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Divaricoside Exerts Antitumor Effects, in Part, by Modulating Mcl-1 in Human Oral Squamous Cell Carcinoma Cells

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ABSTRACT

Cardiac glycosides (CGs), prescribed to treat congestive heart failure and arrhythmias, exert potent antitumor activity. In this study, divaricoside (DIV), a CG isolated from *Strophanthus divaricatus* was examined for its antitumor potency in oral squamous cell carcinoma (OSCC) cells. Cell growth was inhibited by DIV in a dose- and time-dependent manner in SCC2095 and OECM-1 OSCC cells using MTT assays. DIV induced S and G2/M phase arrest accompanied by downregulation of phosphorylated CDC25C, CDC25C, and CDC2 in SCC2095 cells. In addition, DIV induced apoptosis by activating caspase-3 and downregulating the expression of Mcl-1. Furthermore, overexpression of Mcl-1 partially reversed DIV-induced death in SCC2095 cells. Additionally, western blot and transmission electron microscopy analyses also indicated that DIV induced autophagy in SCC2095 cells. However, the combination of autophagy inhibitor did not affect DIV-mediated apoptosis in SCC2095 cells. Together, these findings suggest that translational potential of DIV to be developed as a therapeutic agent for OSCC treatment.

1. Introduction

Oral squamous cell carcinoma (OSCC) continues to remain a global health burden with increasing morbidity and mortality. The economic burden of OSCC treatment has been estimated at $16.9 billion in South Asia [1]. Tobacco smoking, alcohol consumption, and betel quid use are risk factors causing an increase in mortality rates due to OSCC [2]. The dysregulation of multiple genes, including genes encoding the Bcl-2 family of proteins and cyclooxygenases, plays an important role in oral carcinogenesis [3]. In addition to surgery, radiation therapy, chemotherapy, and molecular targeted therapy play a central role in the treatment of OSCC. However, the overall survival rates of OSCC patients continue to remain low at approximately 50–60% [4]. An effective strategy is therefore, required for the treatment of OSCC.

Multiple plant products have been shown to present chemopreventive properties or therapeutic potential against various diseases including cancer [5]. For example, resveratrol, a polyphenolic compound found in grapes, induces apoptosis by modulating PTEN/Akt signaling in doxorubicin-resistant gastric cancer cells [6]. Moreover, paclitaxel, a compound isolated from the Pacific yew tree, *Taxus brevifolia*, has been routinely used for the treatment of breast and endometrial cancers for several years [7].

Strophanthus divaricatus, an indigenous plant found in Asia including Taiwan, contains pollution-free organic pesticides [8]. The *Strophanthus* genus is well known for being a rich source of alkaloids, flavonoids, steroids, and cardiac glycosides (CGs) that have been used for the treatment of congestive heart failure and arrhythmias for century [9–11]. Blocking the cardiac Na^+/K^+-ATPase pump is the main mechanism of action for CGs. However, accumulating evidence shows that CGs induced apoptosis in several cancer cell lines, including colon, breast, and osteosarcoma cells [12–14]. For example, oleandrin induces apoptosis by the activation of caspases and upregulation of Bax expression in colon cancer cells [13]. In addition, digoxin, bufalin, and ouabain have been reported to inhibit cell growth by regulating multiple signaling pathways including topoisomerases I and II [15], hypoxia-inducible

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factor 1 [16], PI3K/Akt [17], and Bcl-2/Bax [18]. Furthermore, PBI-0524 and Anvirzel have been evaluated for their antitumor potency in clinical trials for the treatment of solid tumors and have been proven to be safe and effective [19,20]. In this study, we investigated the anti-tumor activity and the underlying mechanism of action of divaricoside (DIV) (Fig. 1A), a CG isolated from *S. divaricatus* [21]. In addition to elevating intracellular free calcium levels in guinea pigs [22], the mechanism underlying anticancer properties of DIV remains unknown. We demonstrated that DIV induces apoptosis and autophagy in OSCC cells, which is in part, mediated by reduced levels of the anti-apoptotic protein Mcl-1.

2. Materials and Methods

2.1. Reagents, Antibodies, and Plasmids

Divaricoside (DIV) was isolated from the bark of *S. divaricatus* collected in Pintung County, Taiwan, in June 2013, and a voucher specimen (2013) has been deposited in the Department of Medical Research, China Medical University Hospital (Taichung, Taiwan). The identity and purity of DIV were verified by proton nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry, 2-D NMR spectrometry and HPLC chromatography (supplemental information, Fig. S1-S8) using reported spectral data [21]. Other chemicals and reagents were used in this study were purchased from Sigma-Aldrich unless otherwise noted. All chemicals were dissolved in DMSO, diluted in culture medium, and added to cells at a final DMSO concentration of 0.1%. Antibodies for the following biomarkers were obtained from Cell Signaling Technologies (Danvers, MA): PARP, Bak, caspase-3, Mcl-1, Bcl-xL, LC3B, cyclin E, p-^{216}Ser CDC25C, CDC25C, CDC2, Na^+/K^+-ATPase α1 subunit, Bcl-2, NOXA, Bax, and p62. β-actin antibody, Sigma-Aldrich (St. Louis, MO). Mcl-1 plasmid was obtained from OriGene Technologies, Inc. (Rockville, MD). The enhanced chemiluminescence system for detection of immunoblotted proteins was from GE Healthcare (Little Chalfont, Buckinghamshire, UK).

