ANTICANCER RESEARCH *39*: 3661-3667 (2019)
doi:10.21873/anticanres.13514

Selective Wnt/β-catenin Small-molecule Inhibitor CWP232228 Impairs Tumor Growth of Colon Cancer

JIN YOUNG KIM[1*], GEUMI PARK[2*], MANIGANDAN KRISHNAN[3], EUNYOUNG HA[3] and KYUNG-SOO CHUN[2]

[1]*Department of Internal Medicine, Division of Hematology/Oncology, School of Medicine, Keimyung University, Daegu, Republic of Korea;*
[2]*College of Pharmacy, Keimyung University, Daegu, Republic of Korea;*
[3]*Department of Biochemistry, School of Medicine, Keimyung University Department of Biochemistry, School of Medicine, Keimyung University, Daegu, Republic of Korea*

Abstract. *Background/Aim: To explore the possibility of a selective small-molecule β-catenin inhibitor, CWP232228, as a potential therapeutic drug in the treatment of colorectal cancer (CRC). Materials and Methods: The effect of CWP2228 on HCT116 cells was analysed in vitro via flow cytometry, western immunoblotting, and luciferase reporter assays. NOD-scid IL2Rgamma^{null} mice were employed for an in vivo xenograft study to validate the in vitro studies. Results: CWP232228 treatment decreased the promoter activity and nuclear expression of β-catenin and induced a significant cytotoxic effect in HCT116 cells. CWP232228 treatment induced apoptosis and cell-cycle arrest in the G_1 phase of the cell cycle. Furthermore, CWP232228 decreased the expression of aurora kinase A, c-Myc, cyclin D1 and microphthalmia-associated transcription factor. Lastly, CWP232228 also inhibited the growth of xenografted colon cancer cells in mice. Conclusion: Collectively, CWP232228 may be used as a potential therapeutic drug in CRC.*

Cancers of the colonic region or colorectal cancer (CRC) remains a significant life-threating disease worldwide, and in Korea, is the second and third most common cancer diagnosed in men and women, respectively. Conventional therapeutic strategies such as surgery, chemotherapy and radiation therapy, have been challenged with tumor relapse and recurrence, which affect nearly 50% of patients, thus development of new therapeutic agents is required (1).

The molecular pathogenesis that underlies the development of CRC is complex and can be accompanied by the actions of genetic and epigenetic alterations such as Wnt/β-catenin, apoptosis, hedgehog and vascular endothelial growth factor signaling cascades (2, 3). Of these pathways, Wnt/β-catenin signaling plays a crucial role in the development of CRCs (4). A major effector molecule of the Wnt signaling pathway is β-catenin, a cytoplasmic protein involved in the regulation of CRC development. Activation of the Wnt signaling pathway necessitates an interaction between β-catenin and lymphoid enhancer factor-T cell factor (Lef/Tcf) in the nucleus, thereby co-activating transcription of c-Myc and cyclin D1 to exhibit greater tumorigenic potential (5, 6). Alterations in the transcriptional activity of β-catenin have been found in numerous rodent carcinogenesis models, evincing the potential role of β-catenin in the development of colon carcinogenesis (7). Accumulating reports have demonstrated the implications of Wnt/β-catenin dysfunction in several cancer types including colon, breast and ovarian cancers (8).

Interestingly, β-catenin has no enzymatic activity, and is thereby considered as a low affinity target to a drug (9). Wealth of research has reported that several small molecular synthetic inhibitors (such as IWP-2 and XAV939) effectively suppress the oncogenic potential of Wnt signaling by directly targeting β-catenin in multiple cancer types. However, because of their poor *in vivo* pharmacokinetic properties, these inhibitors were not considered for clinical applications (10, 11). Therefore, the development of novel inhibitors that exhibit lower side-effects and improved *in vivo* pharmacokinetic properties towards β-catenin is highly warranted. CWP232228 (Figure 1A), a highly potent and selective small-molecule inhibitor eliciting strong antagonistic effect to β-catenin protein has been developed. Jang *et al*. previously reported that

*These Authors contributed equally to this work.

Correspondence to: Professor Kyung-Soo Chun, College of Pharmacy, Keimyung University, Daegu, Republic of Korea. Tel: +82 53 580 6647, e-mail: chunks@kmu.ac.kr; Professor Eunyoung Ha, Department of Biochemistry, School of Medicine, Keimyung University, Daegu, Republic of Korea. Tel: +82 535803852, e-mail: eunyoungha@kmu.ac.kr

Key Words: Colorectal cancer, CWP232228, β-catenin inhibitor, apoptosis, cell cycle.

CWP232228 suppresses tumor formation and metastasis through inhibition of cancer stemness properties of breast cancer models *in vitro* and *in vivo* (12). Also, its modulatory effect on Wnt/β-catenin-mediated liver cancer stemness has been reported (13). In this study, we exploited the possibility of a selective small molecule β-catenin inhibitor, CWP232228, as a potential therapeutic drug in the treatment of CRC.

