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Diosgenin inhibited the expression of TAZ in hepatocellular carcinoma

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ABSTRACT

Emerging evidence has supported that TAZ (transcriptional co-activator with PDZ binding motif), one transcription co-activator in Hippo signaling pathway, plays an oncogenic role in liver carcinogenesis. Targeting TAZ could be a potential therapeutic approach for liver cancer patients. In the current study, we aim to determine whether diosgenin could be an inhibitor of TAZ in liver cancer cells. We found that diosgenin inhibited the expression of TAZ in liver cancer cells. Moreover, we found that diosgenin inhibited cell growth, induced apoptosis, suppressed cell migration and invasion in part via inhibition of TAZ in liver cancer cells. Our study provides the evidence to support that diosgenin could be a potential agent for treating human liver cancer.

1. Introductions

Natural plant products have been known to exhibit tumor suppressive activities in human cancer cells, which are characrized as chemopreventive or therapeutic agents [1,2]. Diosgenin, the product of hydrolysis of saponins and extracted from the tubers of wild yam (*Dioscorea villosa*) has exhibited multiple bioactivities including anti-tumorantitumor [3], anti-inflammatory [4], and anti-diabetes [5]. Recently, the anti-tumor properties of diosgenin have been demonstrated by some studies. For example, diosgenin was found to inhibit cell growth and induce apoptosis and cell cycle arrest in chronic myeloid leukemia cells [6], squamous cell carcinoma [3], gastric cancer cells [7], oral squamous cancer [8], prostate cancer cell line [9], and breast cancer cells [10]. Moreover, diosgenin was reported to retard cell migration and invasion in a variety of human cancers including gastric cancer cells [7], breast cancer cells [10], and oral squamous cancer [8]. The function of diosgenin in liver cancer cells remains unclear.

Diosgenin has been validated to regulate the multiple genes and several signaling pathways in human cancers. For example, diosgenin was found to induce reactive oxygen species (ROS) generation, activation of caspase-3, and inhibition of Bcl-2 and Bcl-xL proteins in chronic myeloid leukemia cells [6]. Diosgenin suppressed expression of matrix metalloproteinases (MMPs) and blocked ERK, JNK and PI3K/Akt signaling and NF-kappaB activity in prostate cancer cells [11]. Diosgenin governed the expression of E-cadherin, integrin-alpha 5 and integrin beta 6 in gastric cancer cells [7]. Diosgenin inhibited VEGFR2 and AKT/MAPK signaling pathways in colon cancer cells [12]. In addition, diosgenin suppressed PI3K/Akt/mTOR signaling pathway in prostate cancer cells [9]. The Hippo signaling pathway plays an important role in liver tumorigenesis. It is known that Mst1/2 (mammalian Ste20-like kinases) bind to their cofactor Sav1 (Salvador) and subsequent phosphorylate and activate the Last1/2 (large tumor suppressor), which in turn phohphorylate and inhibit the transcription co-activators, YAP (Yes-associated protein) and TAZ (transcriptional co-activator with PDZ binding motif) [13]. Therefore, YAP and TAZ are key oncogenic driver in liver carcinogenesis, and targeting YAP/TAZ might have clinical utility in cancer therapy [14]. In supporting of this notion, TAZ was observed to be highly expressed in hepatocellular carcinoma tissues [15]. Similarly, TAZ overexpression in liver cancer tissues was associated with a lower overall survival rate of cancer patients [16]. Another study further revealed that TAZ expression was related to a poor prognosis in liver cancer cells [17]. Moreover, TAZ enhanced cell proliferation and induced EMT (epithelial-mesenchymal transition) in liver cancer cells [16]. Overexpression of TAZ promoted cell growth, migration and invasion and inhibited

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apoptosis, while down-regulation of TAZ suppressed cell proliferation, motility in vitro and tumor formation in vivo in liver cancer [17]. Without a doubt, inhibition of TAZ could be helpful to treat patients with liver cancer.

In the current study, we aim to determine the effects of diosgenin on cell growth, cell apoptosis, cell cycle, migration and invasion in liver cancer cells. We also dissect whether diosgenin could inhibit the expression of TAZ in liver cancer cells. Moreover, we will define whether diosgenin exhibits its anti-cancer activity via down-regulation of TAZ. Our study will provide the evidence to support that diosgenin could be a potential agent for treating human liver cancer.

2. Methods and materials

2.1. Cell culture

Human liver cancer cell line HepG2 was bought from Chinese Academy of Science (Shanghai, China) and cultured in DMEM (Dulbecco's modified Eagle's medium, Gibco, Grand Island, NY, USA). The culture DMEM was added with 10% fetal bovine serum and 100 U/ml penicillin and streptomycin (HyClone). The cells were incubated in a humidified atmosphere with 5% CO_2 at 37 °C.

