Apoptotic Effects of Diosgeninlactoside on Oral Squamous Carcinoma Cells in Vitro and in Vivo

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Diosgenyl saponins possess a variety of biological functions. Herein, we demonstrate a new type of diosgenyl saponin derivatives that inhibit cellular proliferation of oral squamous cell carcinoma (OSCC) cell lines. Thereafter, we analyzed these cells’ expression of apoptosis-related proteins. Crucial proteins that participate in apoptosis regulation including caspases 8, 9, and 3, and cleaved Bid were activated and upregulated accompanied by increased concentrations of diosgenyl saponins. Meanwhile, Bel-2 was downregulated and mitochondrial membrane potential decreased. In our mice model of OSCC, compound 1 showed potent inhibition of solid tumor growth and salient antitumor activity. Diosgenyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside might induce OSCC cell line apoptosis through extrinsic and intrinsic pathways, and might provide a mechanistic background for the development of this new type of diosgenylsaponin derivatives into anti-cancer agents against OSCC.

Key words  diosgenyl saponin; oral squamous cell carcinoma (OSCC); apoptosis

Oral cancer is one kind of the head and neck tumors, and is the eighth most common occurring cancers in the whole world. The factors related to this disease are reported to be tobacco usage, betel quid chewing, excessive alcohol drinking, dietary micronutrient deficiency, and human papilloma virus infection, etc. Its high incidence can be found worldwide, including nearly all the continents, and caused serious public health threats. More than 90% of all oral cancers are attributed to oral squamous cell carcinoma (OSCC). OSCC most commonly appears in tongue and the floor of the mouth, whereas it less frequently occurs in other locations such as lip, buccal mucosa, retromolar area, gingiva, soft palate and the back of the tongue and hard palate. It is generally accepted that OSCC is caused by mutations of oral keratinocyte: DNA mutations increase hyperplasia of keratinocyte and become pre-malignant or potentially malignant disorders (which are metastases, to the majority of patients with advanced cancer, chemotherapy is an indispensable therapy. However, the resistance to chemotherapy agent was reported to be the most critical problem, thus a wide array of structurally diverse drugs with explicit mechanism are badly needed. Furthermore OSCC is a highly complex multifactorial process involving numerous and multi-step genetic events, personalized therapy became a focus of attention. As a consequence, the search of new type of compounds against OSCC and the elucidation of their mechanisms have high scientific and practical values.

Diosgenyl saponins, mainly obtained as plant extracts, showed many bioactivities ranging from anti-fungi to anti-inflammation. Recently, such type of saponins also gained attentions in the area of anticancer researches. For instance, a series of diosgenyl saponins isolated from Dioscorea zingiberensis Wight, a Chinese medicinal plant, were reported to inhibit a panel of cancer cell lines to varying extents. But the use of naturally isolated diosgenyl saponins as anticancer drugs has been hindered because of the difficulty of scaling up those compounds to sufficient quantities. To circumvent this problem, lots of efforts have been put into the synthesis of different diosgenyl saponin analogues carrying various sugar residues.

The authors declare no conflict of interest.

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MATERIALS AND METHODS

Chemical Reagents and Antibodies Diosgenyl β-α-galactopyranosyl-(1→4)-β-α-glucopyranoside (compound 1) was provided by the Laboratory of Ethnopharmacology, Institution for Nanobiomedical Technology and Membrane Biology, Regenerative Medicine Research Center of West China Hospital, Sichuan University, China. 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyldetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were purchased from Sigma (St. Louis, MO, U.S.A.). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody was obtained from Abcam (Cambridge, U.K.). Antibodies for caspase 3, caspase 8, caspase 9, Bcl-2 and Bid were purchased from Cell Signaling Technology Company (Beverly, MA, U.S.A.). Annexin V-fluorescein isothiocyanate (FITC) apoptosis detection kit was obtained from KeyGen Tec (Nanjing, China). 4,6-Diamidin-2-phenylindol-dihydrochloride (DAPI) and mitochondrial membrane potential detection kit (JC-1) were obtained from Beyotime (Jiangsu, China). DeadEnd® Fluorometric TUNEL System was purchased from Promega (Madison, WI, U.S.A.).

