Taiwanin C inhibits proliferation and enhances apoptosis of arecoline and 4-NQO-induced oral cancer cells via the suppression of EGFR/PI3K pathway

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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 T28 cancer cells. The treatment of natural herbal product from Taiwania cryptomerioides Hayata, Taiwanin C significantly inhibited the cell viability of T28 cells in a dose dependent manner, but no cytotoxicity effect on N28 normal cells. Taiwanin C activated P21 and P27 proteins and reduced the Cyclin A, Cyclin B1, Cyclin D1 and Cyclin E cell cycle regulatory proteins, which resulted in G2/M cell cycle arrest in T28 cells. Besides, by TUNEL assay and Flow-FITC measurement, Taiwanin C strongly enhanced T28 oral cancer cells apoptosis in a dose dependent manner. Taiwanin C also decreased anti-apoptotic protein Bcl-2 and p-Bad, increased pro-apoptotic protein Bax, and down-regulated p-PI3K, p-AKT survival protein levels in T28 oral cancer cells. Moreover, we observed that Taiwanin C inhibited p-Tyr1068EGFR and COX-2 protein expressions in T28 cells. Taken together, Taiwanin C down-regulated the p-Tyr1068EGFR/p-AKT signaling pathway to inhibit COX-2 expression and activated P21/P27 to induce G2/M cell cycle arrest, and also up regulated the expression of pro-apoptotic Bcl-2 family members to induce cell apoptosis, which resulted in the proliferative suppression and apoptosis promotion of T28 primary oral squamous cancer cells. We believe the Taiwanin C can apply as a potential treatment candidate for arecoline-induced oral cancer.