探討 5,7,2’-trihydroxyflavone 及 5,7-dimethoxyflavone 藥物對於內生性 CBF1 所媒介的 Notch 訊息路徑之調控作用

The regulatory effects of the 5,7,2’-trihydroxyflavone and 5,7-dimethoxyflavone on the endogenous CBF1-dependent Notch signaling

中文摘要

Notch 訊息路徑廣泛參與在不同組織細胞形成的過程中，包括維持幹細胞與前驅細胞處於未分化狀態，同時影響細胞之分化走向、增殖與細胞凋亡。本實驗室先前利用基因轉殖方法，著手建立可經由內生性 CBF1 所媒介的 Notch 訊息傳遞路徑誘發冷光酶表現的穩定單一細胞株 K562/CBF1-RE-Luc。在此細胞株平台進行多種藥物的篩選後，發現 5,7,2’-trihydroxyflavone 及 5,7-dimethoxyflavone 兩種藥物，在 50 microM 的濃度處理 24 小時後對於內生性 CBF1 所媒介的 Notch 訊息路徑具有劑量與時間效應的活化作用。另外，經由及時定量聚合酵素連鎖反應結果得知，兩種 flavonoids 藥物的活化作用，並不是藉由影響 Notch1 受體 mRNA 的表現達成。

在流式細胞儀、聯苯胺染色法、細胞群落分析以及西方轉印法分析中發現，這兩種 flavonoids 藥物皆使 K562 細胞株之細胞週期之 G0/G1 時期分佈增加，同時抑制細胞群落形成的能力，並促使 K562 細胞往紅血球分化能力提升。在西方轉印法中進一步觀察到與調控細胞週期相關的 Rb、ppRb 蛋白與 cyclin A 蛋白表現量都增加，而 E2F-4 蛋白質表現量下降。另外，利用短暫轉染之冷光酵素報導基因分析中得知：50 microM 之 5,7-dimethoxyflavone 藥物處理 24 小時後，也可活化非 CBF1 所媒介的 Notch 訊息之目標基因：c-Myc 基因之啓動子的活性；並且在活化 NF-kappa B response element 活性的作用當中，與 Notch 訊息之間似乎存在加乘作用。

綜合以上抑制細胞生長、促進分化以及活化 NF-kappa B 基因的結果，呼應了這兩種天然存在的抗癌藥物其作用和功效。但是更詳細的作用機制可能必須要經過更多的實驗佐證以及進一步的研究，方能了解藥物與 Notch 訊息傳遞路徑之間的交互作用的機制全貌。

英文摘要

Notch signal pathway plays the critical roles in cell proliferation, differentiation, and apoptosis. In the prevailing model for Notch signaling, the activated Notch receptors enhance the expression of their target genes via both CBF1-dependent and -independent pathways. Deregulated expression of Notch receptors, ligands, and their targets is observed in numerous malignancies.

Recently, the platform to screen the drugs modulating the endogenous Notch signal pathway had been established. In the present study, 5,7,2'-trihydroxyflavone and 5,7-dimethoxyflavone were demonstrated to activate the endogenous CBF1-dependent Notch signaling with the dose-dependent and time-dependent manners in K562 cell. In addition, the Notch1 mRNA expression was not affected after the treatment with these two flavonoids. Furthermore, the activation of Notch signaling by these two flavonoids was reversed after the treatment of the gamma-secretase inhibitor MG132. Therefore, the control of Notch1 transcript might not be involved in the activation of endogenous CBF1-dependent Notch signaling by these two flavonoids.

In flow cytometry analysis, the results revealed G0/G1 arrest by these flavonoids in K562 cells. Furthermore, these two flavonoids increased the expression of the cell cycle-related proteins such as hyperphosphorylated Rb, Rb, and cyclin A, and decreased the expression of E2F-4 protein. Additionally, erythroid differentiation of K562 cells was promoted by 5,7,2'-trihydroxyflavone and 5,7-dimethoxyflavone. By colony-forming assay in soft agar, the colony number was decreased after the treatment with these flavonoids in K562 cells.

By reporter gene assay, 5,7-dimethoxyflavone also enhanced the promoter activity of c-Myc gene which is activated by the CBF1-independent Notch signaling. In addition, the activity of reporter gene containing NF-kappa B response elements was also up-regulated in the presence of 5,7-dimethoxyflavone but not 5,7,2'-trihydroxyflavone.

Taken together, both 5,7,2'-trihydroxyflavone and 5,7-dimethoxyflavone exhibit the therapeutic potential in the tumorigenesis of K562 cells. However, it needs to be further dissected the regulating mechanism of endogenous CBF1-dependent Notch signaling by 5,7,2'-trihydroxyflavone and 5,7-dimethoxyflavone.