Materials and Methods

Materials. CWP232228, a Wnt/β-catenin signaling inhibitor was designed and provided by JW Pharmaceutical Corporation (Seoul, Republic of Korea). Radio immuno-precipitation buffer (RIPA), nitrocellulose membrane and Bradford's reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents of analytical grade were purchased from Merck (Darmstadt, Germany). The antibodies directed towards Bcl-2, Bcl-xl, cytochrome C, cyclin D1, cyclin D2, cyclin D3, survivin, c-Myc, microphthalmia-associated transcription factor (MITF), aurora kinase A, β-catenin, β-actin, cleaved PARP (c-PARP) and cleaved caspases-9, -7 and -3 (c-caspase-9, -7, and -3) were purchased from Cell Signaling Technology (Beverly, MA, USA). The secondary antibodies conjugated with horse radish peroxidase (HRP) were purchased from Santa Cruz Biotechnology (Paso Robles, CA, USA).

Cell culture. The human colon cancer cell line HCT116 was procured from American Type Culture Collection (Manassas, VA, USA). The cell lines were cultured in RPMI media (Thermo Fischer Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 mg/ml streptomycin at 37˚C in a humidified 5% CO_2 incubator. The HCT116 cells were routinely passaged twice a week and then used for experiments after attaining 80% confluence with a normal morphology.

Assessment of cell death. The effect of CWP232228 on HCT116 cell death was measured using MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] cell viability assay kit (Promega, Madison, WI). Briefly, cells (2×10^3) were seeded in a 96-well plate in triplicate. After incubation with 0.1, 1.0 and 5.0 μM of CWP232228 for 24, 48 and 72 h, MTS solution was added (20 μl/well) and then incubated for 60 min. Absorbance corresponding to cell viability was read at 492 nm using an ELISA plate reader (Tecan Trading AG, Männedorf, Switzerland).

Measurement of apoptotic index. Quantitative determination of apoptotic index on CWP232228 treated HCT116 cells was performed by (Fluorescein isothiocyanate (FITC)-Annexin V staining kit (BD Biosciences, San Jose, CA, USA) and then assessed for apoptosis using flow cytometry (BD Biosciences).

Cell cycle analysis. The HCT116 cells were treated with varying concentrations of CWP232228 for 24 h. Cells were harvested, washed with PBS, resuspended and fixed with cold 70% ethanol overnight at 4˚C. Samples were subsequently subjected to propidium iodide (PF) probing (PI/RNase Staining Buffer), and analyzed for cell cycle distribution by flow cytometry.

Luciferase reporter gene assay for β-catenin activity. To investigate the transcriptional activity of β-catenin in CWP232228 treated HCT116 cells, cells were seeded into 12-well plates at a density of 5×10^4 cells/well 24 h prior to transfection. Cells were transfected with β-catenin-TA-luc (Clontech, Palo Alto, CA, USA) or control vector using Genefectin transfection reagent (Genetrone Biotech, Seoul, Republic of Korea). Twenty-four h after transfection, cells were treated with CWP232228 for an additional 24 h, and the lysates were incubated with luciferase substrate. Luciferase activity was measured by a luminometer (Tecan Trading AG). The β-galactosidase assay (Promega, Madison, WI, USA) was preformed according to the manufacturer's recommendations, for normalizing the luciferase activity and the results were expressed as fold transactivation.

Protein isolation and immunoblot analysis. After treatment, cells were lysed in RIPA buffer and the total protein concentrations of cell lysates were quantified using bicinchoninic acid (BCA) protein assay kit (Pierce Biotechnology, Rockford, IL, USA) and stored at –20˚C till further use. Similarly, nuclear fractionation was performed using the NE-PER Nuclear and Cytoplasmic Extraction Reagents kit (Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's protocol. The protein samples (30 μg) were separated by SDS-PAGE using a 12% (w/v) polyacrylamide resolving gel, transferred to PVDF membrane, and blocked using 3% (w/v) bovine serum albumin (BSA) for 1 h at room temperature (RT). The membranes were subsequently incubated with primary antibodies at 4˚C overnight, followed by incubation with their respective secondary antibody for 1 h at RT. The protein-antibody complexes were visualized using Super-signal pico chemiluminescent substrate or dura-luminol substrate (Thermo Fischer Scientific) according to manufacturer's instruction and visualized with imagequant™ LAS 4000 (Fujifilm Life Science, Ushijima, Japan). Densitometric analysis of signals was performed using the Image J (NIH, Bethesda, MD, USA) software.

In vivo xenograft. Eight-week-old male NOD-scid IL2Rgamma^{null} (NSG) mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and maintained in accordance with the institutional guidelines for care and use of laboratory animals of Keimyung University, College of Medicine. All animal studies were carried out according to approved experimental protocols (KM-2016-06). The mice were held in individually ventilated cages (IVC) under sterile and standardized environmental conditions (25±2˚C, 12 h light-dark cycle). HCT116 cells (1.0×10^6 cells) from an *in vitro* passage were transplanted subcutaneously (*s.c.*) into the left flank region of mice on day zero. Mice were randomly distributed to the experimental groups (6 mice per group). When the tumors have grown to 500 mm^3 in volume, treatment was initiated. Mice were treated intraperitoneally (*i.p.*) with either CWP232228 (100 mg/kg), or PBS vehicle daily for five days followed by two days rest for 2 weeks. Tumor size was measured twice per week in two dimensions using a caliper. Individual tumor volumes (V) were calculated by the formula $V=[\text{length} \times (\text{width})^2]/2$.

