. BCA represses the expression of the β-catenin-dependent genes. (A) SW480, HCT116, and SNU475 cells were incubated with the vehicle (DMSO) or BCA (5, 10, and 20 μM) for 15 h and cell extracts were immunoblotted using anti-axin2, anti-cyclin D1, and anti-c-myc antibodies. The blots were re-probed with anti-actin antibody as a loading control, and the results indicated are representative of three independent experiments. (B) SW480, HCT116, and SNU475 cells were incubated with indicated concentrations of BCA and cell viability was determined using the CellTiter-Glo assay. To calculate growth inhibition, the value at time 0 was subtracted. The results represent the mean ± S.D. of three independent experiments. *$P < 0.01$ compared with the vehicle control group.

at a BCA concentration of 20 μM (Fig. 6B). Moreover, incubation of these colon and liver cancer cells with BCA enhanced the activity of caspase-3/7, consistent with increased apoptosis (Fig. 6C). Finally, treatment of SW480, HCT116, and SNU475 cells with BCA promoted the formation of active caspase-3 (cleaved form), which in turn catalyzed the cleavage of PARP, a biochemical marker of apoptosis (Fig. 6D and Supplemental Fig. S6). These results suggest that apoptosis contributes to the BCA-mediated inhibition of proliferation in colon and liver cancer cells.

4. Discussion

Medicinal plants are potential resources for the identification of therapeutics for various human diseases. *B. papyrifera,* a traditional medicinal plant, produces various polyphenolic compounds, such as, chalcones, flavonols, flavans, and benzofluorenones (Ryu et al., 2010). It also produces BCA, a chalcone, which exhibits numerous biological effects, including anti-platelet aggregation, anti-inflammatory properties, and anti-cancer activity. Recently, Park et al. (2018) reported that BCA inhibits the activity of human cytochrome P450 2J2, which is overexpressed in human cancer tissue and cell lines and activates Foxo3 that reportedly participates in the inhibition of cell growth, thereby suppressing the proliferation of hepatoma cells. Here, we demonstrated for the first time that BCA promotes the degradation of oncogenic β-catenin, which plays an essential role in the development and progression of certain human cancers, thereby inhibiting the growth of colon and liver cancer cells.

The scaffolding protein Axin usually associates with APC, GSK-3β, CK1, and β-catenin via separate domains to form a destruction complex (Hart et al., 1998) and coordinates the sequential phosphorylation of β-catenin at Ser45 by CK1 and then at Ser33/37/Thr41 by GSK-3β, resulting in β-catenin degradation via a ubiquitin-dependent mechanism (Aberle et al., 1997). However, molecular lesions in the N-terminal CK1/GSK-3β phosphorylation sites of β-catenin or in components of the destruction complex (APC or Axin) result in slow turnover of β-catenin (Barker and Clevers, 2000). Several findings observed in the present study indicate that BCA-induced β-catenin degradation differs from the destruction complex-dependent pathway. First, the levels of β-catenin with mutations at the CK1 phosphorylation site (Ser45) were reduced by treatment with BCA in HCT116 cells. Second, BCA reduced intracellular β-catenin levels even in the presence of a GSK-3β inhibitor. Finally, BCA stimulated the degradation of β-catenin in SNU475 cells with *Axin* mutation and SW480 cells with *APC* mutation.

The results of several studies have demonstrated that cyclin-dependent kinase 2 (CDK2)/cyclin A and protein kinase Cα (PKCα) catalyzes β-catenin phosphorylation at Ser33/37/Thr41 residues and then promotes its degradation via a mechanism independent of β-catenin destruction complex (Park et al., 2004; Gwak et al., 2006, 2009). In this study, we observed that BCA promoted β-catenin phosphorylation at Ser33/37/Thr41 residues and downregulated the level of non-phospho-β-catenin in the presence of Wnt3a-CM, which inhibits GSK-3β-mediated β-catenin phosphorylation in the destruction complex, thereby increasing β-catenin ubiquitination. Therefore, in addition to GSK-3β, other kinases, including CDK2 and PKCα, may be involved in BCA-mediated β-catenin phosphorylation and ubiquitin-dependent degradation.

