多酚類化合物誘導皮膚癌細胞之凋亡機制探討

The mechanism of Flavonoids-induced apoptosis in skin cancer cells

中文摘要

Flavonoids 廣泛存在於自然界中，屬於多酚類化合物其中一類，文獻指出 flavonoids 具有抗發炎、癌症預防發生以及抑制腫瘤等功用。本研究的目的在於探討結構相類似之 flavonoids 於皮膚癌細胞所造成細胞毒性的機制爲何。三種惡性程度不同的皮膚癌細胞 A431（人類上皮細胞癌）、RPMI 7951（人類惡性黑色素細胞瘤）以及 Hs 695T（人類非黑色素細胞之黑色素瘤）處理 17 種結構相類似之 Flavonoids（Flavanone, 2’-OH Flavanone, 4’-OH Flavanone, 6’-OH Flavanone, 7’-OH Flavanone, Naringenin, Narigin, Taxifolin; Flavone, 3-OH Flavone, 5-OH Flavone, 7-OH Flavone, Baicalein, Kaempferol, Quercetin, Morin and Myricetin）後均有不同程度的毒性反應，其中以 2’-OH Flavanone 及 3-OH Flavone 對該三種癌細胞最具細胞毒性。Acridine orange 染色得知 2’-OH Flavanone 及 3-OH Flavone 會誘導癌細胞發生染色質濃染的情形。此外本實驗發現 2’-OH Flavanone 會造成 sub-G1 比例升高、caspase-3 及 PARP 的活化情形，而利用西方點墨法亦檢測出三株皮膚癌細胞的 Bcl-2 表現量會隨時間增加下降，而在 p53、p21、BclxL、Bax、Bad 及 Mcl-1 等則無明顯變化。上述的情況在 3-OH Flavone 組並無被觀察到同樣的情形。以 DCFHDA 方式偵測 2’-OH Flavanone 與 3-OH Flavone 對細胞的自由基含量是否有影響的結果顯示 2’-OH Flavanone 會促進自由基產生，而 3OH Flavone 會降低細胞內自由基的含量。在動物實驗方面，在 BALB/c-Hfh11nu mice 臀部兩側皮下注射 106/site A431 細胞，人工誘發腫瘤生長約至 50 mm3 後以塗抹方式及直接注射腫瘤的方式分別給予 25 mg/site, 及 50 mg/site 之 2’-OH Flavanone。結果顯示直接注射 2’-OH Flavanone 之 25mg/site 及 50 mg/site 明顯抑制癌細胞之生長。結果推論爲 2’-OH Flavanone 可能是經由產生自由基活化 caspase-3 及 PARP 最後造成 A431、RPMI 7951 及 Hs 695T 皮膚癌細胞之細胞凋亡。而在動物實驗證實 2’-OH Flavanone 的確能抑制皮膚癌細胞的生長。

英文摘要

Flavonoids are a class of polyphenolic compounds widely distributed in the plant kingdom, which display a variety of biological activities, including anti-inflammatory activity, chemoprevention and tumor growth inhibition. The aim of the study was to investigate the mechanism of structurally related flavonoids-induced cytotoxicity in skin cancer cells. Three different malignant cell lines, human epidermoid carcinoma (A431), human malignant melanoma (RPMI 7951) and amelanotic melanoma (Hs

695T) were treated with 17 structurally related Flavonoids including Flavanone, 2’-OH Flavanone, 4’-OH Flavanone, 6’-OH Flavanone, 7’-OH Flavanone, Naringenin, Narigin, Taxifolin; Flavone, 3-OH flavone, 5-OH Flavone, 7-OH Flavone, Baicalein, Kaempferol, Quercetin, Morin and Myricetin. 2’-OH Flavanone and 3-OH Flavone showed obvious cytotoxic effects in three cancer cell lines by MTT assay. Chromatin condensation in using acridine orange stain was observed in A431, RPMI 7951 and Hs 695T with 2’-OH Flavanone and 3-OH Flavone. Treatment with 2’ - OH Flavanone but not 3- OH Flavone causes caspase-3 activation and cleavage of poly(ADP) ribosepolymerase (PARP); with higher sub-G1 ratio by flow cytometric analysis. ROS（Reactive oxygen species）was detected in 2’ - OH Flavanone treated cells by DCHF-DA assay. Waf-1/p21 protein was induced only in 2’-OH Flavanone-treated A431 cell line. Moreover, 2’-OH Flavanone-induced apoptosis is involved in down regulation of the level of Bcl-2, but not Bcl-xL, Mcl-1, Bax and Bad. We conclude the cytotoxic effects induced by 2’-OH Flavanone and 3- OH Flavone through different mechanisms. In vivo study, BALB/c-Hfhllnu mice were injected with A431 cells 106/ site side by side. when tumor size grows to 50 mm3, mice were received 25 mg/ site and 50 mg/ site 2’ -OH Flavanone by topical treatment（each day） and local injection（twice a week） for 2 weeks. The data indicated that tumor size was obviously decreased when the mouse was injected 50 mg 2’ -OH Flavanone. In conclusion, 2’ -OH Flavanone plays as a pro-oxidant and induces apoptosis in A431, RPMI 7951 and Hs 695T skin cancer cells. In vivo study, the result indicated that 2’-OH Flavanone inhibited the growth of skin cancer cells.