Cell Culture HSC-3, a human tongue squamous cell carcinoma cell line was obtained from Japan JCRB cell bank, Cal-27, a human tongue squamous cell carcinoma cell lines, OSCC cell line derived from C3H mice SCC-7, were obtained from the American Type Culture Collection (ATCC), Cal-33 cell line was obtained from the European Collection of Cell Cultures (ECACC), NOK-SI cell line was kindly provided by Dr. J. S. Gutkind (National Institute of Dental and Cranio-facial Research). All the cell lines were stored by the State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, China. Cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) (Gibco BRL, Grand Island, NY, U.S.A.) supplemented with 10% heat inactivated fetal bovine serum (FBS, Gibco, Auckland, New Zealand), 100 U of penicillin G and 100 U of streptomycin sulphate under 5% CO2, and humidified air atmosphere at 37°C.

MTT Assay for Cell Viability The OSCC cell lines HSC-3, Cal-27 and Cal-33, a OSCC cell line derived from C3H mice SCC-7, and normal oral keratinocytes spontaneously immortalized (NOK-SI) cell line in the logarithmic growth phase were seeded in 96-well plates (BD) 3×104/mL per well. After culturing for 24 h, the cells were treated with varying concentrations (0, 2, 4, 6, 8, 10, 12, 16, 20 and 24 µM) of compound 1 for various hours (12, 24, 36 and 48h). Then, they were treated with 20µL of MTT (5mg/mL stock) and incubated at 37°C for 4h. Absorbance was recorded at 570 nm by microplate reader (MDC). For all in vitro assays, the compound 1 was dissolved in DMSO as a stock solution, and the final concentration of DMSO was less than 0.1% (v/v) in the culture medium. Independent experiment was repeated for three times.

Morphological Analysis The HSC-3 cell in the logarithmic growth phase were seeded in six-well plates (BD) 1×105/mL per well and cultured overnight. They were treated with various concentrations (0, 4, 8 and 12 µM) of compound 1, and the cells was observed in an inverted microscope (Olympus, Tokyo, Japan) after 24h. Fluorescent microscope observation was carried out after DAPI nuclear staining at the wavelength of 330–385 nm.

Apoptosis Analysis with Annexin V/Propidium Iodide (PI) Staining Surface exposure of phosphatidylserine in apoptotic cells was quantitatively detected with Annexin V-FITC/PI apoptosis detection kit (KeyGenTec). After treatment with different concentrations of compound 1 (0, 4, 8 and 12 µM) for 24h, the cells were suspended in binding buffer and stained with Annexin V-FITC and PI for 15 min at room temperature. Then they were detected by a flow cytometer (Beckman Coulter) to differentiate apoptotic cells (annexin- and PI-negative) from necrotic cells (annexin- and PI-positive).

Western Blotting Analysis After treated with different concentrations of compound 1 (0, 4, 8 and 12 µM) for 24h, cells were lysed on ice RIPA (Beyotime) lysis buffer (1×106 cells/100 µL) containing 1% phenylmethylsulfonyl fluoride (PMSF) and 1% Cocktail protease inhibitors. BCA protein assay kit (Pierce) was used for measuring the protein content. An equal amount of protein was denatured for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (10%) electrophoresis at 100 V for 1.5 h, then transferred to polyvinylidene difluoride (PVDF) membrane (Milipore), blocked with 5% skim milk in TBST at room temperature for 1 h. Then, anti-caspase 3 (1 : 1000), anti-caspase 8 (1 : 1000), anti-caspase 9 (1 : 1000), anti-Bcl-2 (1 : 1000), anti-Bid (1 : 1000) and anti-GAPDH (1 : 5000) antibodies were used as primary antibody to probe the membranes at 4°C overnight. After washing with TBST, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies. Immunoblot signals were visualized using an ECL Western blotting detection reagent (Millipore).

Measurement of Mitochondrial Membrane Potential The loss of mitochondrial membrane potential (ΔΨm) was assessed by flow cytometry using the lipophilic cationic probe JC-1 dye (5,5′,6′,6′-tetrachloro-1,3,3′,4′-tetraethylbenzimidazo carbocyanine iodide) detection Kit. After being treated with different concentrations of compound 1 (0, 4, 8 and 12 µM) for 24h, the cells were incubated with JC-1 for 20 min. The cells were washed with cold buffer and observed with fluorescence microscope at 460–480 nm and 510–550 nm to detect green and red fluorescence respectively. Red emission of the dye represented a potential-dependent aggregation in the mitochondria, reflecting ΔΨm. Green fluorescence represented the monomeric form of JC-1, appearing in the cytosol after mitochondrial membrane depolarization. With the same exposure time, the proportion of red and green fluorescence intensity could express the mitochondrial membrane potential of HSC-3 cells.