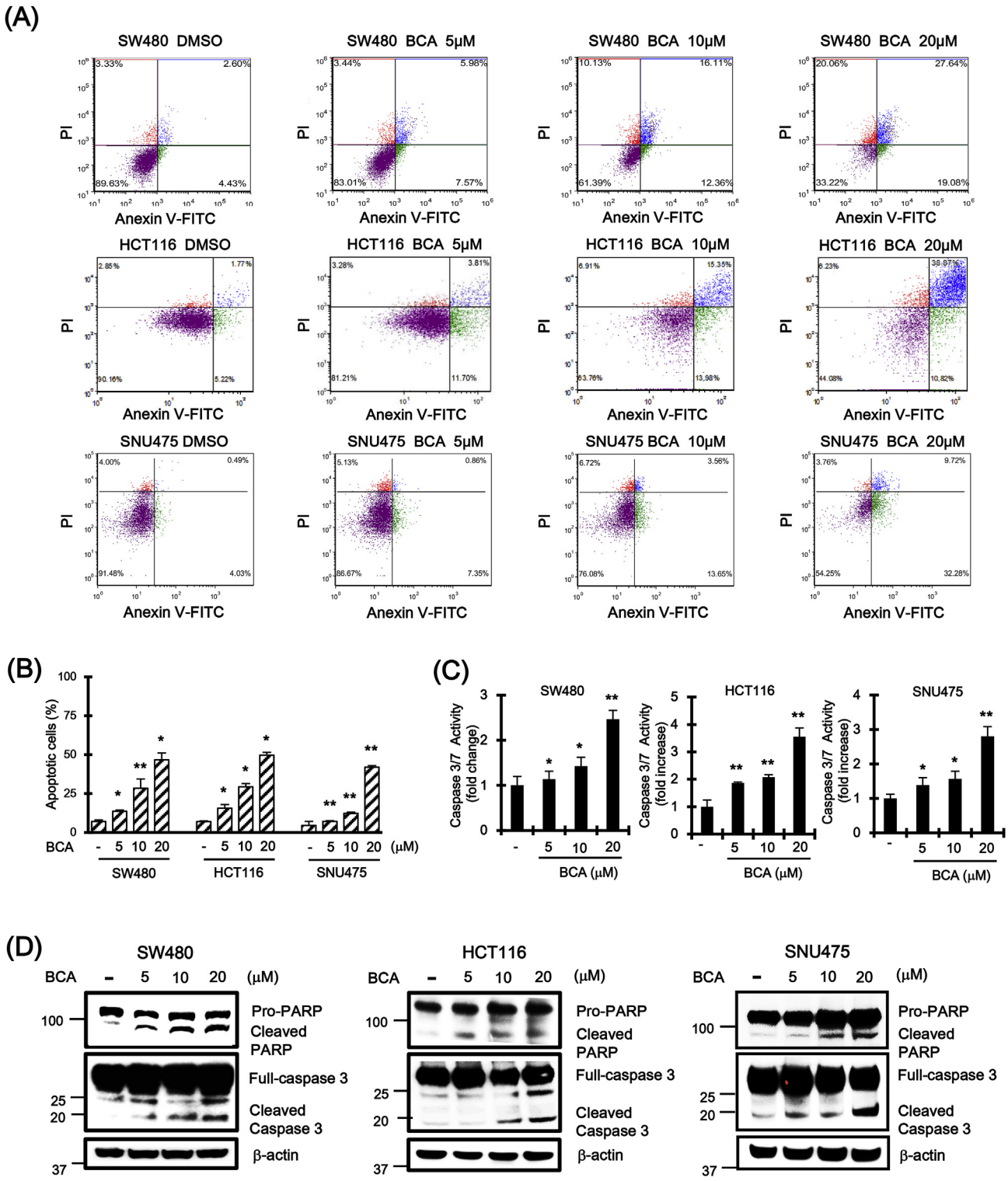

Fig. 6. BCA induces apoptosis in β-catenin-responsive transcription-positive cancer cells. (A) and (B) SW480, HCT116, and SNU475 cells were incubated with the vehicle (DMSO) or BCA (5, 10, and 20 μM) for 48 h. After incubation, the cells were harvested and stained with annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) and analyzed using a Cellometer cytometer. The x-axis indicates the Annexin V-FITC intensity and the y-axis indicates the PI fluorescence. (C) Activation of caspase-3/7 by BCA. SW480, HCT116, and SNU475 cells were incubated with the indicated concentrations of BCA. After 48 h, the firefly luciferase activity was measured. standard deviations. The results represent the mean ± S.D. of three independent experiments. *$P < 0.05$ and **$P < 0.01$, compared with the vehicle control group. (D) Cell extracts from SW480, HCT116, and SNU475 cells treated with either vehicle (DMSO) or the indicated concentrations of BCA for 48 h were immunoblotted with anti-caspase-3 and anti-poly(ADP) ribose polymerase (anti-PARP) antibodies. The blots were re-probed with anti-actin antibodies as a control, and the results indicated are representative of three independent experiments.

Recently, small molecules that accelerate pathogenic β-catenin turnover have been identified. We previously demonstrated that hexachlorophene stimulates β-catenin degradation by activating the expression of Siah-1, an E3 ubiquitin ligase, in HCT116 and LS174T colon cancer cells carrying β-catenin mutations at the CK1/GSK-3β phosphorylation sites, but not in SW480 and DLD-1 colon cancer cells with APC mutations (Park et al., 2006). In contrast, CGK062 promotes protein kinase Cα-mediated β-catenin phosphorylation and degradation, thereby inhibiting the proliferation of SW480 and SNU475 colon cancer cells (Gwak et al., 2012b). In the present study, BCA induced β-catenin degradation via a destruction complex-independent mechanism and suppressed the proliferation of HCT116, SW480, and SNU475 cancer cells with β-catenin, APC, and Axin mutations, respectively.

In conclusion, we have unveiled an anticancer mechanism of BCA against colon and liver cancer cells by using a chemical biology approach. BCA promoted β-catenin phosphorylation at Ser33/37/Thr41 residues and accelerated the turnover of oncogenic β-catenin, which contributes to the development of colon and liver cancer, through the ubiquitin-dependent pathway, thereby inducing apoptosis in colon and liver cancer cells. Therefore, BCA can be developed into a chemopreventive agent against cancers that contain mutations in β-catenin, APC, and Axin.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.05.058.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2019.05.058.

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