2.2. Cell Culture

SCC2095 cells (American Type Cell Culture, human tongue primary tumor) were kindly provided by Professor Susan R. Mallery (The Ohio State University). OECM-1 (human gingival epidermoid carcinoma; papilloma virus negative) cells and dysplastic oral keratinocyte (DOK) cells were kindly provided by Dr. Chih-Wen Shu (I-Shou University). SCC2095 cells were maintained in DMEM/F12 (Invitrogen, Carlsbad,

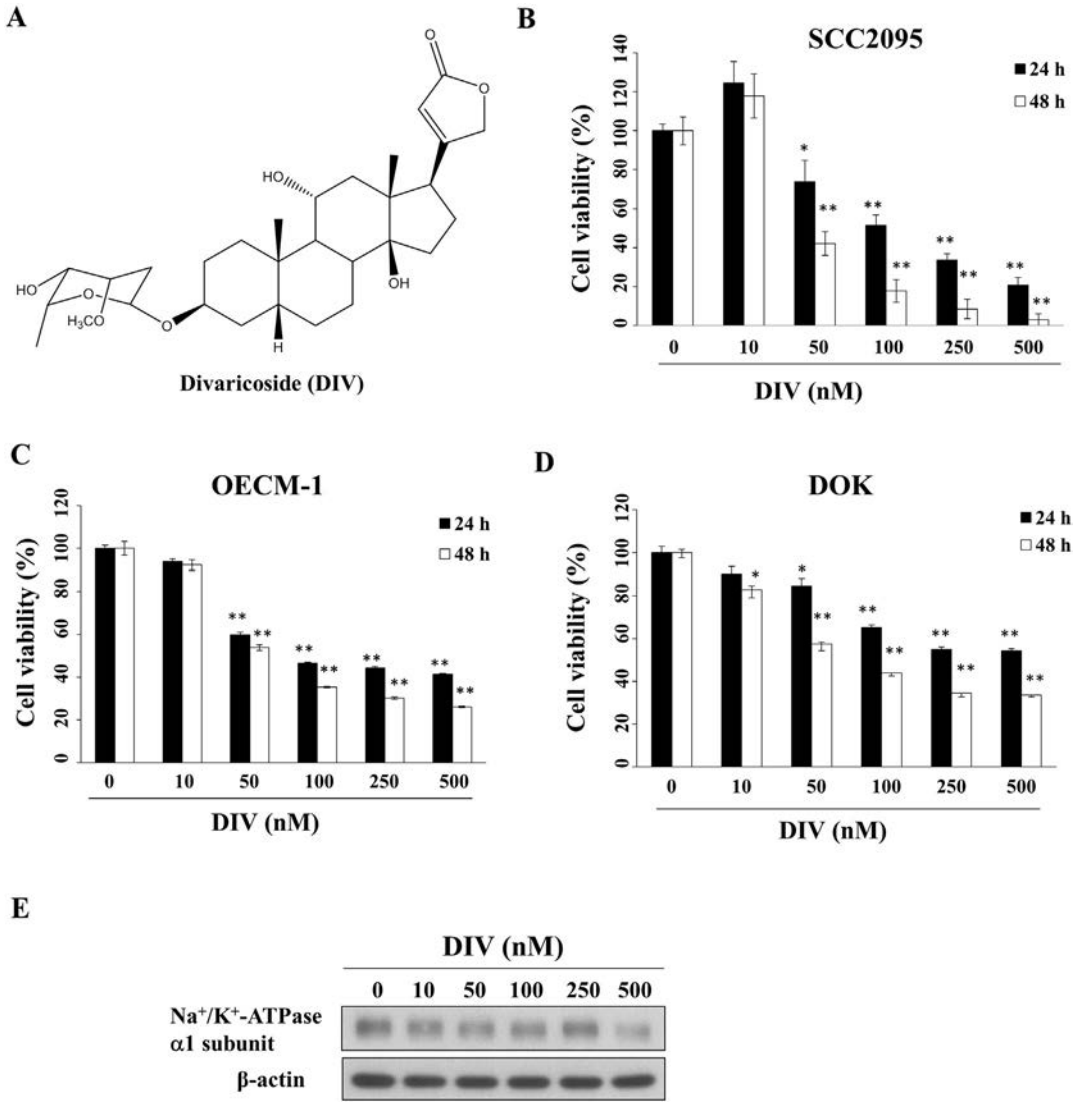

Fig. 1. Effect of divaricoside (DIV) on the viability of oral cancer cells (SCC2095 and OECM-1) and DOK cells. (A) The chemical structure of DIV. (B) SCC2095, (C) OECM-1, and (D) DOK cells. Cells were seeded into 96-well plates, after 24 h of incubation, cells were treated with DIV for 24 h or 48 h, and cell viability was detected by MTT assays. *Points* represent means; *bars* represent S.D. ($n = 3$–6). $^*P < 0.05$, $^{**}P < 0.01$ compared to the control group. (E) SCC2095 cells were treated with DIV and detected for the protein expression by Western blot analysis.

CA); and OECM-1 and DOK cells were maintained in DMEM (Invirtogen), supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco, Grand Island, NY), and cells were cultured at 37 °C in a humidified incubator containing 5% CO_2 and 95% relative humidity.

2.3. Cell Viability Analysis

To assess cell viability, cells were seeded in 96-well plates and incubated overnight, and then cells were exposed to drug for 24 h or 48 h [23]. Subsequently, cells were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In brief, 200 μL medium containing 0.5 mg/mL of MTT was added to each well and incubated for 4 h. Supernatants were aspirated from the wells, and 200 μL of DMSO was added to each well to dissolve the crystal formazan dye. The absorbance was measured at 570 nm using a plate reader.

2.4. Immunoblotting

Western blot analysis was performed as reported previously [23]. Briefly, treated cells were lysed with RIPA buffer (50 mM Tris, 150 mM NaCl, 1 mM PMSF, 1.0 mM EDTA, 1% sodium deoxycholate, 0.1% Triton X-100). Proteins were resolved on 10–15% SDS-PAGE, then transferred to PVDF (Bio-Rad) membranes. After blocking, the membranes were incubated with primary antibodies at 4 °C overnight, then incubated with secondary antibodies at room temperature for 1 h. The immunoblots were visualized by enhanced chemiluminescence.

2.5. Flow Cytometry

Cells (2×10^5/3 mL) were treated with drug at the indicated concentration or DMSO for 24 h. For cell cycle analysis, after being washed twice with ice-cold PBS, cells were fixed in 70% cold ethanol for 4 h at 4 °C. ROS production was detected using the fluorescence probe 5-(and-6)-carboxy-2′,7′-dichlorodihydrofluoresceindiacetate (carboxy-DCFDA) [24]. For apoptosis evaluation [23], cells were stained with Annexin V and PI (1 μg/mL), counted on a BD FACSAria flow cytometer, and analyzed by ModFitLT V3.0 software program (Becton Dickinson, Germany).

