Molecular Mechanisms of Taiwanin C to Inhibit Proliferation and Enhance Apoptosis of Arecoline and 4-NQO-induced Oral Cancer Cells

2012 The 27th Joint Annual Conference of Biomedical Sciences
第27屆 生物醫學聯合學術年會
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Shu-Huai Wang[1], Wei-Wen Kuo[2] , Da-Tian Bau [3]and Chih-Yang Huang[1, 4]

[1]Graduate Institute of Basic Medical Science, China Medical University, Taichung
[2]Department of Biological Science and Technology, China Medical University, Taichung
[3]Graduate Institute of Clinical Medical Science, China Medical University, Taichung
[4]Graduate Institute of Chinese Medical Science, China Medical University, Taichung

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. Arecoline may induce proliferative activity, through activate of the EGF receptor and promote downstream protein COX2 over expression. 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 C significantly inhibited the cell viability of T28 cells in dose dependent manners, but no effect on N28 normal cells. Taiwanin C active P27 cell cycle regulatory protein and reduce the cyclin A and cyclin E in T28 cells. Besides, Taiwanin C can even enhance T28 oral cancer cells apoptosis in dose dependent manners. Moreover, we observed that Taiwanin C inhibit p-Tyr^{1068}EGFR and reduceβ-catenin nuclear translocation via induced active-form GSK-3-β and suppress p-ser^{9} GSK-3-β protein level, then down-regulated its downstream cyclin D1, Tbx3 and c-Myc proteins in T28 cells. Taken together, Taiwanin C not only activated GSK-3-β, suppressed β-catenin nuclear translocation to inhibit OSCC metastasis, but also inhibited p-Tyr^{1068}EGFR , p-AKT to suppress cell cycle and induce cell apoptosis.

Results

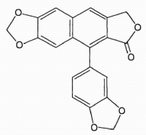


Figure 1. Molecular structure of Taiwanin C (Molecular weight : 348) .

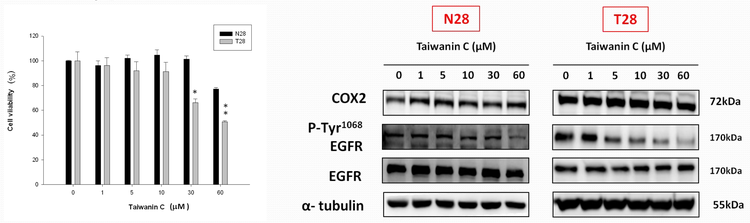


Figure 2. Taiwanin C inhibits oral cancer cells proliferation. Normal oral cells (N28) and oral cancer cells (T28) were treated with different concentrations of Taiwanin C at 0, 1, 5, 10, 30 and 60uM individually for 24 hr. Cell viability as measured using MTT assay. Columns, Repeated by three separated experiments. *p < 0.05 and **p < 0.01 represent significant differences when compared with the control group.

Figure 3. Modulation EGFR signal pathway proteins following Taiwanin C treatment in normal oral cells (N28) and oral cancer cells (T28). Normal oral cells (N28) and oral cancer cells (T28) were treated with Taiwanin C for 24 hr. Cell lysates were analyzed for the expression of EGFR, P-Tyr^{1068} EGFR and COX2 by Western blot using specific antibodies. The blots were reprobed with α-tubulin antibody for comparison of protein loading.

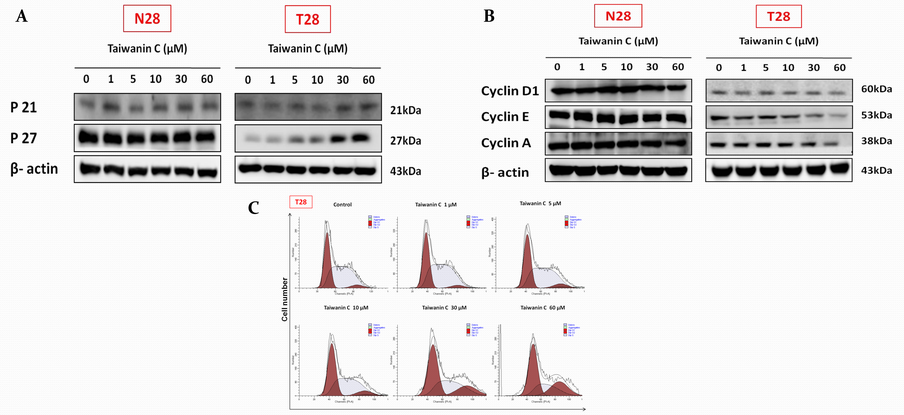


Figure 3. Cell cycle progression inhibition by Taiwanin C in normal oral cells (N28) and oral cancer cells (T28). (A) And (B) Normal oral cells (N28) and oral cancer cells (T28) were treated with Taiwanin C for 24 hr. Cell lysates were analyzed for the cell cycle controlling proteins expression of P21 and P27, Cyclin A, D1 and E by Western blot using specific antibodies. The blots were reprobed with β-actin antibody for comparison of protein load.(C) Oral cancer cells (T28) were treated with Taiwanin C for 24 hr, and stained with propidium iodide. DNA content was analyzed by flow cytometry. Results were represented as percent of cell population in G1, S, and G2-M phases of the cell cycle.