Statistical analysis. All statistical analyses were performed using the GraphPad Prism Software (Version 5.0, San Diego, CA, USA). The IC_{50} concentrations were determined using non-linear curve fit. One-way ANOVA was performed to compare the differences between treated groups and control. For multiple comparisons, two-

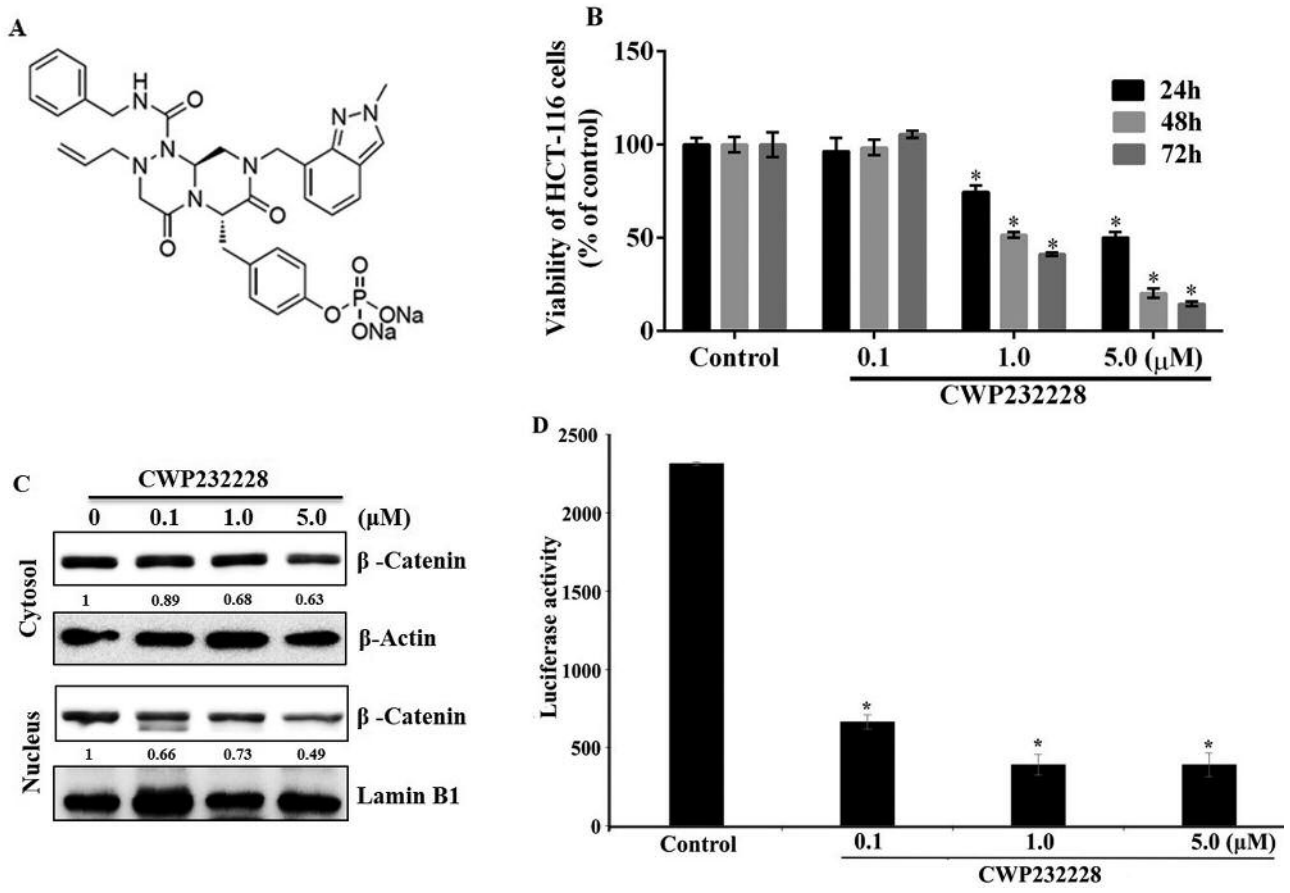

Figure 1. *CWP232228 induced cytotoxic effect on HCT116 cells. (A) Chemical structure of CWP232228, (B) Cell viability of HCT116 cells treated with CWP232228 (0.1, 1, 5 μM) for 24, 48 and 72 h by MTS assay. (C) Western blot analysis of the expression of β-catenin levels after treatment with CWP232228 (0.1, 1, 5 μM) for 24 h. Values were expressed as fold changes relative to HCT116 control, and normalized to β-actin. Data are representative of three independent experiments. (D) Luciferase activity in CWP232228-treated HCT116 cells transfected with β-catenin-TA-luc construct or control vector. Values are represented as mean±SD of three replicates and are statistically significant (*p<0.05) as compared with control.*

way ANOVA (cell cycle distribution) was used. Differences between control and CWP232228 treated mice were analyzed using a Student's *t*-test. $p<0.05$ was considered to indicate statistical significance. All results are presented as the mean±S.D. of three independent experiments.

Results

CWP232228 exhibits a cytotoxic effect and targets the Wnt/β-catenin pathway in human colon cancer cells. The cytotoxic effect of CWP23222 (Figure 1A) on human HCT116 cells was examined by the MTS assay. After exposure of HCT116 cells with varying concentrations (0.1, 1.0 and 5.0 μM) of CWP232228 for 24, 48 and 72 h, a significant concentration-dependent cytotoxic effect of CWP232228 on HCT116 cells was observed (Figure 1B). The concentrations required to inhibit 50% of tumor cells survival were 4.81, 1.31 and 0.91 μM in 24, 48 and 72 h, respectively, which suggest that CWP232228 was cytotoxic towards colon cancer cells.