2.6. Transient Transfection for Overexpression

For overexpression of Mcl-1, SCC2095 cells (2×10^5/3 mL) were transfected with Fugene HP (Roche) according to the manufacture's protocol [23] and then cultured in a six-well plate for 24 h. Plasmids expressing vector (pCMV6-Entry) and Mcl-1 (Myc-DDK-tagged) were purchased from OriGene Technology (Rockville, MD, USA). Proteins were collected for Western blot analysis.

2.7. Detection of Autophagosome Formation with Acridine Orange by Flow Cytometry

To detect the presence of acidic vesicular organelles (AVO) [23], a flow cytometer (BD FACSCanto II) with red (650 nm, stained by cytoplasmic vesicles) *vs* green (510–530 nm, stained nuclei) fluorescence (FL3/FL1) from cells illuminated with blue (488 nm) excitation light were measured. A minimum of 10,000 cells within the gated region were analyzed. The fluorescence intensity is proportion to both the degree of acidity and the volume of the cellular acidic compartment.

2.8. Transmission Electron Microscope

Transmission Electron Microscope was performed as described previously [24]. Briefly, SCC2095 cells were fixed in a solution containing 0.2 M sodium cacodylate, 2% paraformaldehyde, and 2.5% glutaraldehyde for 1 h. Then, cells were suspended in a buffered solution containing 1% osmic acid for 1 h, followed by dehydration in a graded ethanol series, washing with acetone and embedding into EPON epoxy resin. Thin sections (60–80 nm) were contrasted with lead citrate and uranyl acetate. All sections were observed and photographed with a Hitachi H-600 transmission electron microscope (Hitachi, Tokyo, Japan).

2.9. Statistical Analysis

All experiments were performed in three replicates. Statistical significance was determined with Student's *t*-test comparison between two groups of data sets. Differences among group means of ROS generation were analyzed for statistical significance using one-way ANOVA followed by the Neuman-Keuls test for multiple comparisons. Differences between groups were considered significant at $^*P < .05$, $^{**}P < .01$.

3. Results

3.1. DIV Suppresses the Viability of OSCC Cells

We first examined the viability of two oral cancer cell lines, SCC2095 and OECM-1 and dysplastic oral keratinocyte (DOK) cells after DIV treatment using the MTT assay. As shown in Fig. 1B-D, DIV decreased cell viability in a dose- and time-dependent manner in all cell types. The IC_{50} values at 24 h were 143.8, 133.6, and 291.1 nM for SCC2095, OECM-1, and DOK cells, respectively. At 48 h, the IC_{50} values were 78.5, 98.5, and 111.0 nM for SCC2095, OECM-1, and DOK cells, respectively. The cell viability were slightly increased after the treatment of 10 nM DIV at 24 h and 48 h in SCC2095 cells (Fig. 1B, $^*P = 0.101$, $^*P = 0.103$) which is similar to the cardiac glycosides at low concentration in cancer cells [25,26]. Notably, the cell viability for DOK was higher than for the oral cancer cells at 24 h. We further examine the effect of DIV on Na^+/K^+-ATPase using Western bloting. As shown in Fig. 1E, DIV down-regulated the expression of Na^+/K^+-ATPase α1 subunit in SCC2095 cells.

3.2. DIV Induces Cell Cycle Arrest in OSCC Cells

Bufalin, another CG, has been reported to inhibit cell growth by inducing cell cycle arrest and lowering cyclin D1 and cyclin E expression in pancreatic cancer cells [27]. To determine whether the cell cycle regulation was related to DIV-mediated inhibition of cell growth, SCC2095 cells were treated with DIV for 48 h and stained with propidium iodide (PI) to analyze the cell cycle profile. Flow cytometry analysis indicated a dose-dependent increase in both S and G2/M phase cell populations after DIV treatment manner (Fig. 2A). Compared to control, the cell population in the S phase increased from 19.7 ± 1.3% to 51.1 ± 1.9% after DIV (50 nM) treatment for 48 h, whereas the cell population in the G2/M phase also increased from 14.9 ± 1.1% to 29.0 ± 6.1% (Fig. 2B). In addition, we examined the effect of DIV on the levels of some cell cycle-regulating proteins, such as cyclins, and cell division cycle proteins (CDCs) by western blot analysis. As shown in Fig. 2C, DIV inhibited the expression and phosphorylation of cyclin E, CDC25C, and CDC2 in SCC2095 cells.

3.3. DIV Induces Apoptosis in OSCC Cells

To further examine whether DIV induces apoptosis in SCC2095 cells, PI/annexin V staining was performed. As shown in Fig. 3A and B, the percentage of annexin V-positive cells increased after DIV treatment for 48 h in a dose-dependent manner. Furthermore, DIV treatment increased PARP cleavage and promoted caspase-3 activation (Fig. 3C). Hence, we conclude that DIV induced caspase-3 dependent apoptosis in SCC2095 cells.