In Vivo Efficacy Evaluation 5×106 SCC-7 cells were injected subcutaneously at right flank regions of female Balb/c mice (purchased from the animal center of Chengdu dashuo Bio-Technology) between 4 and 6 weeks of age. All experiments conformed to the animal care and use guidelines of the Institute’s Animal Care and Use Committee. When the volume of tumors were around 50mm3 about one week after implant, the animals were randomized into three groups: compound 1 high-dose group (0.4 mg/20 g, n=5), compound 1 low-dose group (0.2 mg/20 g, n=5) and control group (n=5). All mice received intratumoral injection every three days. Each mouse of compound 1-treated group received of compound 1 dissolved in absolute ethyl alcohol and then diluted by normal saline, while mice of control group were given the same volume of solvent. Body weight of the mice was measured every three days. The mice were sacrificed after 14d treatment.
autopsies were performed. Tumor weight and tumor volume were recorded at the conclusion of treatment, and tumor volume was calculated using the modified ellipsoid formula: $0.52 \times A \times B^2$, where A is the longer axis and B is the axis perpendicular to A.\textsuperscript{30} Each tissue was fixed in formalin and processed for paraffin embedding according to standard histological procedures. We prepared 4-μm-thick sections and stained them with hematoxylin and eosin (H&E) and observed by two pathologists in a blinded manner under a microscope.

DNA Nick End Labeling by Terminal Deoxy-Nucleotidyl Transferase-Mediated Deoxyuridine Triphosphated Nick-End Labeling (TUNEL) Formalin-fixed paraffin-embedded tissues were made into 4-μm sections. DeadEnd\textsuperscript{TM} Fluorometric TUNEL System (Promega) were used to detect the apoptotic cell nuclei in situ labeling with a TdT-mediated incorporation of fluorescein-dUTP into the 3' ends of DNA fragments. The positive cells were measured and photographed under a fluorescent microscope. Three equal-sized fields (at 400× magnification) were chosen randomly and the mean number of positive cells green fluorescence-positive cells was counted.

Statistical Analysis Differences were evaluated by Student’s t-test or one-way ANOVA followed by LSD multiple comparison tests. A $p$ value of $<0.01$ or $<0.05$ was regarded as a significant difference.

RESULTS

The Effects of Compound 1 on Cell Viability Compound 1 for the cell viability test, kindly provided by Laboratory of Ethnopharmacology, Regenerative Medicine Research Center of West China Hospital, is a $O$-glycoside with the diosgenin as aglyconemoeity and the lactose as the disaccharide residue and its chemical structure is shown as in Fig. 1. To assess the growth inhibition effect of compound 1, HSC-3 line was cultured with compound 1 at concentrations in the range of 4–24 μM for 12, 24, 36 and 48h. Cal-27 and Cal-33 cell lines were treated with compound 1 at concentrations in the

Fig. 1. Chemical Structure of Compound 1

Fig. 2. Effects of Compound 1 on the Cell Viability of OSCC Cell Lines

A: Treating HSC-3 cells with varying concentrations of compound 1 (2, 4, 6, 8, 10, 12, 16, 20, 24 μM) for 12, 24, 48h, respectively and treating Cal-27, Cal-33, SCC-7, NOK-SI cell lines with varying concentrations of compound 1 for 48h, resulted in a significant dose-dependent reduction in cell viability. The asterisk * ($p<0.05$) or ** ($p<0.01$) indicated a significant difference between the test group and control group. B: HSC-3 cells were treated with compound 1 for 0, 12, 24, 36 and 48h resulted in a time-dependent reduction in cell viability. Each point represents the mean of the data from three parallel experiments.
Cell viabilities were analyzed by MTT assay and IC_{50} values were determined. The IC_{50} value of HSC-3 cell line is around 9.4 \mu M for 24 h treatment, and 8.1 \mu M for 48 h treatment (Fig. 2). The results showed the effect of inhibition of compound 1 trend to be in a dose-dependent and time-dependent manner. The results of the other two cell lines were similar to that of HSC-3 cell lines (IC_{50} for 48 h treatment are around 6.8 \mu M for Cal-27, 7.0 \mu M for Cal-33).