Translocation of β-catenin into the nucleus has been implicated as a distinct hallmark of Wnt/β-catenin pathway activation. CWP232228 treatment decreased nuclear expression of β-catenin (Figure 1C). Furthermore, CWP232228 treatment significantly decreased the transcriptional activity of β-catenin as evinced by the measurement of the luciferase activity (Figure 1D) in human colon cancer cells.

CWP232228 inhibits human colon cancer cell growth through induction of apoptosis and cell cycle arrest. The mechanism of CWP232228-induced apoptosis was then examined. Annexin V and PI staining revealed that 1.0 and 5.0 μM CWP232228 significantly induced apoptosis compared with control untreated cells (Figure 2A and B). Immunoblot analysis

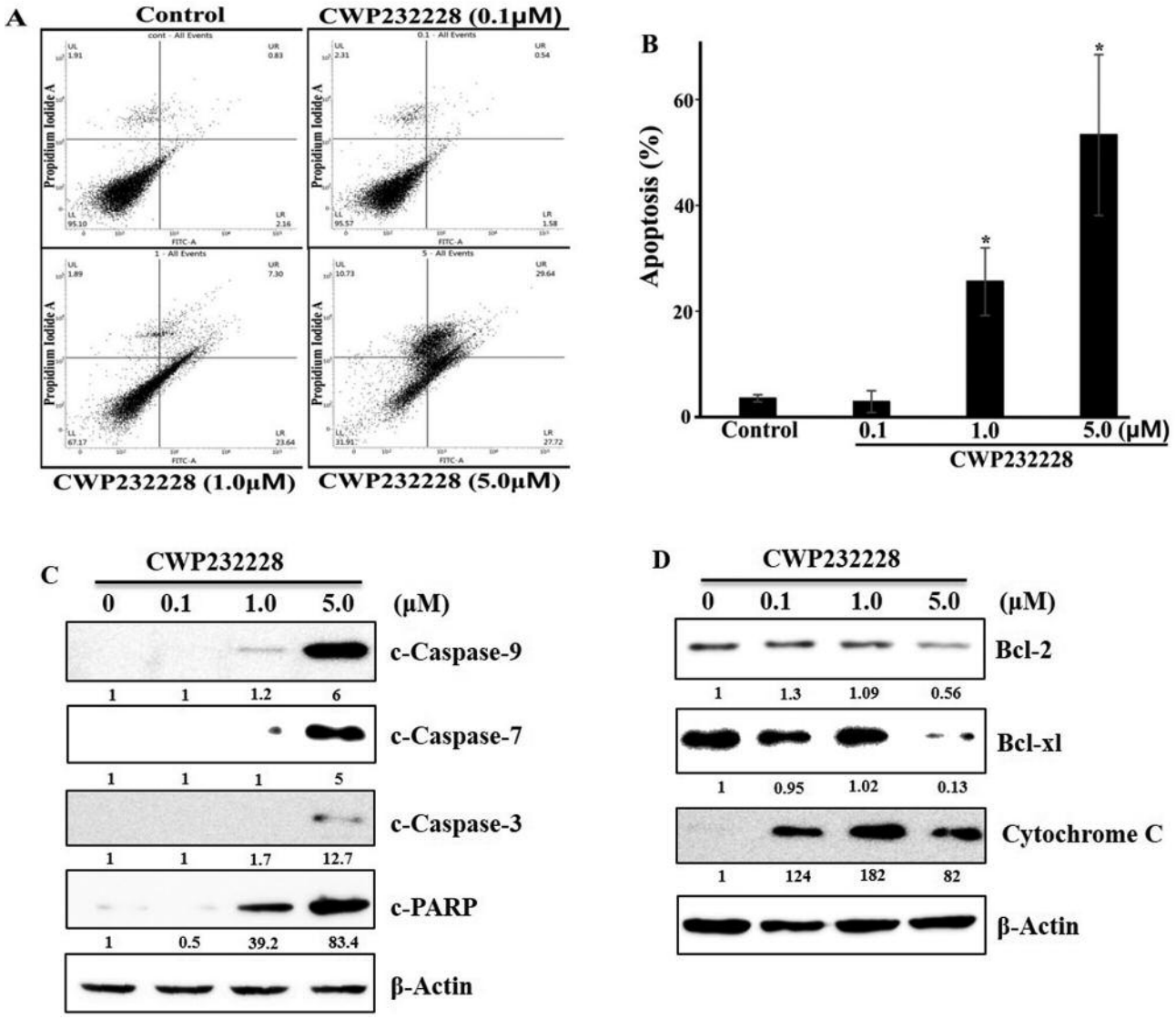

Figure 2. *Effect of CWP232228 on cellular markers of apoptosis in HCT116 cells. (A) Apoptosis inducing capacity of CWP232228 was examined by Annexin V and PI staining. (B). Quantitative data show the percentage of apoptosis induced by CWP232228 in all experimental groups of cells and the values are represented as mean±SD of three independent experiments (*p<0.05 compared to control). (C and D) Immunoblot analysis of cleaved caspases-9, and 3, cleaved PARP, Bcl-2, Bcl-xl, Bax, and cytochrome c levels on CWP232228-treated HCT116 cells. The intensities were determined by densitometry and the fold changes of proteins are shown in each lane. The data are representative of three independent experiments. β-Actin was used as a loading control.*