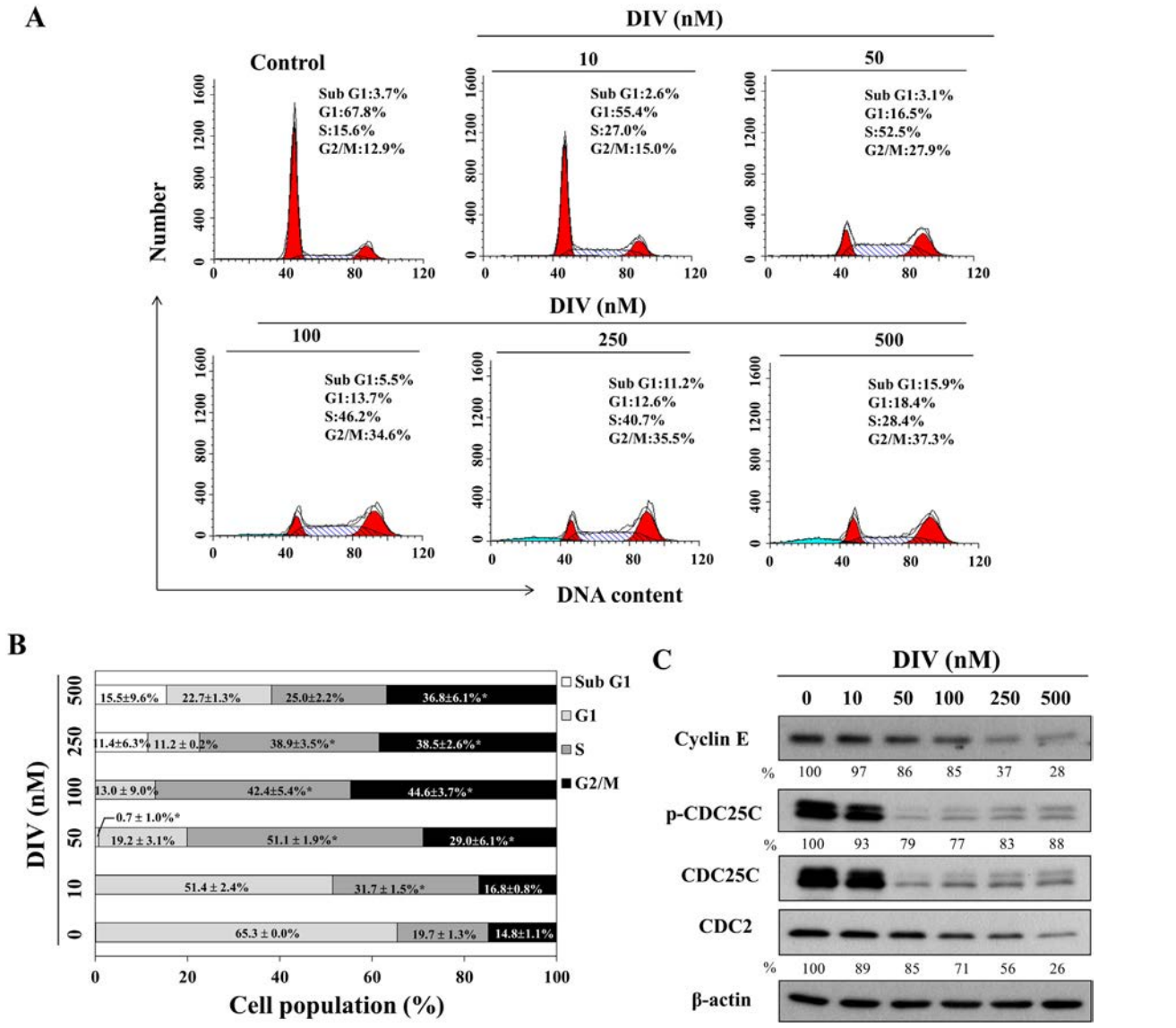

Fig. 2. Effect of divaricoside (DIV) on cell cycle and cell cycle-regulating proteins. (A) Cell cycle analysis showed an increase in S and G2/M phase population after treatment with DIV at the indicated concentrations for 48 h in SCC2095 cells, followed by propidium iodide (PI) staining. Three independent experiments were performed; and data are presented in (B) as mean ± S.D. (C) Western blot analysis of DIV-treated SCC2095 cells indicating the phosphorylation and expression of cyclin E, CDC25C, and CDC2. Cells were treated with DIV in 5% FBS-supplemented DMEM/F12 medium for 48 h, and cell lysates were immunoblotted as described in the Materials and Methods section. The values in percentage denote the relative intensity of protein bands of drug-treated samples to that of the respective DMSO vehicle-treated control after being normalized to the respective internal reference (total respective protein or β-actin).

3.4. DIV Induces Reactive Oxygen Species (ROS) Generation

Previous studies have shown that an imbalance in ROS contributes to the development of OSCC and resistance to chemotherapy [28,29]. We report that DIV leads to an increase in ROS generation in SCC2095 cells after 24 h of treatment (Fig. 4A, H_2O_2 was used as a positive control). Pretreatment with antioxidants, *N*-acetylcysteine or glutathione for 1 h, partially rescued DIV-induced ROS generation, confirming that DIV treatment leads to an increase in ROS levels (Fig. 4B).

3.5. DIV Induces Autophagy in OSCC Cells

Several studies have reported that CGs bufalin, digoxin, and ouabain induce autophagy in glioma cells and lung cancer cells [30,31]. We further investigated the potential interplay between autophagy and DIV-mediated antitumor effects. Firstly, transmission electron microscopy was performed to examine the ability of DIV to induce the formation of autophagosomes (arrows), which are indicative of autophagy induction (Fig. 5A). Secondly, flow cytometry analysis of DIV-treated SCC2095 cells demonstrated an increase in cells stained with acidic vesicular organelles (Fig. 5B, rapamycin was used as a positive control). Furthermore, western blot analysis of DIV-treated SCC2095 cells indicated a dose-dependent increase in the expression levels of autophagy markers LC3B-II [32] and p62 [32] (Fig. 5C). To investigate the role of autophagy in DIV-induced cell death, we examined the apoptotic response to the autophagy inhibitor 3-methyladenine (3-MA) or chloroquine (CQ) in DIV-treated SCC2095 cells. The cytotoxicity of 3-MA and CQ in SCC2095 cells were showed in Fig. S9. Analysis of PI/annexin V staining showed no significant change in the apoptotic cell count upon combined treatment of DIV and 3-MA or CQ, in comparison to that upon DIV treatment alone (Fig. 5D). These results suggest that autophagy and apoptosis might be independently induced in DIV-treated SCC2095 cells.

