A109 Comparative effects of flavonoidal phytochemicals on membrane fluidity and tumor cell growth

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Flavonoids contained in medicinal plants potentially interact with plasma membranes and membraneous organelles to alter the physicochemical property of lipid bilayers. Such an interaction has been suggested to be responsible for various pharmacological activities of flavonoids. The effects of flavonoidal phytochemicals on the fluidity of artificial and cellular membranes and on the growth of tumor cells were comparatively studied to address the structure-activity relation on membrane-mediating action. Liposomes prepared with cholesterol and three phospholipids (the lipid composition corresponding to tumor cells) were treated with 25 representative flavonoids (0.1–50 μM), and then fluorescence polarization was measured with a set of probes to determine the fluidity changes in different membrane regions. Liposomal membranes were rigidified by almost all of the tested flavonoids, in which flavones were more effective than isoflavones and flavanones. In comparison of flavones (each 10 μM), the potency to rigidify membranes was galangin > quercetin > kaempferol > myricetin > morin = fisetin, chrysin > luteolin > apigenin, galangin > chrysin, quercetin > luteolin, and kaempferol > apigenin ($p < 0.01$). Myricetin, kaempferol, quercetin and galangin acted in increasing order of intensity on the hydrophobic regions of lipid bilayers to make membranes rigid at sub-μM levels. The membrane effects of structurally modified quercetin varied in intensity as being quercetin dimer > quercetin > tamarixetin > quercetin-4'-glucoside > isoquercitrin = rutin ($p < 0.01$). 3-, 5- and 7-Hydroxylation of flavones is essential to membrane rigidification, which is also influenced by hydroxyl groups in the B ring. The membrane-acting flavonoids (10–100 μM) like galangin, quercetin, quercetin dimer, kaempferol and genistein inhibited the growth of mouse myeloma cells 24–48 h after incubation as well as a reference anti-tumor drug, doxorubicin, and their inhibitory effects correlated to the rigidifying effects on liposomal membranes. They rigidified tumor cell membranes during 1–48 h incubation simultaneously with cell growth inhibition. The interaction with membrane lipids underlies the anti-tumor action of flavonoidal phytochemicals. The analysis of membrane fluidity using liposomes is useful for the first screening of medicinal plants with the potent anti-tumor activity.

A110 Efficacy validation of medicinal plant using functional genomics and metabolomics approaches

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Anoectochilus formosanus (AF) has been used widely as functional food and folk medicine in Taiwan and other Asian countries. Significant cytotoxicity on MCF7 cells, a human breast carcinoma, has been detected for the crude extract and its derived fractions of AF plant, based on MTT colorimetric and ^{3}H-thymidine incorporation assays. Based on a "bioactivity-guided fractionation principle", we have obtained an enriched E fraction that exhibits the most significant inhibiting effect on the proliferation of MCF7 cells among the test extracts. Metabolite profiling and candidate index compounds of this E fraction were well characterized using mainly HPLC and NMR spectrometric analyses. Treatment of MCF7 cells with the E fraction resulted in the release of mitochondrial cytochrome C into cytosol and the cleavage of poly(ADP-ribose)polymerase. Western blot analysis showed that the levels of proliferating cell nuclear antigen (PCNA) and p53 (a tumor suppressor protein) decreased with the increase in incubation time of MCF7 cells with E fraction. However, the expression of Bcl2 and Bax proteins were not changed after the treatment with AF extracts. Differential protein expression and activities of caspases, *e.g.* caspases 2, 7 and 8, have been observed using western-blot and enzymatic activity assays. In summary, this study provides evidences that plant extract of *A. formosanus* can induce apoptosis of human mammary carcinoma cells, via specific cellular mechanism(s). The signaling pathway in the event of programmed cell death of MCF7 cells, induced by the treatment of *A. formosanus* extract, are proposed.