revealed that CWP232228 treatment increased the release of cytochrome C while decreased Bcl-xl expression (Figure 2D). Cytochrome C dependent activation of apical (caspase-9) and effector caspases (caspase-7 and -3) was also observed, resulting in increased cleavage of PARP (Figure 2C). Since apoptosis is linked to cell cycle regulators (14), we next investigated the effect of CWP232228 on the distribution of cells in each cell cycle phase by flow cytometry. CWP232228 treatment induced cell accumulation at G_1 phase (77±0.49%) compared to the control (63.75±2.29%), thereby significantly reducing the transition of cells to the G_2/M phase (15±2.18%) compared to control (33.18±5.9%) (Figure 3A). However, no significant changes were observed in the S phase (Figure 3A). In addition, CWP232228 attenuated the expression of cyclin D2, D3, and survivin (Figure 3B). To further validate the efficacy and specificity of CWP232228 to arrest cell cycle progression at the G_2/M phase, we analyzed the expression levels of proteins involved in the Wnt/β-catenin pathway, and found that CWP232228 inhibited expression of aurora kinase A, cyclin D1, c-Myc and MITF in HCT116 cells (Figure 3C).

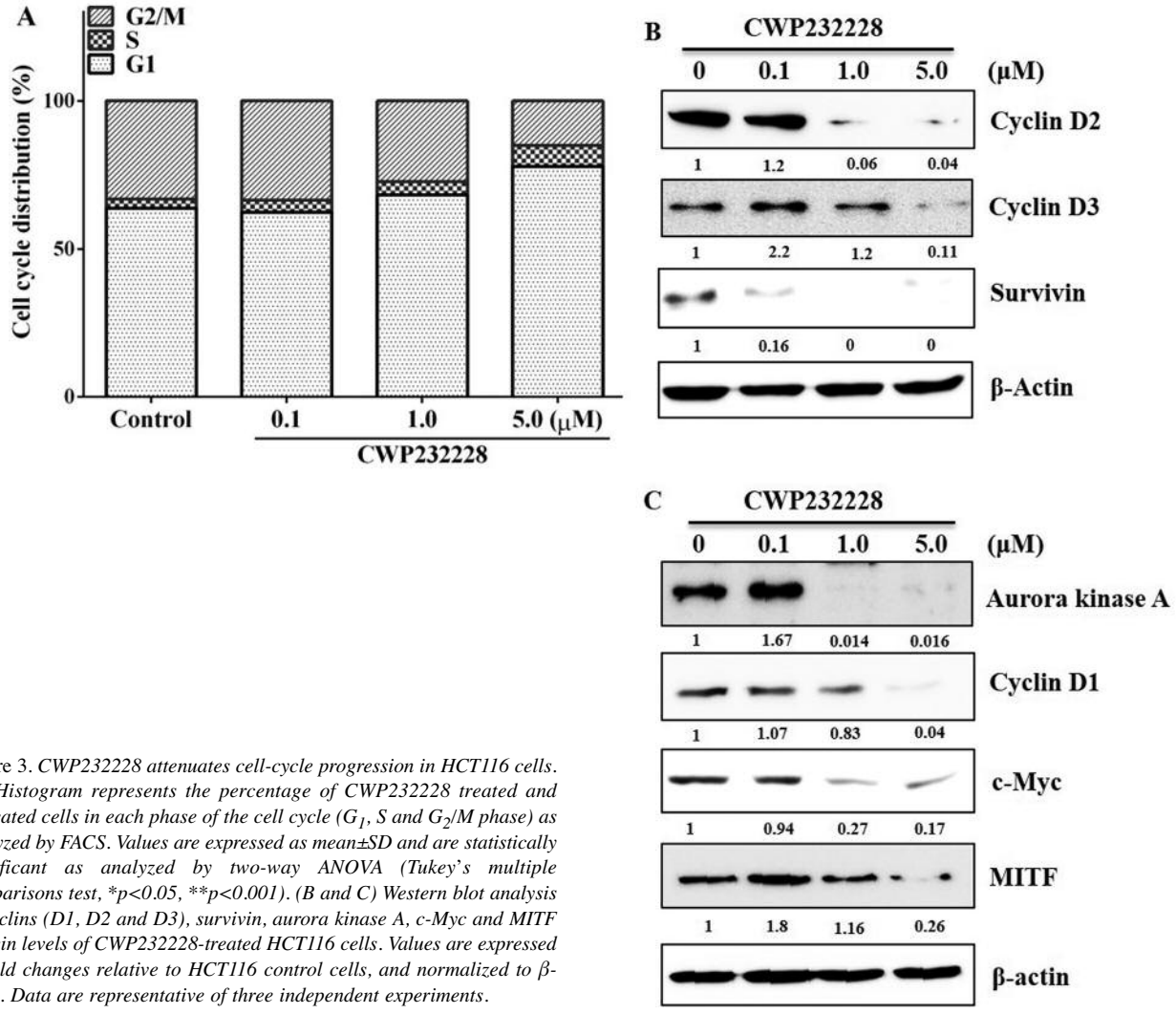

Figure 3. *CWP232228 attenuates cell-cycle progression in HCT116 cells. (A) Histogram represents the percentage of CWP232228 treated and untreated cells in each phase of the cell cycle (G_1, S and G_2/M phase) as analyzed by FACS. Values are expressed as mean±SD and are statistically significant as analyzed by two-way ANOVA (Tukey's multiple comparisons test, *p<0.05, **p<0.001). (B and C) Western blot analysis of cyclins (D1, D2 and D3), survivin, aurora kinase A, c-Myc and MITF protein levels of CWP232228-treated HCT116 cells. Values are expressed as fold changes relative to HCT116 control cells, and normalized to β-actin. Data are representative of three independent experiments.*