3.6. Mcl-1 is Involved in DIV-Mediated Cell Death

Recent evidence suggests that Mcl-1, a Bcl-2 family protein, plays an important role in ouabain- and bufalin-induced apoptosis in lung cancer cells [33,34]. To evaluate the same for DIV, we first tested if DIV-induced apoptosis involved the regulation of Bcl-2 family proteins in SCC2095 cells. As shown in Fig. 6A, DIV decreased the expression of Bcl-xL and Bcl-2, and a greater extent, Mcl-1 in a dose-dependent manner, accompanied by increases in the proapoptotic proteins NOXA and Bax, whereas the expression of Bak remained unaltered after DIV treatment. In addition, time-course experiments revealed that Mcl-1 expression reduced after 12 h of DIV treatment, whereas the expression of Bcl-xL decreased at higher concentration of DIV at 48 h (Fig. 6B). To further confirm that reduced levels of Mcl-1 contributed to DIV-induced apoptosis and cytotoxicity, we transiently transfected myc-tagged-Mcl-1 in SCC2095 cells and observed that Mcl-1 overexpression abrogated DIV-induced caspase-3 activation and cytotoxicity in these cells.

4. Discussion

Natural products have served as source of antitumor drugs for several centuries [35]. Although the narrow therapeutic window has been a major safety clinical concern for CGs in treating congestive heart failure, the promising antitumor effects of CGs have encouraged their repurposing for new indications including cancer [36]. For example, lanatoside C, an FDA-approved CG, induces apoptosis by attenuating Wnt/β-catenin/c-Myc signaling in gastric cancer cells [37]. Therefore, the other CGs have proved to be potential candidates for the investigation of their antitumor activity and underlying pharmacological mechanisms. In the present study, we report that DIV, a CG isolated from *S. divaricatus*, shows anticancer activity by inducing apoptosis in OSCC cells by partially regulating Mcl-1 levels.

Incubation of SCC2095 cells with low DIV concentration of 10 nM for 24–48 h slightly increased cell count by 15–18% (Fig. 1B). These results correlate well with the data on the activation and inhibition of cell growth by low and high concentration of CGs in prostate cancer cells

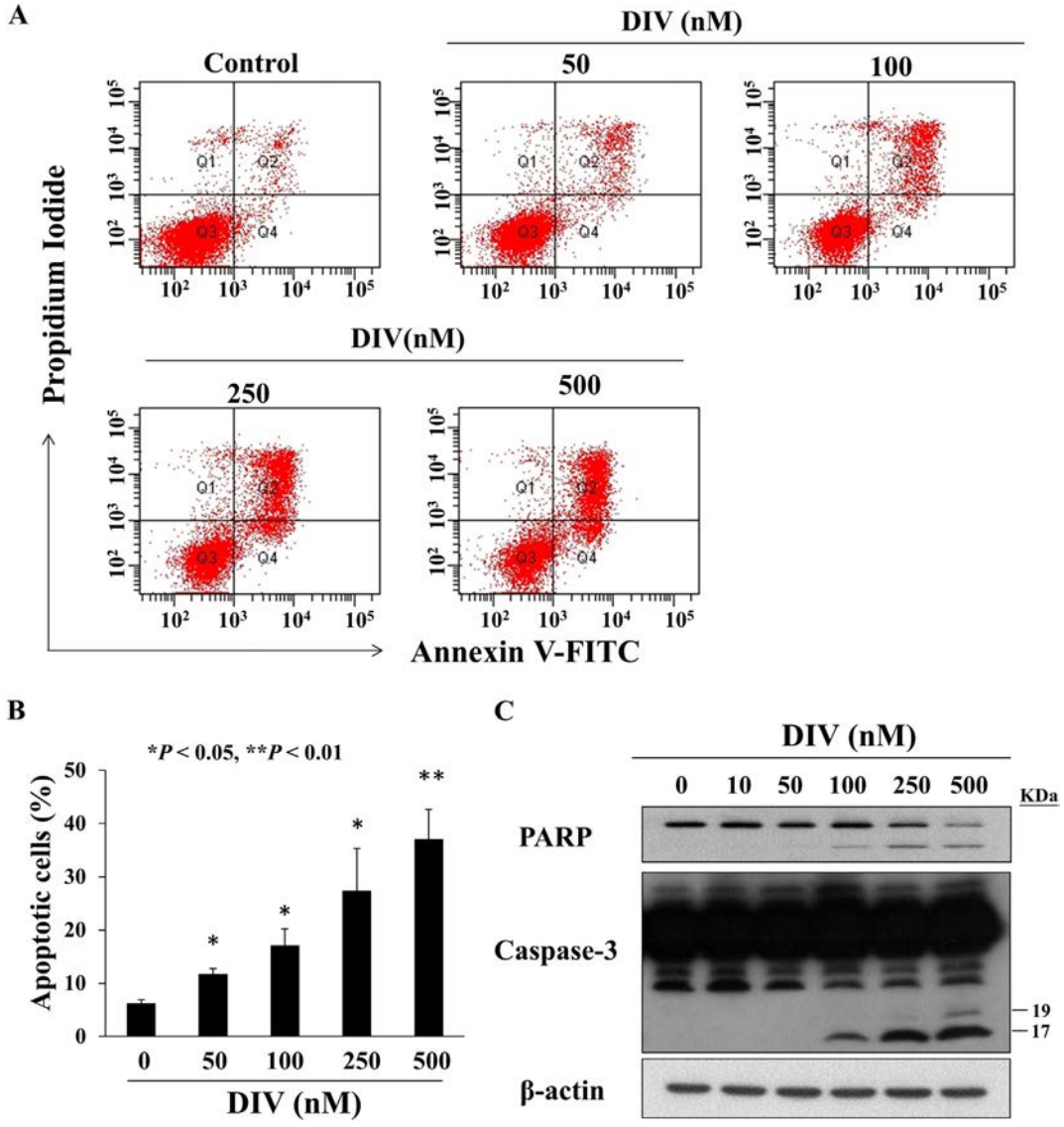

Fig. 3. Effect of divaricoside (DIV) treatment on apoptosis. (A) SCC2095 cells were treated with DMSO or DIV in 5% FBS-supplemented DMEM/F12 medium for 48 h and stained with propidium iodide (PI)/annexin V. (B) The percentage of apoptotic cells (Q2 + Q4) after the treatment with DMSO or DIV for 48 h. Cells were analyzed by flow cytometry after staining with fluorescein-conjugated annexin V and PI. *Columns* represent means; *bars* represent S.D. ($n = 3$). $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the control group. (C) Concentration-dependent effect of DIV on caspase-3 activation and PARP cleavage in SCC2095 cells after 48 h treatment in 5% FBS-supplemented DMEM/F12 medium.