The IC_{50} value of SCC-7 and NOK-SI cell lines were 16.8 \mu M and 9.7 \mu M, respectively for 48 h treatment. The efficiency of compound 1 was comparable with positive control drug 5-FU whose IC_{50} values are around 7.9 \mu M and 4.1 \mu M for 24 h and 48 h, respectively. Considering it is the first saponin derivative against OSCC cell, we would like to further investigate its anticancer mechanism. HSC-3 was chosen as the standard substrate for further research.

Fig. 3. Compound 1 Induced Apoptosis in Vitro

A: Morphological changes of HSC-3 cells after treatment with varying concentrations of compound 1 for 24 h. Phase contrast microscope (magnification \times 200). B: Fluorescence microscope view after DAPI staining (magnification \times 200); a and a', the control group; b and b', treated with 4 \mu M of compound 1; c and c', treated with 8 \mu M of compound 1; d and d', treated with 12 \mu M of compound 1. C: Flow cytometer analysis of apoptotic HSC-3 cells using the Annexin V-FITC apoptosis assay kit after treatment with varying concentrations of compound 1 (0, 4, 8, 12 \mu M) for 24 h. The lower right quadrant indicates the percentage of early apoptotic cells, and the upper right quadrant indicates the percentage of late apoptotic cells. Data are the representative of three parallel experiments.
Morphological Changes of HSC-3 Cells To elucidate whether the growth-inhibitory effect of compound 1 was related to the induction of apoptosis, we carried out morphological observation. The cells were observed under a fluorescence microscope. Control cells without treatment were fusiform and adherent (Fig. 3a), and stained nuclei were oval and intact with sharp and smooth edges (Fig. 3a’). Treated cells showed smaller size, deformation and shrinkage with the concentration-dependent changes (4, 8, 12 µM). Chromatin aggregation, marginalization, decreased or disappeared nucleolus, nuclear membrane lysis, apoptotic bodies and other typical features of apoptosis were also observed after treatment for 24h (Fig. 3c–d). The morphological changes were also concentration-dependent. These results indicated that the growth inhibition of HSC-3 by compound 1 may be related with apoptosis.

Apoptosis Rate of HSC-3 Cell Line As the treated cells showed typical morphological changes of apoptosis, we stained the cells with Annexin V and PI to detect the externalization of phosphatidylserine (PS) on the cell membrane by flow cytometry. As shown in Fig. 3C, after treating HSC-3

Fig. 4. Effects of Compound 1 on the Expression of Caspases, Bcl-2 and Bid after Treating HSC-3 Cells with Compound 1 (0, 4, 8, 12 µM) for 24h

Fig. 5. JC-1 Mitochondrial Membrane Potential Assay

After being treated with different concentrations of compound 1 (0, 4, 8 and 12 µM) for 24h, the HSC-3 cells was stained with JC-1 for 20min. In fluorescent microscopic images, red dot-like images reflecting JC-1 aggregation within the mitochondria were observed in cells with high ΔΨ, and in cells with low ΔΨ, the red color turned into diffuse green fluorescence, reflecting the monomeric state of JC-1.
cells with compound 1 at 0, 4, 8 and 12\(\mu M\) concentrations for 24h, the proportions of cells displaying early stage (A+ P−) apoptosis was 0%, 4.0%, 8.2%, and 10.9% while the late stage (A+ P+) apoptosis was 0%, 5.3%, 32.2% and 34.5%, respectively. Compared with the control group, the rate of total apoptosis cells were significantly increased with the rise of compound concentrations, which supported that compound 1 induced apoptosis efficiently in HSC-3 cell line.

The Regulation of Apoptosis-Related Protein
To further investigate the molecular mechanisms in the response of HSC-3 cells to compound 1 treatment, the activation of intrinsic and extrinsic caspase cascades was investigated by Western blot analysis. The cells were treated with compound 1 at concentrations of 4, 8 and 12\(\mu M\) for 24h, and the total lysates from the cells were subjected to Western blot analysis using caspase antibodies. GAPDH was used as a loading control. As the result shown in Fig. 4, the compound 1 increased the expression of cleaved caspase 8, caspase 9, caspase 3 and Bid while decreased the expression of Bcl-2. The changes in the protein expression revealed us that compound 1 may activate caspase 9 cascade to induce apoptosis.