CWP232228 inhibits xenografted human colon cancer cell growth. Using a xenograft tumor model, we next analyzed the anti-tumor efficacy of CWP23228 on HCT116 cells-xenografted mice. Figure 4A and B demonstrates that CWP232228-treated mice exhibited reduced tumor growth after 2 weeks of treatment (268.0±259.0 mm^3) when compared with vehicle-treated control mice (614.0±423.0 mm^3). This result clearly suggests the anti-tumor effect of CWP232228 against HCT116 cells-injected mice.

Discussion

It has been well documented that, in most CRC patients, diagnosis is made at an early stage where the tumor can be surgically removed. However, tumor recurrence and relapse are likely to occur due to undetectable residual cancer cells (15). Several chemotherapeutic drugs including cetuximab, bevacizumab, 5-fluorouracil (5-FU) and others are used for targeting CRC (16). Among these, 5-FU remains the gold standard in the treatment of CRC but its application remains limited due to its adverse side effects (17). Although other targeted agents have been developed, advanced CRC is still regarded as an incurable malignancy because of its progressive gain of chemoresistance and distal metastasis (18). Thus, identification and development of suitable targeted therapeutics is greatly warranted.

Evidence has indicated the significant impact of dysregulated Wnt/β-catenin signaling in CRC progression (19). Typically, β-catenin acts as a common signaling molecule by forming complexes with cadherin to initiate transcription of target genes *via* interacting with Tcf/Lef-1 proteins. Mutations in the β-catenin gene are considered as critical events in the early stages of CRC progression, where it interacts with growth promoting genes such as c-Myc,

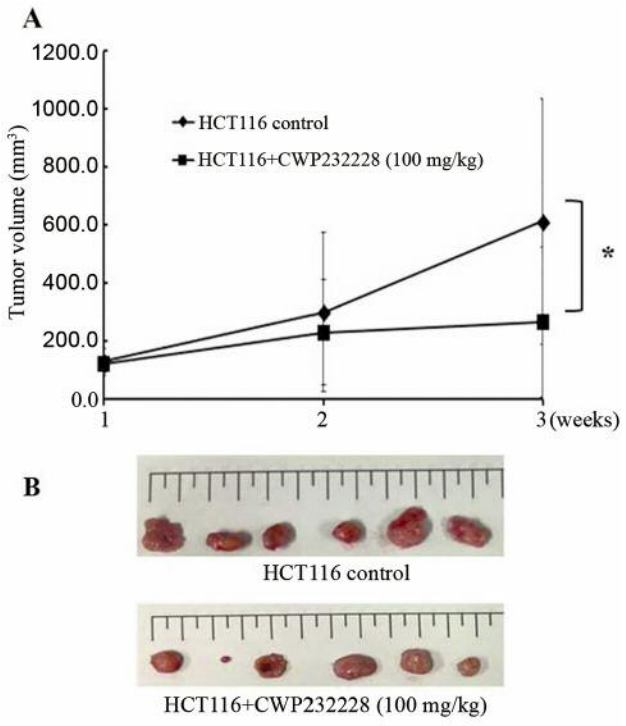

Figure 4. *CWP232228 delays colorectal tumor growth in xenograft mice model of CRC (A) Graphical data represent the tumor volume in control (HCT116) and CWP232228-treated mice for 3 weeks. (B) Photomicrograph showing the tumor sizes of all experimental mice. Values are expressed as mean±SD (n=6) and are statistically significant at p<0.05 as analyzed by t-test.*

cyclin D1, suggesting that this pathway is critical for tumorigenesis (20).

In the current study, we demonstrated that CWP232228, a potent small selective inhibitor of β-catenin, acts as a promising anti-tumor therapeutic agent in CRC. CWP232228 treatment clearly caused a potent cytotoxic effect against human colon cancer cells, HCT116, in a concentration-dependent manner. The anti-tumor effect of CWP232228 is mediated by the induction of apoptosis. In addition to the induction of apoptosis, CWP232228 decreased the expression of cell survival genes, including cyclins D2, D3, and survivin, which play critical roles in apoptosis and cell cycle progression (21).

Based on the results of this study, CWP232228 induced apoptosis as well as cell cycle arrest in HCT116 cells. Cyclin D1 and c-Myc are transcriptional target genes of β-catenin that regulate cell cycle progression (G1 to S phase) (22). Aurora kinase A is a serine threonine kinase that targets GSK-3β thereby activating Wnt signaling (23). Its function is involved in cell cycle regulatory processes including centrosome separation, microtubule formation, mitotic entry, chromosome arrangement and cytokinesis, and its overexpression is associated with CRC progression (24, 25). MITF is a well-established oncogene in melanoma, one of the key roles of which is cell cycle regulation *via* cyclin-dependent kinase 2 (26). Interestingly, a recent study demonstrated an unrecognized role of MITF as a driver of lysosomal biogenesis which is stabilized by Wnt (27).