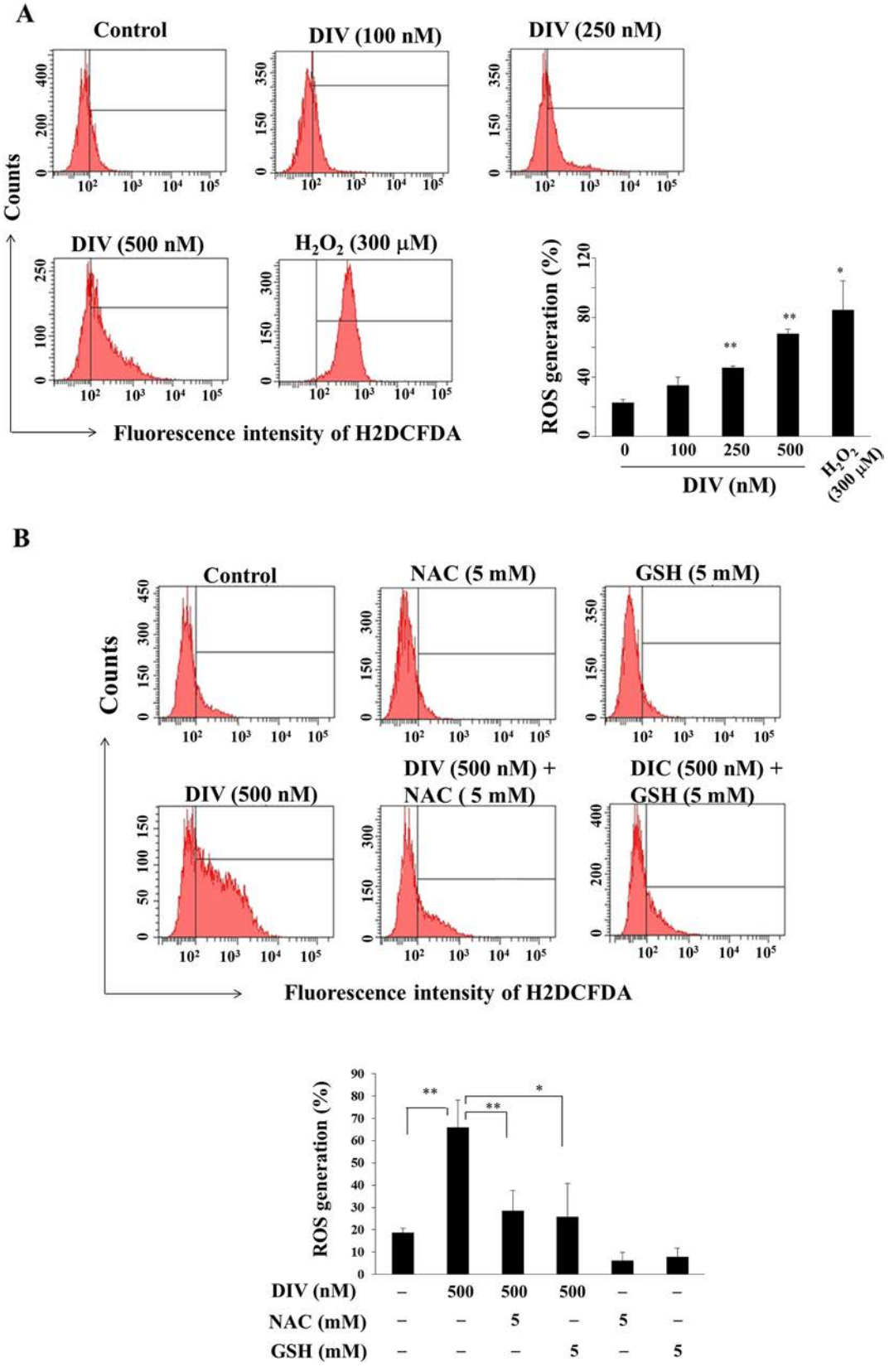

Fig. 4. Reactive oxygen species (ROS) analysis in SCC2095 cells. (A) Left panel, cells were treated with DMSO or divaricoside (DIV) at the indicated concentration for 24 h and were stained with carboxy-DCF-DA. Right panel, data are presented as the mean ± S.D. ($n = 3$). $^{*}P < 0.05$, $^{**}P < 0.01$. (B) Upper panel, cells were treated with DMSO or 500 nM DIV for 24 h and were stained with carboxy-DCF-DA. *N*-acetylcysteine (NAC) or glutathione (GSH) was used to inhibit ROS production. Lower panel, data are presented as the mean ± S.D. ($n = 4$). $^{*}P < 0.05$, $^{**}P < 0.01$.