Mitochondrial Membrane Potential of HSC-3 Cell Line Decreased by Treat with Compound 1
The indirect assessment of mitochondrial membrane potential was detected through the JC-1 fluorescent probe trace. When the mitochondrial membrane potential is high, JC-1 accumulates in the mitochondrial matrix to form a polymer that can emit red fluorescence. When the mitochondrial membrane potential is lower due to the damage, JC-1 could not gather in the mitochondrial matrix leading to the formation of only JC-1 monomers which produce green fluorescence. According to Fig. 5, red and green fluorescence ratio is lowered in compound-treated group in a concentration-dependent manner. Most cells in the view field are only green fluorescence as observed under the concentration of 12\(\mu M\). These results suggested that compound 1 causes mitochondrial damage, resulting in the decrease of mitochondrial membrane potential.

Antitumor Activity of Compound 1 in Vivo
In the mice model of oral squamous cell carcinoma xenografts, the female Balb/c mice received \(5\times10^6\) SCC-7 cells via subcutaneously into the right flank regions. As the result shows, both of the compound 1 treated groups, were significantly reduce the growth of tumors compared with control group, tumors of control group and low-dose-treated group reached mean volume of 663.52 mm\(^3\) and 12.48 mm\(^3\), respectively. More interestingly, we found tumors of the mice with high-dose-treatment group were disappeared (Figs. 6A, D). H&E coloration of pathological section showed the area of tumor cells in low-
dose-treated group was smaller compared with that of control group (Fig. 6B). Consistent with the data of tumor volume, the mean weight of tumors of mice in low-dose-treated group was 0.15±0.0.07 g, accounting for 8.88% of that of mice in control group (1.69±0.14 g) (Fig. 6C).

**Systematic Toxicity in the Compound 1 Treated Mice**

H&E histological staining of the heart, liver, spleen, lung, and kidney indicated no significant differences between compound 1-treated and the control mice (Fig. 7A). No significant differences of body weight were obtained between compound 1-treated and the control mice (Fig. 7B). In addition, no conspicuous adverse effects in gross measures were observed, such as appetite, feeding, ruffling of fur, behavior change, etc.

**Compound 1-Induced Apoptosis in Vivo**

The cytotoxic effects mediated by compound 1 were also analyzed to evaluate whether the cytotoxic mechanisms of the constructs included the element of apoptosis in the SCC-7 tumor model. We performed the in TUNEL assay to evaluate the influence of compound 1 low-dose-treated groups on apoptosis in tumor cells. We observed an apparently elevated apoptosis rate of mice in low-dose-treated group (Figs. 8A, B, p<0.05). Therefore, compound 1 could lead to increased apoptosis in vivo, which associated with inhibition of tumor growth.

**DISCUSSION**

Previously, diosgenyl saponins have drawn much attention due to their multiple bioactivities. In addition to natural resources, many synthetic efforts were also put into this area in order to overcome the bottleneck of scaling up and meanwhile to introduce more structural diversity, especially from the sugar constituents. Many diosgenyl saponins bearing various sugar residues have been investigated and it was revealed that the identity and the number of sugar residues of the saccharide chain have significant impacts on the compound’s bioactivities. Surprisingly, so far the reports regarding lactose acting as the sugar counterpart were very few. Compared with others, compound 1 is an interesting saponin derivative. First, because of its low price and other supreme properties, lactose is hugely used in the food and pharmaceutical industries. Second, the synthesis of compound 1 can be achieved rather straightforward, a one-pot reaction by condensation of per-O-acetyl-D-lactose with diosgenin and deprotection, unlike the cases of multistep synthesis routes of other diosgenyl saponins bearing 3 or 4 monosaccharide residues. Concerning the advantages of this compound and the urgent need of new compound to treat OSCC, we carried out the current research.

