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Effect and Mechanism of Sophoridine to suppress Hepatocellular carcinoma in vitro and vivo

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ABSTRACT

Aim: The aim of this study is to explain effect and mechanism of Sophoridine to suppress Hepatocellular carcinoma in vitro and vivo.
Methods: In vitro experiment, the HepG2 cells were divided into 5 groups: 0 μg/mL Sophoridine treated group (0 μg/mL group); 10 μg/mL matrine treated group (10 μg/mL group); 20 μg/mL matrine treated group (20 μg/mL group) and 10 μg/mL Paclitaxel treated group (Positive drug group). Measuring the cell proliferation of difference groups by MTS assay; evaluating cell apoptosis of difference by flow cytometry; the cell invasion and migration abilities of difference HepG2 cells were measured by transwell and wound healing testing; measuring the relative proteins expression in difference groups. In vovo experiment, the nude mice were divided into 5 groups: 0 μg/mL, 5 μg/mL, 10 μg/mL, 20 μg/mL and Positive drug groups, after executing, taking the tumor tissue from nude mice of difference groups, measuring the tumor volume and weight; evaluating the PTEN protein expression in tumor tissue by Immunohistochemistry (IHC).
Results: In the cell experiments, Compared with 0 μg/mL group, cell proliferation rates were significantly reduced, cell aopotosis were significantly increased and invasion and wound healing abilities were significantly decreased in marine treated groups with dose-dependent ($P < 0.05$, respectively). In the nude mice experiment, the tumor volume and weight of matrine treated groups were significantly decreased compared with 0 μg/mL group with dose-dependent ($P < 0.05$, respectively). And the PTEN protein expression of Sophoridine treated groups were significantly decreased compared with 0 μg/mL group with dose-dependent ($P < 0.05$, respectively).
Conclusion: Sophoridine had anti-cance effects to suppress HepG2 activities by regulation PTEN/PI3K/AKT, Caspase-3/-9 and MMP-2/-9 signaling pathway.

1. Introduction

Cancer is a serious threat to human health, currently, cancer mortality is second only to cardiovascular disease, and is the second leading cause of mortality; Hepatocellular carcinoma is a common digestive disease with high mortality in cancer [1]. At present, Surgical treatment is the first choice for the treatment of liver cancer, but the prominent problem is the high rate of postoperative recurrence and low resection rate. Natural medicine is an important source of human prevention and treatment of diseases, the use of active ingredients to develop new drugs is a shortcut to study anti-tumor drugs. Sophoridine is monomer alkaloids which extracts from *Sophora alopecuroides L*, is considered to be a potential antitumor natural product with high application value [2–5]. In our study, We studied the effecs and mechanism of Sophoridine to suppress tumor activities in vitro and vovo.

2. Materials and methods

2.1. Materials

The Human hepatocellular carcinoma cell line HepG2 cells were purchased from ATCC. The cells were cultured in DMEM culture medium which contained 10% fetal bovine serum in 37 °C and contained 5% CO_2 incubator. The cells were digested by EDTA trypsin, and were passage cultured in cell culture bottle. The nude mice were proved from Nanjing Medical University Animal Center.

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Sophoridine (Sigma, USA), MTT kit, Transwell kit, cell cycle and apoptosis assay kit (BD Biosciences, USA), ECL immunoblotting substrate kit (Millipore, USA), Relative anti-body (PTEN, PI3K, AKT, Caspase-3, Caspase-9, MMP-2 and MMP-9) were purchased from Abcam (USA), Flow cytometry (BD, USA), Microplate Reader (Bio-Rad, USA).

2.2. Cell culture and grouping

Hep G2 cells were cultured in petri dish, and stayed in 37 °C, 5% CO_2 and saturated humidity incubator, culture medium was 90% DMEM contained 10% fetal bovine serum (FBS). 0.25% Pancreatin-EDTA digestion passage, Logarithmic growth phase cells were used in all experiments. The Hep G2 cells were divided into 5 groups, 0 μg/mL group: The cells were treated with nothing; 5 μg/mL group: The cells were treated with 5 μg/mL concentration Sophoridine, 10 μg/mL group: The cells were treated with 10 μg/mL concentration Sophoridine, 20 μg/mL group: The cells were treated with 5 μg/mL concentration Sophoridine. Postive drug group: The cells were treated with Paclitaxel (10 μg/mL).

2.3. Evaluating cell proliferation in difference groups

Taking 100 μL logarithmic growth phase cells into 96 wells plate as 3×10^4 cells/ml. Depending on the grouping, the cells were treated by difference methods, After that, treated for 72 h, Sucking out the medium, adding MTS reagent according to kit instructions, Finally, measuring the absorbance by enzyme labeling instrument at 490 nm, and the cell proliferation rate was calculated.

