Investigating the effects of Taiwania cryptomerioides Hayata Extracts against Arecoline and 4-NQO-induced Oral Cancer Cells

Pei- Jie Liu [1,#], Zhong-Ren Jiang[1] , Chih-Yang Huang[2,*]

1Department of Medical Laboratory Science and Biotechnology ,China Medical University ,Taichung

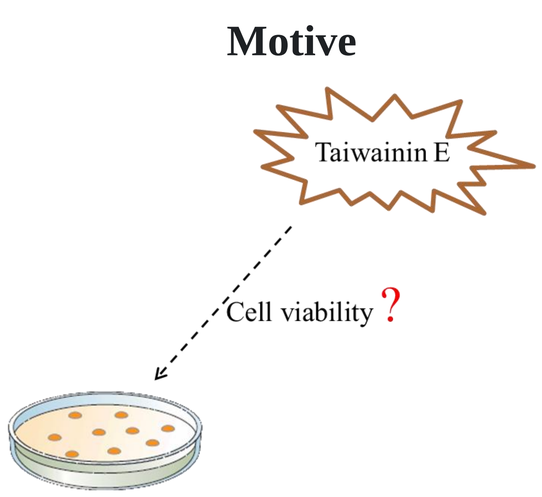
2Graduate Institute of Chinese Medical Science, China Medical University, Taichung

cyhuang@mail.cmu.edu.tw

Abstract

Oral cancer is the major life-threatening oral diseases. Chewing Areca nut (AN) is a popular oral habit in Taiwan and Asia, arecoline is a potent carcinogen in Areca nut. Chronic exposure to Arecoline carcinogens in the upper aerodigestive tract causes genetic changes in the epithelial cells of the oral mucosa. The OSCC model in C57BL/6J Narl mice is generated by 0.5mg/mL arecoline plus 0.2mg/mL 4NQO carcinogen in drinking water for 8 and 28 weeks to mimic the etiology of oral cancer patient in Asia. Mice were sacrificed and cell were cultured as T8 and T28 cancer cells. The treatment of nature herbal product from Taiwania cryptomerioides Hayata, Taiwanin E significantly inhibited the cell viability of T28 cells in dose dependent manners, but no effect on N28 normal cells. Taiwanin E actived P21 and P27 cell cycle regulatory protein and reduced the Cyclin A, Cyclin D1 and Cyclin E, also inhibit cell proliferation in T28 cell. Besides, Taiwanin E can even enhance T28 oral cancer cells apoptosis in dose dependent manners. Taiwanin E decreased anti-apoptotic protein Bcl-X_L, also increased pro-apoptotic protein Bax in T28 oral cancer cells.

Motive



N28,T8 and T28

Materials and Methods

Cell culture

The OSCC model in C57BL/6J Narl mice is generated by 0.5mg/mL arecoline plus 0.2mg/mL 4NQO carcinogen in drinking water for 8 and 28 weeks to mimic the etiology of oral cancer patient in Asia. Mice were sacrificed and cell were cultured as T8 and T28 cancer cells .T8 and T28 Were grown in Dulbecco's modified Eagle's medium (DMEM).supplemented with 10%FBS.Cells were seeded in cell culture flasks and maintained in a humidified incubator at 37 ◦C with 5% CO_2 .

MTT assay

[3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltertrazolium-bromide]

Adherent cells in 24 well plates. Make a solution of 5 mg/ml MTT dissolved in PBS and filter sterile. Add 500 ul of MTT solution from step one to each well containing cells. Incubate the plate in a CO_2 incubator at 37 ◦C for 2 hours. Remove media with pipette. Add 500 ul of isopropanol to each well and pipette up and down to dissolve crystals. Transfer to plate reader and measure absorbance at 570nm.