The cytotoxicities of compound 1 were detected by MTT assay and we found it indeed inhibited cell proliferation of HSC-3, Cal-27, Cal-33, SCC-7 and NOK-SI in a dose-dependent manner, with a comparable efficiency of 5-FU. As the treated cells showed typical morphological characteristics of apoptosis in phase contrast microscope and fluorescence view, we supposed one of the possible mechanisms to inhibit the cell proliferation might be attributed to apoptosis-inducing effect. The increasing apoptosis rate, which was detected by phosphatidylserine valgus and membrane integrity through flow cytometry, was positively correlated with the concentrations of compound 1. The result supported this supposition. To date, there are two recognized pathways of inducing apoptosis: death receptor pathway and mitochondria pathway, which activate different cascade reaction of caspase family. Both pathways will converge at the caspase 3. The activation of caspase 3 will lead to the cutting of board nucleus, causing...
inner nuclear plate cracking, chromosome condensation, activating caspase-dependent endonucleases, the fragmentation of nuclear DNA and eventually the death of cells. In our case, cleaved caspase 3, the crucial downstream apoptotic protein, was detected to be up-regulated by increasing the concentration of compound 1. This confirmed the compound 1 indeed go through the apoptosis pathway. Formerly, the related deltonin isolated from Dioscorea zingiberensis was proved to go through the mitochondrial apoptosis to inhibit cancer cells.\(^2^4\) To see whether compound 1 induced apoptosis of HSC-3 through the same pathway, the level of Bcl-2, the key apoptotic protein of mitochondrial pathway, was examined. It’s considered that Bcl-2 may regulate the calcium balance of endoplasmic reticulum and mitochondrial and inhibit the release of cytochrome c to cause inhibition of apoptosis. The down-regulation of Bcl-2 will lead to the decrease in mitochondrial membrane potential (ΔΨm) of the cells treated with compound 1 was obviously reduced compared to the control group. Theoretically, damage-caused mitochondrial membrane potential decreasing will lead to the releasing of cytochrome c which subsequently activate Apaf-1-mediated activation of the caspase 9.\(^3^8,3^9\) We found that caspase 9 was indeed activated: the cleaved caspase 9 increased upon the addition of compound 1 in parallel with the decreasing of the full-length caspase 9 (Fig. 5). Therefore, the apoptosis-inducing effect of compound 1 really go through this route. However, another way of apoptosis, death receptor pathway could not be excluded by these results. Activation of this pathway occurs through the complex interactions of binding of death receptors (Fas, DR4 and DR5) with their respective ligands (FasL and TRAIL).\(^4^0,4^1\) Since many protein families are involved in this network, it might be very difficult to identify which one is involved in this pathway exactly. Anyway, this route will converge to the crucial protein caspase 8. So we also carried out western bolt to detect the expression level of caspase 8 in order to see whether compound 1 also go through the death receptor pathway. The result showed that

Fig. 8. Compound 1 Induced Apoptosis in Vivo

Induction of apoptosis was indicated by TUNEL assay. The TUNEL-positive cells display dark green nuclei and are observed under a fluorescence microgroup (×400 magnification). TUNEL-positive nuclei were significantly increased in compound 1 treated tissues compared with those in the control groups (**p<0.01).
cleaved caspase 8 was also up-regulated, and confirmed the involvement of compound 1 in this pathway as well. It is well known that when caspase 8 is activated, a downstream protein Bid, the key factor mediating cytochrome c release from mitochondria in response to activation of cell surface death receptors, will be activated subsequently, which is consistent with our results.42,43 (Fig. 4).

Taken all the above results into consideration, we found compound 1 could efficiently inhibit the proliferation of OSCC cells and compound 1 induced the apoptosis of HSC-3 cells by both extrinsic pathway and intrinsic pathways through activating some key proteins from both routes such as: caspase 8, caspase 9, caspase 3, Bcl-2, and cleaved Bid with HSC-3 cells. Furthermore, in our mice model of OSCC cell line, compound 1 showed a potent efficiency in inhibiting solid tumor growth, which exhibited salient antitumor activity. Since NOK-S1 cell line was used as control in vitro and found it is sensitive to compound 1. Therefore we gave the mice intratumoral injection instead of systemic treatment. Considering the good tumor-suppressive effect and no significant evident toxic effects were found in the organs of mice in our model. We suggest that the compound 1 might be an interesting and potential cancer therapy choice in inoperable tumor. And DNA nick end labeling by TUNEL suggested compound 1 make contribution to induce tumor apoptosis in vivo as well. As toxicity to normal tissues exists in most launched anti-tumor drugs, such as renal toxicity and ototoxicity of cisplatin. This preliminary elucidation of its actions may provide a mechanistic background for the introduction and further development of this new type of diosgenyl saponin derivatives as anti-oral cancer agent against OSCC especially OSCC inoperable tumor.

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