We demonstrated the therapeutic mechanism of action of CWP232228 *via* inhibiting cell cycle progression and showed that CWP232228 treatment markedly attenuated the expression of aurora kinase A, cyclin D1, c-Myc, and MIFT. Also, the *in vivo* xenograft model confirmed the *in vitro* anti-tumor therapeutic effect of CWP232228 in NSG mice.

In summary, our current data indicate that CWP232228 exerts its potent anti-tumor effect primarily through induction of apoptosis and G_2/M cell-cycle arrest in human colon cancer cells *in vitro* and *in vivo*, suggesting the potential therapeutic usage of CWP232228 in CRC.

Conflicts of Interest

The Authors declare no conflicts of interest regarding this study.

Authors' Contributions

Jin Young Kim designed the research and wrote the article. Geumi Park performed majority of the experiments. Manigandan Krishnan contributed to *in vivo* animal experiments. Eunyoung Ha and Kyung-Soo Chun supervised this study.

Acknowledgements

This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2017R1C1B5076611, 2018R1A2B6006175) and the Ministry of Science, ICT and Future Planning (2017R1A2B4009831).

References

1 Kanwar SS, Poolla A and Majumdar AP: Regulation of colon cancer recurrence and development of therapeutic strategies. World J Gastrointest Pathophysiol *3(1)*: 1-9, 2012. PMID: 22368781. DOI: 10.4291/wjgp.v3.i1.1

2 Roper J and Hung KE: Priceless gemms: Genetically engineered mouse models for colorectal cancer drug development. Trends Pharmacol Sci *33(8)*: 449-455, 2012. PMID: 22739258. DOI: 10.1016/j.tips.2012.05.001

3 Tiwari A, Saraf S, Verma A, Panda PK and Jain SK: Novel targeting approaches and signaling pathways of colorectal cancer: An insight. World J Gastroenterol *24(39)*: 4428-4435, 2018. PMID: 22739258. DOI: 10.1016/j.tips.2012.05.001

4 Deitrick J and Pruitt WM: Wnt/beta catenin-mediated signaling commonly altered in colorectal cancer. Prog Mol Biol Transl Sci *144*: 49-68, 2016. PMID: 27865466. DOI: 10.1016/bs.pmbts.2016.09.010

5 Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B and Kinzler KW: Activation of beta-catenin-tcf