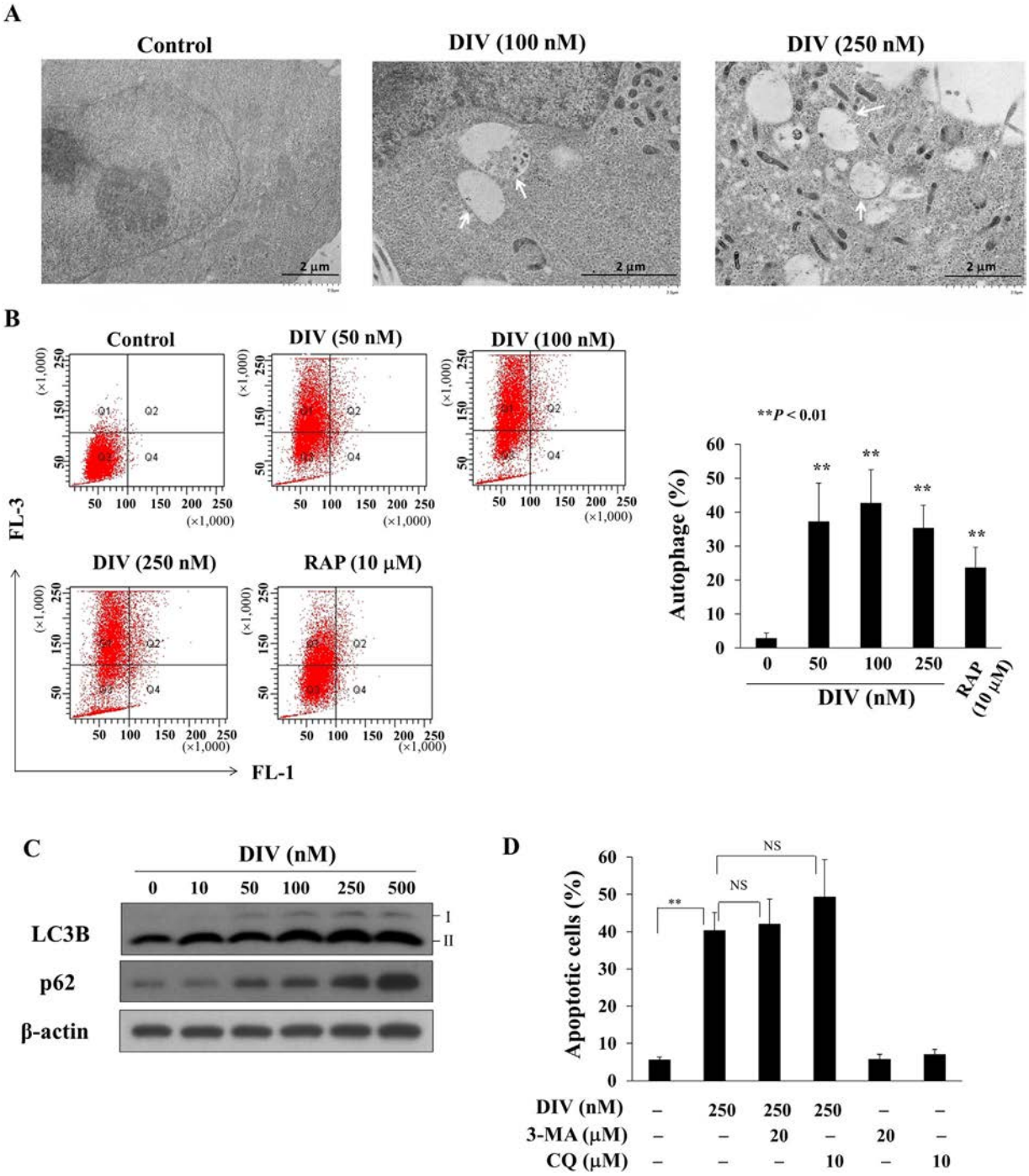

Fig. 5. Divaricoside (DIV) induces autophagy. (A) Electron microscopy analysis of autophagosome formation in DMSO- or DIV-treated SCC2095 cells as described in the Materials and Methods section; arrows indicate autophagosomes. (B) SCC2095 cells were treated with DMSO or DIV or rapamycin (RAP) for 48 h. Cells were harvested and stained with acridine orange to determine the ratio of autophagy using flow cytometry; arrows indicate autophagosomes. (C) The expression levels of LC3B-II and p62 after DIV treatment for 48 h in SCC2095 cells. (D) Left, SCC2095 cells were treated with 250 nM DIV alone or in combination with 20 μM 3-methyladenine (3-MA) or 10 μM chloroquine (CQ) for 48 h, and dual staining with propidium iodide (PI)/annexin V-FITC was performed. Percentages in the graphs are representative of cell percentage in the respective quadrants (n = 3). *Columns* represent means; *bars* represent S.D. $^{**}P < 0.01$. NS, indicates not significant when comparing DIV alone treatment and the combined treatment of DIV and 3-MA or CQ.

and HUVECs [25,38]. Liu et al. showed that bufalin inhibited cell proliferation by inducing cell cycle arrest at the S phase and downregulating cyclin D1 and cyclin E in pancreatic cancer cells [27]. Bufalin and hellebrigenin induced G2/M arrest and apoptosis by reducing phosphorylated CDC25C, CDC25C, and also CDC2 levels in hepatocellular carcinoma cells [39,40]. Cdk2/Cyclin E complex has been known to promote the transition from late G1 to S phase [41]. Inactivation of CDC25C phosphatase causes the inhibition of CDC2, leading to a G2/M arrest [42]. We observed that DIV treatment led to stalling of cells in the S and G2/M phases in a dose-dependent manner. Consistent with these findings, our results also show that DIV treatment downregulates the cell cycle-regulating gene products cyclin E, CDC25C, and CDC2.

Dysregulation of oxidative stress-related genes and betel quid chewing have been strongly associated with the malignant transformation in the oral cavity [43,44]. However, ROS production also represents an important mechanism of action of chemotherapeutic agents against cancer [45]. For example, paclitaxel suppresses cell proliferation and induces apoptosis by increasing ROS generation in canine mammary