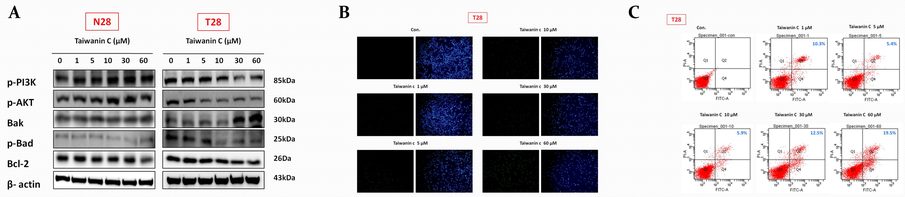
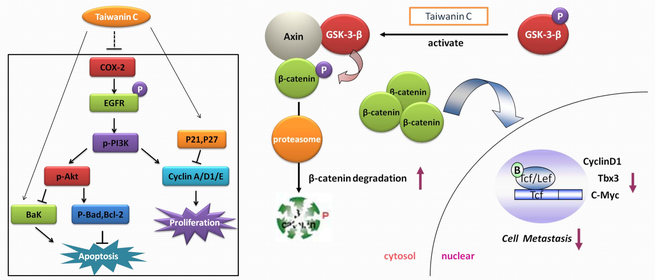


Figure 4. Taiwanin C induced apoptosis in normal oral cells (N28) and oral cancer cells (T28). (A) Normal oral cells (N28) and oral cancer cells (T28) were treated with Taiwanin C for 24 hr. Cell lysates were analyzed for the survival proteins expression of p-PI3K and p-AKT, anti-apoptotic protein expression of BCL2, pro-apoptotic protein expression of BAX and Bcl-2 family protein expression of p-BAD by Western blot using specific antibodies. The blots were reprobed with β-actin antibody for comparison of protein load. (B) Oral cancer cells (T28) were treated with Taiwanin C for 24 hr . DAPI staining (blue) spots in the right panel represent cell nuclei. TUNEL staining (green) spots in the left panel represent apoptotic bodies. (C) Oral cancer cells (T28) were treated with Taiwanin C for 24 hr. The cells were harvested and staining with annexin V-FITC and PI, then analyzed by flow cytometry.

Summary



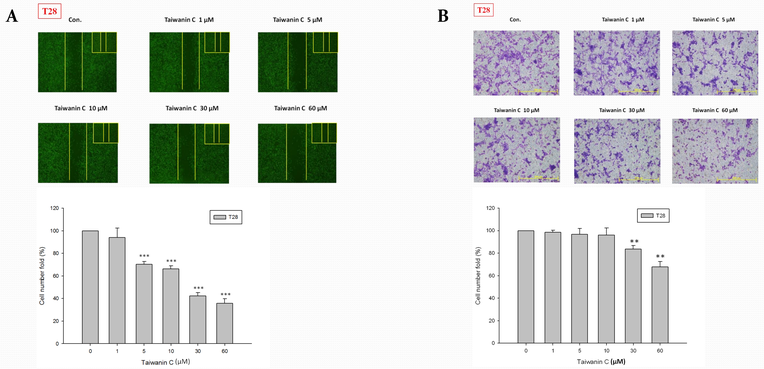


Figure 5. Taiwanin C inhibits oral cancer cells (T28) metastasis in a dose-dependent manner. After serum free 4 h, oral cancer cells (T28) were added by Taiwanin C for 24 hr. (A) The cell migration was assessed by wound healing assay. Overall mean value of invasion cells from triplicate experiments is shown in the bar graph compared with control group. *** indicates the statistically significant difference ($p < 0.001$).(B) The cell migration was assessed by Boyden chambers. Overall mean value of invasion cells from triplicate experiments is shown in the bar graph compared with control group. ** indicates the statistically significant difference ($p < 0.01$).

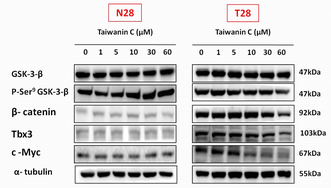


Figure 6. Effect of Taiwanin C on Wnt signaling pathway in normal oral cells (N28) and oral cancer cells (T28). Normal oral cells (N28) and oral cancer cells (T28) were treated with Taiwanin C for 24 hr. Cell lysates were analyzed for the expression of GSK-3-β, P-Ser^{9} GSK-3-β and β-catenin by Western blot. The Wnt β-catenin downstream proteins Tbx3 and c-Myc protein level were analyzed by Western blot. The blots were reprobed with α-tubulin antibody for comparison of protein loading.

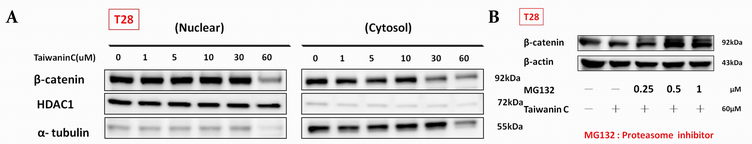


Figure 7. Taiwanin C inhibits the nuclear accumulation effects of β-catenin, and enhances the β-catenin degradation through proteasome-mediated pathway. (A) Normal oral cells (N28) and oral cancer cells (T28) were treated with Taiwanin C for 24 hr. Cytoplasmic and nuclear fractions of oral cancer cells (T28) were subjected to SDS-PAGE and then immunoblotted with anti β-catenin, α-tubulin, and HDAC-1. α-tubulin was used as a cytoplasmic protein loading control, and HDAC-1 was used for nuclear protein loading control. (B) After pre-treat proteasome inhibitor 0, 0.25, 0.5 and 1 μM for 1hr, and added by Taiwanin C 60 μm for 24 hr. β-catennin protein level was analyzed by Western blot.