2.4. Cell apoptosis rate by flow cytometry

Adding 100 μL cells to 6 holes plate by 5×10^6 cells/mL concentration, and incubated overnight at 37 °C, after that, the cells were treated with grouping, and incubated for 24 h at 37 °C, digesting and collecting cells, PI/Annexin V-FITC colorration, detecting cell apoptosis by flow cytometry.

2.5. The cell invasion ability by transwell assay

Taking logarithmic growth phase cells, adding the 200 μL cells to transwell chamber room contained 50 μL Matrigel gel, adding the 500 μL DMEM contained 10% FBS in lower chamber. The cells were treated depending on grouping. Culturing the cells for 24 h, The cells were counted under the microscope after paraformaldehyde fixation and crystal violet staining.

2.6. The cell migration ability by wound healing assay

The 1×10^6 Hep G2 cells were inculated in the 6 holes plate, The cells were cultured in vitro for 90% fusion state, and the cells were starved for the night with the DMEM of 1% calf serum, and the medium was removed and rinsed with PBS for 3 times, using 100 μL Tip head to make 1-shaped sterilization scratches on the culture plate at the bottom, washing by serum-free rinse for 3 times, the cells were added with different concentrations of NaHS fresh DMEM containing 1% calf serum, 37 °C, 5% CO_2 constant temperature incubator, migration of cells under the microscope observation of wound area, respectively 0 and 16 h after scratching by Image J camera and calculate the distance of migration the experiment was repeated 3 times.

2.7. Western blotting assay

Collecting the cells of difference groups and extracting protein from cells. The protein content was measured by BCA methods, took the equal protein to separate by SDS-PAGE methods and trasfer to PCDF film. Using the corresponding antibody (PTEN, 1:800; PI3K, 1:800; AKT, 1:800; Caspase-3, 1:800; Caspase-9,1:800; MMP-2,1:800; MMP-9, 1:800; GAPDH, 1:800) to incubate

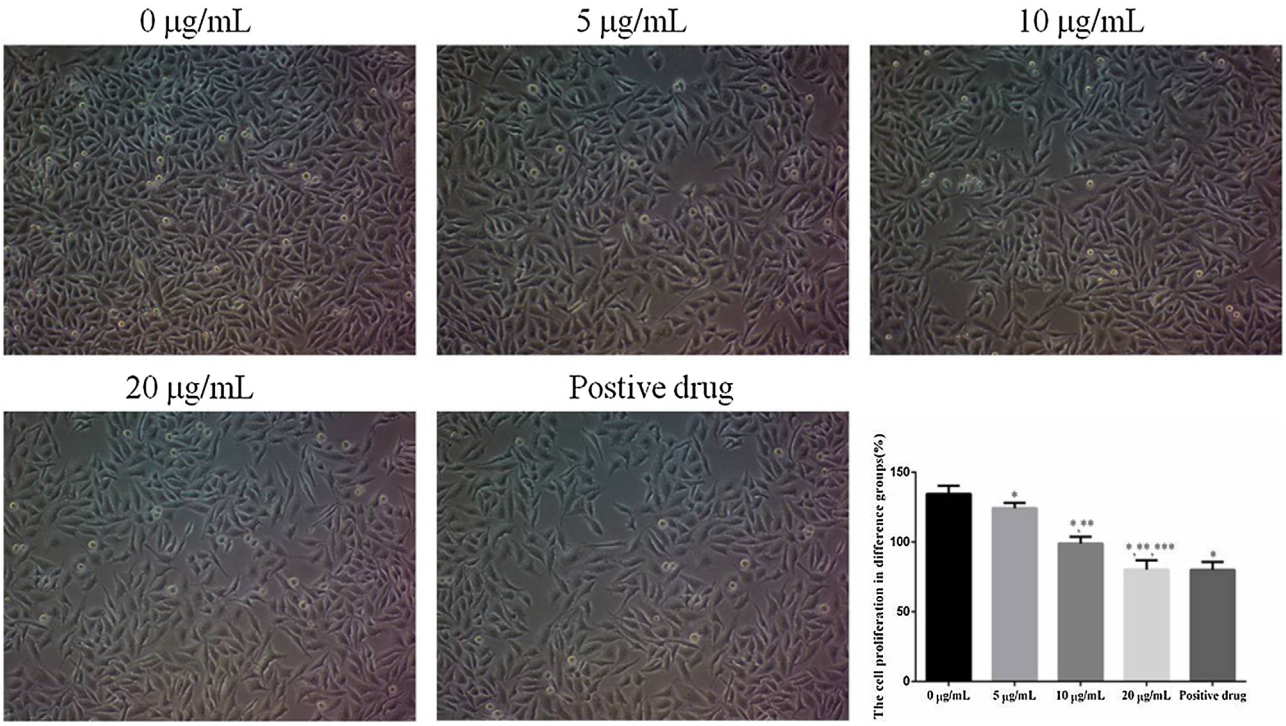

Fig. 1. The cell proliferation rate of difference groups *, $P < 0.05$; *: $P < 0.01$; ***: $P < 0.001$;.