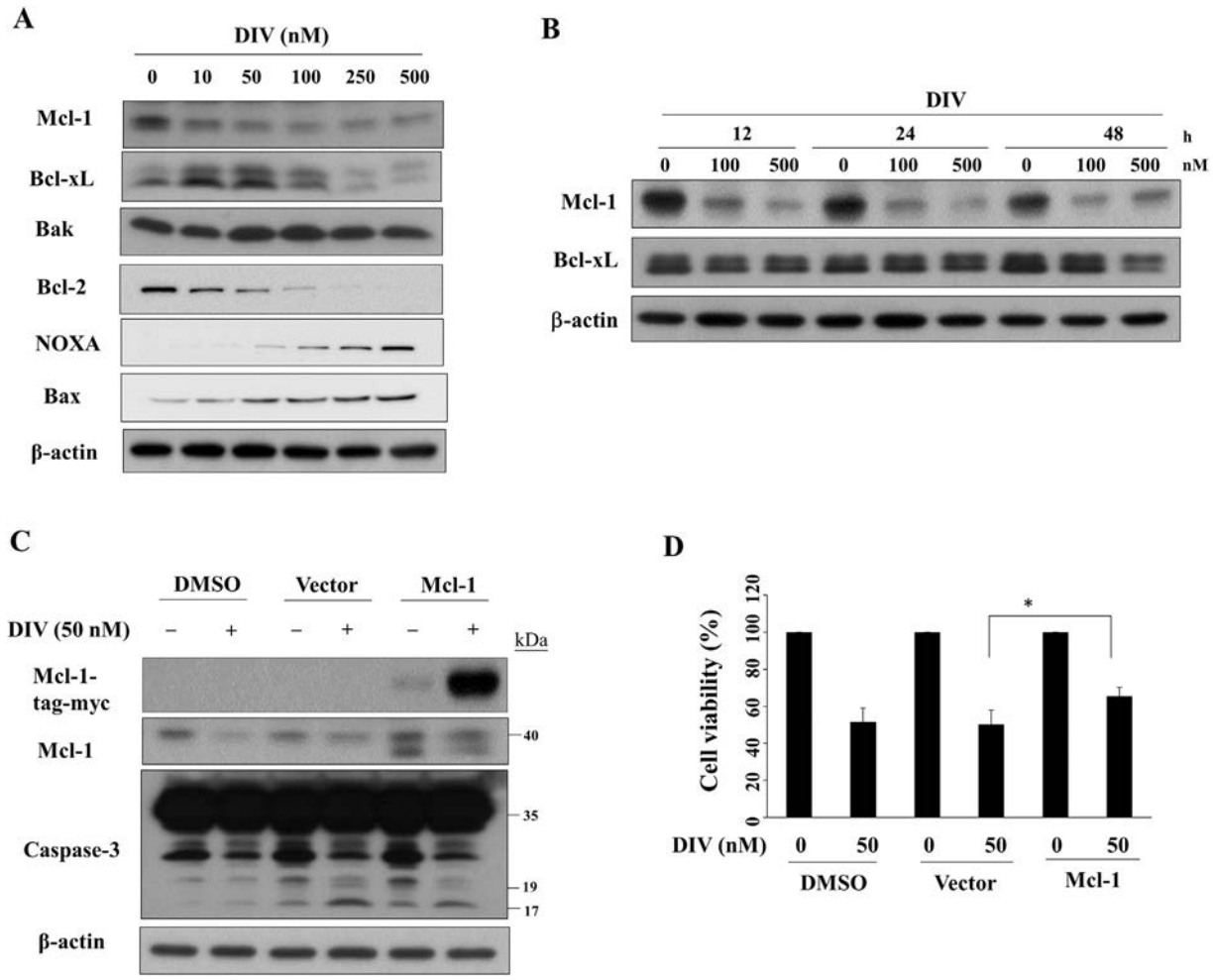

Fig. 6. (A) Effect of DIV treatment on Bcl-2 family of proteins. (A) Effect of DIV treatment on the expression levels of Mcl-1, Bcl-xL, Bak, Bcl-2, NOXA, and Bax in SCC2095 cells. Cells were treated with DIV in 5% FBS-supplemented DMEM/F12 medium for 48 h, and cell lysates were immunoblotted as described in the Material and Methods section. (B) Time-dependent effect of DIV treatment on the expression of Mcl-1 and Bcl-xL. (C) Effect of ectopic Mcl-1 expression after DIV treatment on apoptosis-associated proteins. SCC2095 cells were transfected with control vector or Mcl-1 plasmid for 18 h and then treated with DIV for 24 h. Whole cell extracts were subjected to western blot analysis. (D) Effect of Mcl-1 overexpression on the viability of SCC2095 cells treated with 50 nM DIV for 24 h. After incubation, cells were analyzed using MTT assay. *Columns* represent means; *bars* represent S.D. $^{*}P < 0.05$.

gland tumor cells [45]. Bufalin has also been shown to induce apoptosis *via* reduction in ROS production in tongue cancer cells [46]. Our results showed that DIV exhibits an antitumor effect by increasing ROS generation in SCC2095 cells, and this inhibition can partially be rescued by pretreatment with antioxidants. This discrepancy, however, could be attributed to the differences in cell lines and the drug treatment used.

The effect on DIV treatment on the reduction of anti-apoptotic protein Mcl-1 could be a potential contributor to the antitumor properties of DIV. Reports suggest that down-regulation of Mcl-1 sensitizes OSCC cells to chemotherapeutic agents and radiation [47,48]. Because ectopic expression of Mcl-1 partially protected cells from DIV-induced cell death, we reason that this Mcl-1 downregulation acts in concert to facilitate caspase-dependent apoptosis in DIV-treated OSCC cells.

Multiple lines of evidence suggest that CGs exhibit a contrasting effect on the induction of autophagy. For instance, the CG ophiopogonin D attenuates doxorubicin-induced autophagy by modulating JNK and ERK signaling in cardiomyocytes *in vitro* and *in vivo* [49]. Digoxin and ouabain have been shown to induce autophagic cell death *via* an Src-dependent mechanism, which promotes apoptosis in lung cancer cells [50]. This autophagy-associated apoptosis acts synergistically in bufalin-treated glioma cells [31]. We also report that DIV induces autophagy, as an evident from the accumulation of autophagosomes in the cytoplasm, and the increased levels of LC3B-II and p62 in SCC2095 cells. However, our results show that pretreatment with an autophagy inhibitor, 3-MA or CQ, did not affect apoptosis. Although inhibition of autophagy failed to enhance DIV's apoptotic effect, autophagy as a protective mechanism against DIV-induced apoptosis in oral cancer cells could not be ruled out. The previous findings along with the present data, highlight a complex, cell type-dependent mechanism by which CGs affect cancer cells.

5. Conclusions

In conclusion, our data demonstrate that DIV effectively suppresses the viability of OSCC cells. In addition to ROS generation, DIV induces autophagy and modulates the antitumor activity by lowering Mcl-1 levels in OSCC cells. Our findings suggest a potential mechanism of anticancer activity of CGs and show that DIV can be used as an effective agent in the prevention and treatment of OSCC.

Conflict of Interest

The authors declare no competing financial interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.csbj.2019.01.004.

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