2.2. Reagents

Diosgenin, MTT (3-(4, 5-dimethylthiazol-2- yl)-2, 5-diphenyltetrazolium bromide), anti-tubulin antibody were purchased from Sigma–Aldrich (St. Louis, MO, USA). Fluorescein (FITC)-Annexin V Apoptosis Detection kit, was purchased from Beyotime Biotechnology (Shanghai, China). Calcein-AM, and second antibodies were obtained from Thermo Fisher Scientific (Waltham, MA, USA). Primary antibodies for TAZ, and β-catenin were bought from Cell Signaling Technology (Danvers, MA, USA).

2.3. Cell growth assay

HepG2 cells (5×10^3 cells/well) were cultured in 96-well plates and treated with different concentrations (0, 25, 50, 75, 100 μM) of diosgenin for 48 h and 72 h, respectively. Cell growth was measured by MTT assay as described before [18].

2.4. Cell apoptosis analysis

HepG2 cells (1×10^5 cells/well) were incubated to 6-well plates and were exposed to 0, 50 and 75 μM of diosgenin for 48 h. The annexin V-FITC/PI method for apoptosis measurement was used as described previously [18]. 2.5 *Cell cycle arrest assay.* HepG2 cells (1×10^5 cells/well) were cultured in 6-well plates overnight. Then, cells were treated with 50, 100 μM diosgenin for 48 h. Flow cytometer method was conducted to measure the cell cycle stage as described before [18].

2.5. Wound healing assay

HepG2 cells were cultured in 6-well plates until cells were grown to more than 90% confluence. Then, cell wound with rectangular lesions was created on the cell monolayer via sterile tips. After cells were washed by PBS, diosgenin was added to culture medium for 20 h. The wound area was photographed by an inverted microscope.

2.6. Transwell

Chamber invasion assay. The HepG2 cells were added to 24-well Transwell chambers (8 μm pore size, Corning, NY, USA) with Matrigel coating. Diosgenin and FBS free DMEM were inoculated into the upper chamber of the insert. Diosgenin and DMEM with FBS were inoculated into lower chamber. After 20 h, the cell invasion assay was performed as described before [18].

2.7. Western blotting

HepG2 cells were harvested and lysed in cell lysis buffer. Proteins were subject to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), transferred electrophoretically onto a polyvinylidene fluoride (PVDF) membrane. Then, the Western blotting analysis was conducted as described before [18].

2.8. Transfection

Cells were incubated in 6-well plates, and transfected with TAZ cDNA or TAZ siRNA or empty vector using lipofectamine 2000 as described before [18].

2.9. Statistical analysis

The data were expressed as the mean ± SE. The differences between groups were assessed by ANOVA. $p < 0.05$ was considered to be significant.

3. Results

3.1. Diosgenin suppressed the cell growth

To determine whether diosgenin could inhibit cell growth in liver cancer cells, HepG2 cells were treated with different concentrations of diosgenin for 48 h and 72 h. Our MTT results

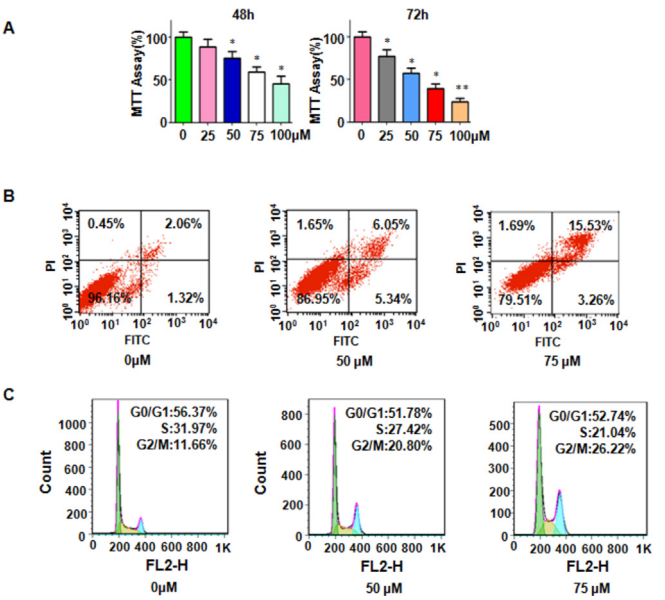

Fig. 1. Diosgenin inhibited cell growth and induced apoptosis and cell cycle arrest.
A. HepG2 cells were seeded in 96-well plates and treated with different concerntrations of diosgenin for 48 h and 72 h. MTT assay was carried out to detect the cell viabilities after diosgenin treatment. *P < 0.05, **P < 0.01 compared to the control groups treated with DMSO.
B. HepG2 cells were cultured in 6-well plates and were treated with 0, 50 and 75 μM of diosgenin for 48 h. Annexin V-FITC/PI staining and flow cytometry assay were performed to detect the apoptotic cell death.
C. Diosgenin-induced cell cycle arrest was detected by PI staining and flow cytometry.

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