signaling in colon cancer by mutations in beta-catenin or apc. Science *275(5307)*: 1787-1790, 1997. PMID: 9065402.
6 Shtutman M, Zhurinsky J, Simcha I, Albanese C, D'Amico M, Pestell R and Ben-Ze'ev A: The cyclin d1 gene is a target of the beta-catenin/lef-1 pathway. Proc Natl Acad Sci USA *96(10)*: 5522-5527, 1999. PMID: 10318916. DOI: 10.1073/pnas.96.10.5522
7 Basu S, Haase G and Ben-Ze'ev A: Wnt signaling in cancer stem cells and colon cancer metastasis. F1000Res 5(2016). PMID: 27134739. DOI: 10.12688/f1000research.7579.1
8 Zhan T, Rindtorff N and Boutros M: Wnt signaling in cancer. Oncogene *36(11)*: 1461-1473, 2017. PMID: 27617575. DOI: 10.1038/onc.2016.304
9 White BD, Chien AJ and Dawson DW: Dysregulation of wnt/beta-catenin signaling in gastrointestinal cancers. Gastroenterology *142(2)*: 219-232, 2012. PMID: 22155636. DOI: 10.1053/j.gastro. 2011.12.001
10 Chen B, Dodge ME, Tang W, Lu J, Ma Z, Fan CW, Wei S, Hao W, Kilgore J, Williams NS, Roth MG, Amatruda JF, Chen C and Lum L: Small molecule-mediated disruption of wnt-dependent signaling in tissue regeneration and cancer. Nat Chem Biol *5(2)*: 100-107, 2009. PMID: 19125156. DOI: 10.1038/nchembio.137
11 Huang SM, Mishina YM, Liu S, Cheung A, Stegmeier F, Michaud GA, Charlat O, Wiellette E, Zhang Y, Wiessner S, Hild M, Shi X, Wilson CJ, Mickanin C, Myer V, Fazal A, Tomlinson R, Serluca F, Shao W, Cheng H, Shultz M, Rau C, Schirle M, Schlegl J, Ghidelli S, Fawell S, Lu C, Curtis D, Kirschner MW, Lengauer C, Finan PM, Tallarico JA, Bouwmeester T, Porter JA, Bauer A and Cong F: Tankyrase inhibition stabilizes axin and antagonizes wnt signalling. Nature *461(7264)*: 614-620, 2009. PMID: 19759537. DOI: 10.1038/nature08356
12 Jang GB, Hong IS, Kim RJ, Lee SY, Park SJ, Lee ES, Park JH, Yun CH, Chung JU, Lee KJ, Lee HY and Nam JS: Wnt/beta-catenin small-molecule inhibitor cwp232228 preferentially inhibits the growth of breast cancer stem-like cells. Cancer Res *75(8)*: 1691-1702, 2015. PMID: 25660951. DOI: 10.1158/0008-5472.CAN-14-2041
13 Kim JY, Lee HY, Park KK, Choi YK, Nam JS and Hong IS: Cwp232228 targets liver cancer stem cells through wnt/beta-catenin signaling: A novel therapeutic approach for liver cancer treatment. Oncotarget *7(15)*: 20395-20409, 2016. PMID: 26967248. DOI: 10.18632/oncotarget.7954
14 Guo M and Hay BA: Cell proliferation and apoptosis. Curr Opin Cell Biol *11(6)*: 745-752, 1999. PMID: 10600713.
15 Moertel CG, Fleming TR, Macdonald JS, Haller DG, Laurie JA, Tangen CM, Ungerleider JS, Emerson WA, Tormey DC, Glick JH, Veeder MH and Mailliard JA: Fluorouracil plus levamisole as effective adjuvant therapy after resection of stage iii colon carcinoma: A final report. Ann Intern Med *122(5)*: 321-326, 1995. PMID: 7847642.
16 Geng F, Wang Z, Yin H, Yu J and Cao B: Molecular targeted drugs and treatment of colorectal cancer: Recent progress and future perspectives. Cancer Biother Radiopharm *32(5)*: 149-160, 2017. PMID: 28622036. DOI: 10.1089/cbr.2017.2210
17 Morawska K, Goirand F, Marceau L, Devaux M, Cueff A, Bertaut A, Vincent J, Bengrine-Lefevre L, Ghiringhelli F and Schmitt A: 5-fu therapeutic drug monitoring as a valuable option to reduce toxicity in patients with gastrointestinal cancer. Oncotarget *9(14)*: 11559-11571, 2018. PMID: 29545919. DOI: 10.18632/oncotarget.24338
18 Gener P, Gouveia LP, Sabat GR, de Sousa Rafael DF, Fort NB, Arranja A, Fernandez Y, Prieto RM, Ortega JS, Arango D, Abasolo I, Videira M and Schwartz S, Jr.: Fluorescent csc models evidence that targeted nanomedicines improve treatment sensitivity of breast and colon cancer stem cells. Nanomedicine *11(8)*: 1883-1892, 2015. PMID: 26238079. DOI: 10.1016/j.nano.2015.07.009
19 Zardawi SJ, O'Toole SA, Sutherland RL and Musgrove EA: Dysregulation of hedgehog, wnt and notch signalling pathways in breast cancer. Histol Histopathol *24(3)*: 385-398, 2009. PMID: 19130408. DOI: 10.14670/HH-24.385
20 Jaiswal AS, Marlow BP, Gupta N and Narayan S: Beta-catenin-mediated transactivation and cell-cell adhesion pathways are important in curcumin (diferuylmethane)-induced growth arrest and apoptosis in colon cancer cells. Oncogene *21(55)*: 8414-8427, 2002. PMID: 12466962. DOI: 10.1038/sj.onc.1205947
21 Yu X, Zhang Y, Cavazos D, Ma X, Zhao Z, Du L and Pertsemlidis A: Mir-195 targets cyclin d3 and survivin to modulate the tumorigenesis of non-small cell lung cancer. Cell Death Dis *9(2)*: 193, 2018. PMID: 29416000. DOI: 10.1038/s41419-017-0219-9
22 Davidson G and Niehrs C: Emerging links between cdk cell cycle regulators and wnt signaling. Trends Cell Biol 20(8): 453-460, 2010. PMID: 20627573. DOI: 10.1016/j.tcb.2010.05.002
23 Dar AA, Belkhiri A and El-Rifai W: The aurora kinase a regulates gsk-3beta in gastric cancer cells. Oncogene *28(6)*: 866-875, 2009. PMID: 19060929. DOI: 10.1038/onc.2008.434
24 Lassmann S, Danciu M, Muller M, Weis R, Makowiec F, Schulte-Monting J, Hopt UT and Werner M: Aurora a is differentially expressed and regulated in chromosomal and microsatellite instable sporadic colorectal cancers. Mod Pathol *22(10)*: 1385-1397, 2009. PMID: 19648887. DOI: 10.1038/modpathol.2009.111
25 Sillars-Hardebol AH, Carvalho B, Tijssen M, Belien JA, de Wit M, Delis-van Diemen PM, Ponten F, van de Wiel MA, Fijneman RJ and Meijer GA: Tpx2 and aurka promote 20q amplicon-driven colorectal adenoma to carcinoma progression. Gut *61(11)*: 1568-1575, 2012. PMID: 22207630. DOI: 10.1136/gutjnl-2011-301153
26 Hartman ML and Czyz M: Mitf in melanoma: Mechanisms behind its expression and activity. Cell Mol Life Sci *72(7)*: 1249-1260, 2015. PMID: 25433395. DOI: 10.1007/s00018-014-1791-0
27 Ploper D, Taelman VF, Robert L, Perez BS, Titz B, Chen HW, Graeber TG, von Euw E, Ribas A and De Robertis EM: Mitf drives endolysosomal biogenesis and potentiates wnt signaling in melanoma cells. Proc Natl Acad Sci USA *112(5)*: E420-429, 2015. PMID: 25605940. DOI: 10.1073/pnas.1424576112

Received May 7, 2019
Revised June 2, 2019
Accepted June 3, 2019