Ceramide 經由 GSK-3β及 Endoplasmic reticulum stress 導致細胞死亡之探討

Involvement of GSK-3β and Endoplasmic Reticulum Stress in Ceramide-induced Cell Death

中文摘要

Ceramide (神經醯胺)爲細胞膜的成份之一，且爲一重要的次級訊息傳遞者(second messenger)，參與調控眾多訊息傳遞路徑，並可能導致細胞凋亡(apoptosis)；此外，利用放射線或化學療法處理癌細胞，亦發現 ceramide 的含量上升爲造成細胞死亡的主要原因之一，故 ceramide 被認爲與癌症治療相關。ceramide 可因實驗模式與條件之差異而造成不同的死亡型式，包括細胞凋亡及細胞自噬 (autophagy)。因此本論文探討 ceramide 對惡性膠質瘤 U87-MG 所造成的細胞死亡型式爲何？並研究可能參與之分子訊息。

實驗結果顯示，利用 acridine orange 染劑併用 flow cytometry 偵測 acidic vesicular organelle (AVO)含量，代表 autophagy 之百分比，結果發現 ceramide 處理細胞 24 小時可達最高 autophagy 百分比 (62.1 ± 3.5 %, $p<0.001$)，36 小時下降至 53.0 ± 4.8 % ($p<0.001$)，利用 Western blot 亦可觀察 autophagy 上游分子 LC3 由 type-I 轉變爲 type-II 之現象；另外，利用 Annexin V/Propidium iodide (PI)染劑偵測細胞膜 phosphatidylserine (PS) externalization 與 PI uptake，於 36 小時測得最高 apoptosis 含量 (36.7 ± 1.9 %, $p<0.001$)，推測 ceramide 處理 U87-MG，可在初期造成 autophagy 晚期產生 apoptosis。

研究顯示 endoplasmic reticulum (ER) stress 除了造成 apoptosis 亦能導致 autophagy，因此本論文繼而探討此系統下是否有 ER stress 的產生。Western blot 顯示：ceramide 可誘導細胞內 GADD153 (growth arrest and DNA damage-inducible gene 153)表現及 eukaryotic initiation factor 2α (eIF2α)磷酸化現象，代表 ceramide 可導致 U87-MG 細胞產生 ER stress。亦有研究指出 ER stress 可誘導 glycogen synthase kinase-3β (GSK-3β)的活化而導致 apoptosis，且本實驗室先前之實驗結果顯示 GSK-3β參與導致 autophagy，故續用 Western blot 方式偵測此系統下 GSK-3β之活性變化，實驗結果顯示 GSK-3β Ser9 之磷酸化表現量隨 ceramide 之加入而減少，代表 GSK-3β活化，且利用 lithium chloride 抑制 GSK-3β後，經由 flow cytometry 分析 autophagy 百分比由 63.9 ± 2.9 %降至 40.2 ± 3.7 % ($p<0.001$)，表示 GSK-3β可能參與 ceramide 誘導之細胞自噬。綜合所述，本論文推測 ER stress 及 GSK-3β的活化可能是 ceramide 導致細胞死亡的原因之一。

英文摘要

Ceramide is a structural constituent of cell membrane that has been recognized as an important second messenger implicated in regulating diverse signaling pathways, especially for apoptosis. Moreover, chemotherapy and radiotherapy elicit an increase in the ceramide level. Ceramide causes either apoptosis or autophagy depending on the model systems and experimental conditions. The specific aim of the present study was to investigate the molecular signaling of ceramide-induced cell death in U87-MG, a human malignant glioma cell line.

As revealed by flow cytometry staining with acridine orange to detect the acidic vesicular organelle (AVO), we demonstrated that the percentage of autophagy reached a plateau of 62.1 ± 3.5 % ($p<0.001$) after 24 h exposure of U87-MG cells to 30 μM C6-ceramide, using immunoblot assay we also detected the processing of microtubule-associated protein 1 light chain 3 (LC3). A parallel experiment indicated that apoptosis reached to 36.7 ± 1.9 % ($p<0.001$) after 36 h exposure of 30 μM C6-ceramide, using Annexin V/Propidium iodide (PI) double staining to detect cell membrane phosphatidylserine (PS) externalization and PI uptake, implying that autophagy might be a prelude to apoptosis in ceramide-treated U87-MG cells. Endoplasmic reticulum (ER) stress, which has been shown to be engaged in cell death, was induced by treatment with ceramide using immunoblot to reveal the expression of GADD153 (growth arrest and DNA damage-inducible gene 153) and the phosphorylation of eukaryotic initiation factor 2α (eIF2α). Recent studies indicated that ER stress was able to activate the glycogen synthase kinase-3β (GSK-3β), a serine/threonine kinase. Here, we demonstrated that ceramide induced a decrease of the phosphorylated level of Ser9 of GSK-3β, resulting in elevating its enzymatic activity. Besides, lithium chloride, a GSK-3β inhibitor, was able to reduce the percentage of autophagy from 63.9 ± 2.9 % to 40.2 ± 3.7 % ($p<0.001$), suggesting that GSK-3β might play a pivotal role in ceramide-induced autophagy.

In conclusion, our results indicate that C6-ceramide induced both autophagy and apoptosis. Of which, autophagy was likely to be mediated by ER stress and the activation of GSK-3β.