Terbinafine誘發人類表皮癌細胞A431週期停滯及凋亡之分子機制研究

Studies on the Molecular Mechanisms of Terbinafine-induced G0/G1 Cell Cycle Arrest and Apoptosis in Human Epidermoid Carcinoma A431 Cells

中文摘要

Terbinafine，以下簡稱 TB，是臨床上時常被使用的口服抗黴菌藥物，由於病人服用後藥物會集中在皮膚，於角質層及皮脂達到一相當高之濃度並維持長時期，因此本研究目的在於探討 TB 對人類皮膚癌是否具有治療的潛力以及作用機制。在生長曲線的實驗我發現，相較於 Hs695T 和 RPMI 7951 這兩株人類黑色素瘤細胞，TB 抑制人類表皮癌細胞 A431 的生長效果較為明顯。經由流式細胞儀和 DNA 電泳分析，我也發現 120 M 劑量的 TB 處理 24 和 48 小時會造成 A431 細胞週期 G0/G1、subG1 比例的上升和 DNA 裂片的增加，顯示 TB 引發 A431 細胞死亡是經由 G0/G1 細胞週期停滯導致的計畫性死亡而非壞死。在分析蛋白量變化我同樣也看到 120 M 劑量的 TB 處理 24 和 48 小時促使 A431 細胞內 Rb 表現量增加，Bcl-2 表現量下降，caspase 3、caspase 8、caspase 9 等蛋白活化。利用免疫螢光染色在雷射共軛焦點螢光顯微鏡下觀察，我發現 cytochrome c 和 AIF (apoptosis inducing factor)在經過 TB 刺激 24 小時後會從粒線體分別轉位到細胞質以及細胞核中，也觀察到 p-JNK 和 Bax 分別在 TB 處理 1 和 6 小時轉位到粒線體， Bcl-2 和 14-3-3σ 蛋白位置在 6 小時內沒有變化。以 JC-1 染色發現 TB 會造成粒線體膜電位在 1 小時下降。在 DCFH-DA 染色也發現 TB 處理 5 分鐘細胞內 ROS 量也有增加。綜合我上述發現的結果，我認為 TB 引發人類皮膚癌細胞 A431 週期停滯及凋亡，具有治療皮膚癌的潛力。

英文摘要

TB (Terbinafine) is a clinical antifungal drug. It accumulates in skin tissue soon after taken by patient and then reach a high level for a long period of time. Therefore I exam the possibilities of TB being an anti-skin cancer medicine and forward study the molecular mechanisms. In the growth curve experiment, it is found that TB has caused more significant growth inhibition in A431 human epidermoid carcinoma cells than in Hs695T and RPMI 7951 human melanoma cells. According to the data of flow cytometry and DNA electrophoresis suggesting that TB induces G0/G1 cell cycle arrest and apoptosis instead of necrosis in A431 cells. It is also observed an increase in Rb protein level, decrease in Bcl-2 protein level and caspase 3, 8, 9 cleavage activation in 24hr and 48 hr. Using immunostaining under laser confocal microscopy

observation it is also found that cytochrome c and AIF (apoptosis inducing factor) translocate from mitochondria intermembrane space to cytosol and nuclei respectively in 24hr, and p-JNK and Bax also translocate from cytosol to mitochondria in 1 and 6hr respectively. There is no sign showing Bcl-2 and 14-3-3σ protein translocation in 6hr. TB also causes a loss of mitochondria membrane potential in 1hr by JC-1 staining and a ROS generation in 5 min by DCFH-DA staining. To sum up, TB has agreat potential treatment in clinical skin cancer therapy.