Western blotting

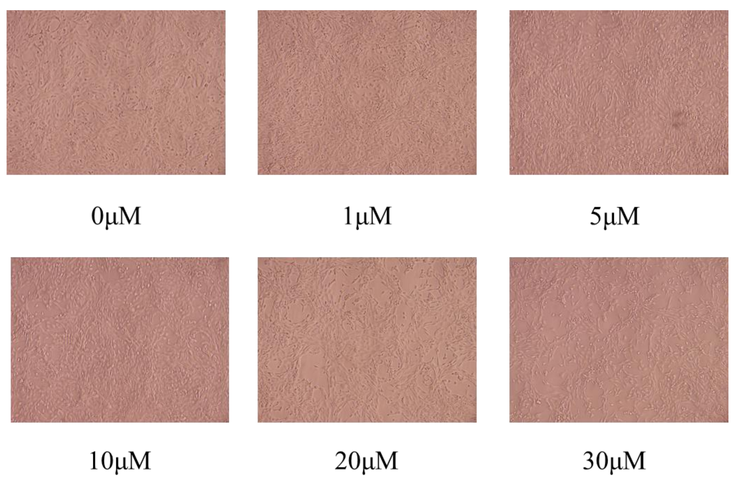
The proteins of cell lysates were analyzed by 8%~12% SDS-PAGE, and proteins were transferred to nitrocellulose(or PVDF) by electrophoresis. Residual protein sites were blocked in Tween/Tris-buffer saline (TBS) containing 5% skin milk. The filters were incubated with primary antibodies in TBS plus 2.5% skin milk at recommended concentrations for 2h or at 4 ◦C overnight and incubated with secondary antibodies for 1h at room temperature. Antibody reaction was visualized with enhanced chemiluminescence (ECL) reagent for Western blotting.

Statistical analyses

Result were expressed as mean+SEM,and data were analyzed one-way ANOVA followed by Student's t test for significant difference. P-value<0.05 was considered statistically significant.

Results

A.



B.

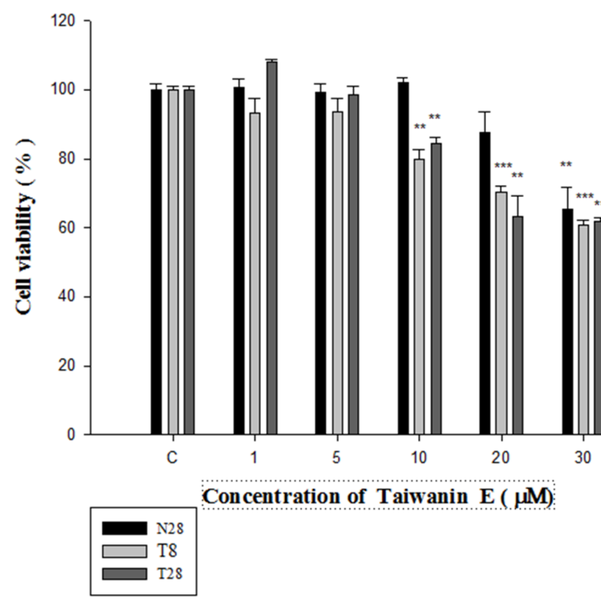


Fig.1 The anti-proliferative effect of Taiwanin E on oral cancer cells

.

(A) Representative photos to show the T28 oral cancer cell morphology after 24hr treated with Taiwanin E at 0,1,5,10,20 and 60μM individually

(B) Oral cancer cells was treated with different concentrations of Taiwanin E at 0,1,5,10,20 and 60μM individually

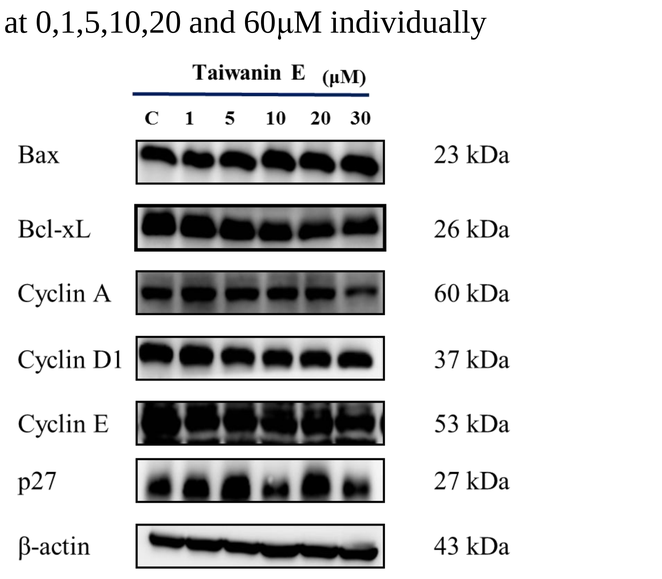


Fig.2 Effets of Taiwanin E on cell cycle arrest-associated protein and apoptosis-associated in oral cancer cells

.

T28 cells were treated with was treated with different concentrations of Taiwanin E at 0,1,5,10,20 and 60μM individually.

.

Summary