TSDH 誘導人類血癌細胞凋亡的分子機制之探討

Study of the pro-apoptotic effects by TSDH in human leukemia cells

中文摘要

TSDH 爲中草藥丹參之萃取物。根據本實驗室近來研究發現，TSDH 能抑制乳癌細胞增生並誘使細胞凋亡，藥物作用機制爲首先使細胞週期調控蛋白 G1 cyclins 的表現量及激脢活性下降，讓細胞停滯在 G1 期，再透過粒線體途徑導致
細胞凋亡。其他研究指出，TSDH 類似物 Tanshinone IIA 可經由 caspase 3 的活化
促使血癌細胞凋亡。本篇論文爲探討 TSDH 作用在人類急性骨髓性血癌第三型細胞 (HL-60) 後細胞凋亡的機制。實驗結果發現，在 1.5 μg/ml 的 TSDH 劑量下，
處理過的 HL-60 細胞即可顯著地加促細胞凋亡的調控分子 Bax、Bad 之表現，並
且活化 caspase 蛋白促使 PARP 裂解，最終導致 HL-60 細胞凋亡。在處理 TSDH 的細胞中可偵測到 caspase-8 及 caspase-9 的活化，這個現象暗示了包含死亡受體
及粒線體媒介的兩條細胞凋亡路徑都有活化的現象。由於 FasL 啓動子區域具有 AP-1 結合位置，且 JNK 的活化具有調節增加 FasL 表現的功用。隨 TSDH 的處
理時間增長發現 JNK 磷酸化程度有加強的趨勢，隨劑量增加也發現 FasL 的表現
量上升。在使用 JNK 抑制劑 SP600125 之後，TSDH 導致的 caspases 活化有略爲
下降；但在 FasL 表現及細胞死亡的程度卻沒有減緩的趨勢。在動物實驗中探討 TSDH 對血癌細胞的研究發現，植入 HL-60 的裸鼠在 TSDH 於 25 mg/kg 之劑量
下可有效抑制腫瘤的形成。根據本篇實驗結果建議，在 TSDH 誘導 HL-60 細胞凋亡的訊息傳遞中，JNK 的活化僅具有部分的調控功能，而 FasL 表現增加則可能扮演相對重要的角色。根據本篇實驗總結，由於 TSDH 在體外及動物體內皆具有促進血癌細胞凋亡的能力，使得 TSDH 具有發展成抗血癌藥物的潛力。

英文摘要

TSDH is a lipophilic compound extracted from traditional Chinese medicine Salvia miltiorrhiza. Our recent studies have shown that TSDH possesses the ability of inhibiting proliferation and can induce apoptosis in human breast cancer cells. First,

TSDH inhibited cell proliferation through down-regulation of G1 cyclins and cell cycle dependent kinase activity and resulting in cell cycle arrest in G1 phase, and then induced cell apoptosis through mitochondria-mediated pathway. Another study reported that TSDH analogue, Tanshinone II A, induces AML cells apoptosis by caspase-3 dependent pathway. In this study, we have investigated the apoptotic effect of TSDH on the human AML type III cell line HL-60. We found that under 1.5 μg/ml of TSDH significantly increased proapoptotic Bax, Bad proteins expression and activated several caspases, thus led to PARP cleavage and resulted in HL-60 cell apoptosis. The activation of caspase-8 and caspase-9 found in TSDH-treated cells suggest that both death receptor and mitochondrion mediated pathways were induced. Due to the AP-1 binding site sits on FasL promoter region, activation of JNK pathway may mediates the regulation of FasL gene expression. Our results indicated that TSDH time-dependently induced JNK phosphorylation and dose-dependently upregulated Fas ligand (FasL) expression. By using JNK-specific inhibitor, SP600125,
can slightly inhibited TSDH-induced caspases activation. However, FasL expression and cell death cannot be reversed. These results suggest that TSDH induced HL-60 cell apoptosis was partially through JNK activation, where the upregulation of FasL may play a more important role. In vivo study of the TSDH effect on HL-60 bearing nude mice indicated that, under TSDH 25 mg/kg treatment efficiently blocked tumor formation. In conclusion, due to the proapoptotic effect in leukemia cells in vitro and in vivo suggest that TSDH has the potential to develop as an anti-leukemia drug in the future.