TSDH 抑制人類乳癌細胞生長之機制探討

Study of the anti-tumoral effect by TSDH in human breast adenocarcinoma cells

中文摘要

乳癌是台灣婦女的十大死因之一。根據行政院衛生署國民健康局公佈的資料顯示，乳癌在女性十大癌症死亡率的排行榜上居第四位。且每年有增加的趨勢。目前，乳癌的治療方式，主要是使用手術治療及化學治療，而化學治療藥物的作用方式，主要爲誘發乳癌細胞產生細胞週期停滯或細胞凋亡。本論文中，我們用 TSDH 去處理人類乳癌細胞(MCF-7 and MDA-MB-231)，發現隨著處理劑量的上升而有細胞存活率下降的現象，故本論文中將針對 TSDH 抑制人類乳癌細胞生長之機制進行探討。我們證實了 TSDH 可誘使 MCF-7 和 MDA-MB-231 細胞發生細胞週期 G0/G1 時期停滯和細胞凋亡現象。以西方墨點法觀察到 p27Kip1 蛋白表現量增加，而 CDK4、D type cyclins 和 cyclin E 的表現量減少，激?活性試驗觀察到 CDK2 和 CDK4 激?活性被抑制，使得 D type cyclins-CDK4 和 cyclin E-CDK2 複合物的活性受到抑制，導致細胞週期停滯於 G0/G1 時期。在 TSDH 造成細胞凋亡的過程中，Bcl-xL 的蛋白表現有被抑制的現象，此外，觀察到 cytochrome c 從粒線體中釋出且轉移至細胞質，然後使得 caspase-9、caspase-3 和 caspase-7 被活化，再去切割 PARP [poly(ADP) ribose polymerase]，可見 TSDH 誘使細胞走向粒線體細胞凋亡路徑。在裸鼠實驗方面，則證實 TSDH 有抑制 MDA-MB-231 細胞誘導之異種移植腫瘤生長的情形，所以在 in vitro 和 in vivo 實驗結果皆顯示 TSDH 可抑制 MCF-7 和 MDA-MB-231 細胞的生長。故本論文證實了 TSDH 可誘使人類乳癌細胞(MCF-7 and MDA-MB-231)生長停滯於細胞週期 G0/G1 時期和發生細胞凋亡現象，因此也許 TSDH 未來可作爲化學治療的藥物。

英文摘要

Epidemiological studies have indicated that breast cancer is one of the leading cancer of death for women in Taiwan. At patient, surgical therapy and chemotherapy are the major strategies for the cure of breast cancer. The chemotherapeutic drugs are usually designed to induce cancer cell death via cell cycle arrest and/or apoptosis pathways. In this study, we used a chemical drug-TSDH to inhibit breast cancer cell proliferation and tumor growth, and investigate the underlying molecular mechanisms. Both human breast cancer cell lines-MCF-7 and MDA-MB-231 are used in this study, and found TSDH significantly decreased cell proliferation by a dose-dependent manner in both cells. Flow cytometry demonstrated that TSDH induced cell cycle arrest at G0/G1 phase in synchronous MCF-7 and MDA-MB-231 cells. When analysis the expression of cell cycle-related proteins, we found that TSDH reduced cyclin D, cyclin E and CDK4 expression, and increased CDK inhibitor p27Kip1 in a dose-dependent manner. In addition, TSDH inhibited the activities of CDK2 and CDK4 by a immunocomplex kinase assay. On the other hand, TSDH also induced apoptosis in both cells, In TSDH treatment cells, the anti-apoptotic protein Bcl-xL was decreased and cytochrome c released into cytoplasm was increased. Moreover, TSDH activated caspase-9, caspase-3 and caspase-7 and resulted in PARP cleavage and cell apoptosis. In nude mice experiment, 10 mg/kg TSDH significantly inhibited the tumor growth of MDA-MB-231 cells, Taken together, these results suggest that TSDH could inhibit human breast cancer cell proliferation and tumor growth, and might be a potential drug for chemothera.