十字花科蔬菜衍生物誘導人類肺腺癌細胞程式凋亡暨其機制探討

Studies on the mechanisms of cruciferous vegetable derivatives - induced apoptosis in human lung adenocarcinoma cells

中文摘要

本研究主要是以人類肺腺癌細胞 (A549 cells)爲實驗模式，探討十字花科蔬菜衍生物中的 phenylethyl isothiocyanate (PEITC)、benzyl isothiocyanate (BITC)與 indole-3-carbinol (I3C)對肺腺癌細胞生長與增殖的影響，並進一步了解 PEITC 與 BITC 誘導 A549 cell apoptosis 其可能的機制。結果顯示，PEITC、BITC (2.5~25 μM)以及 I3C (150~250 μM) 添加於細胞中 1、3、5 天後，可以明顯抑制其細胞生長($p<0.05$)，並隨著劑量與天數的增加，抑制作用亦愈顯著。MTS 細胞毒性分析結果顯示，PEITC 、BITC 與 I3C 可以明顯降低細胞的存活率，並和抑制細胞生長的結果呈現相同的劑量效應($p<0.05$)。以 Flow cytometry 分析細胞週期結果顯示，於細胞中添加 PEITC 與 BITC 24 小時後均可增加 sub G0 細胞 (apoptotic cells) (5~25 μM)與 G2/M (7.5~25 μM)細胞數目的百分比($p<0.05$)，且於 10 μM 時有最多 apoptotic cells 之發生與 G2/M 細胞之堆積；相較於 10 μM 的結果，10 μM 以上的劑量反而會減少 apoptotic cells 數目的百分比，因此以 10 μM 作爲時間效應分析的濃度。時間效應結果顯示，於添加 10 μM PEITC 與 BITC 24、48、72 小時後，均可以明顯增加細胞中 apoptotic cells 數目的百分比($p<0.05$)，並呈現時間的效應。相反地，250 μM I3C 僅可少許增加細胞中 apoptotic cells 數目的百分比且增加的是 G0/G1 細胞數目的百分比。以螢光染色法偵測細胞 apoptosis 與 necrosis 結果顯示，細胞於 10 μM PEITC 與 BITC 培養 24 小時後，確實可誘導細胞 apoptosis 的發生高劑量(25 μM)的 PEITC 與 BITC 誘導細胞 necrosis 勝於 apoptosis 的發生。Western blot 分析與 apoptosis 相關蛋白質結果則指出，PEITC 與 BITC 會增加細胞中 P53 與 P21 蛋白質之表現，但並不會影響 Bax 蛋白質之表現。綜合以上結果得知，10 μM 之十字花科蔬菜衍生物 PEITC 與 BITC 可藉由誘導 apoptosis 的發生而抑制 A549 細胞的生長，同時與增加 P53 及 P21 蛋白質表現有關，但在其過程中增加 Bax 蛋白質表現的增加與否可能不是一個必要的條件。而高濃度 25 μM 之 PEITC 與 BITC 則會誘導較多 necrosis 的發生。另一方面，十字花科蔬菜衍生物 I3C 具抑制 A549 細胞生長的作用，但 apoptosis 並非主要的機作用機制

英文摘要

Glucosinolate derivatives, phenyl ethyl isothiocyanate (PEITC), benzyl isothiocyanate (BITC) and indole-3-carbinol (I3C), are thought to be the bioactive components in cruciferous vegetables. A lot of studies have demonstrated that glucosinolate derivatives are effective inhibitors of tumorigenesis in induced animal models, and of

the growth of many lines of cancer cells. Because lung cancer is the first leading cause of cancer, the aim of the present study was to determine whether PEITC, BITC and I3C inhibit the growth of human lung carcinoma A549cell line, and to investigate the molecular mechanisms of PEITC- and BITC-inducted apoptosis in A549 cells. The results showed that PEITC (5~25μM), BITC (2.5~25μM) and I3C (50~250μM) suppressed the growth and the viability of A549 cells in a dose-dependent manner ($p < 0.05$). Flow cytometric analysis indicated that treat with PEITC (5~25μM) and BITC (7.5~25μM) could increase the percentage of sub G0 cells (apoptotic cells) and G2/M cells in the cell cycle, although higher concentrations of PEITC and BITC (25μM) apparently had less apoptotic cells than the contration of 10μM. PEITC and BITC (10μM) increased the apoptotic cells for 24, 48 and 72 hrs by time dependent manner as well ($p < 0.05$). Compared with PEITC and BITC, fewer apoptotic cells and more G0/G1 cells were induced by 250μM I3C. Fluorescent microscopy showed that apoptosis was more pronomineat at 10μM of PEITC and BITC after 24 hrs treatment, but higher concentration (25μM) of PEITC and BITC induced cell nerosis rather than apoptosis. Western Blot analysis indicated that PEITC and BITC could upregulate the P53 and P21 protein level in dose-and time-dependent manner, but did not affact the Bax protein level. In conclusion,cruciferous vegetable derivatives, PEITC and BITC suppressed A549 cell growth in part by induction of apoptosis which associated with P53 and P21,but not Bax protein expression.I3C also suppressed A549 cell growth, but induction of apoptosis is not the major pathway.