Ceramide 經由 GSK-3β 誘導大鼠腦神經膠質瘤細胞 Autophagy 之探討

The Study of Ceramide-Induced Autophagy in C6 Glioma Cells-the Role of GSK-3β

中文摘要

Autophagy (細胞自噬)爲一演化下高度保留之蛋白質代謝機制，但過度表現亦會導致細胞死亡。阿茲海默症成因爲 amyloid-beta (Aβ，乙型澱粉樣蛋白)之沉積導致腦神經細胞死亡。ceramide (神經醯胺)爲真核細胞膜的成份之一，爲 Aβ之下游代謝物，因此本論文利用 ceramide 模擬 Aβ之細胞毒性。爲探討 ceramide 對大鼠腦神經膠質瘤細胞 (C6 glioma cells)之細胞毒性，以 acridine orange 染色法併用流式細胞儀偵測，發現 autophagy 百分比隨 ceramide 劑量及加藥時間而上升之趨勢，其中以 30 μM ceramide 加入 24 小時後達到最高 47.24 ± 2.73 % ($p<0.001$)的 autophagy，隨即降低，但 apoptosis 則隨之升高，於 36 小時達到 27.3 ± 5.5 % ($p<0.01$)。以西方墨漬法可看出 autophagy 上游蛋白分子，LC3 蛋白之 processing 及 Beclin 1 蛋白的表現量隨時間點往後推移而上升；利用穿透式電子顯微鏡亦可於細胞質觀察到雙層膜之 autophagosomes 構造，顯示 ceramide 可導致 C6 glioma cells 進行 autophagy。此外，若同時加入 autophagy 抑制劑 vacuolar-ATPase inhibitor，bafilomycin A1 (BafA1)，可將 ceramide 所誘導之 apoptosis 由 5.35 ± 0.72 %提高至 23.21 ± 6.87 % ($p<0.05$)，因此推測 autophagy 爲一保護性機制。GSK-3β (肝醣合成酶激酶)爲一 serine/threonine protein kinase，已被證實會參與細胞死亡之調控，但於 autophagy 方面之研究則尚無探討。以西方墨漬法發現 GSK-3β之 Ser-9 位置磷酸化現象隨時間增加而降低；另外，發現加入 GSK 抑制劑，SB216763，可將 autophagy 由 56.51 ± 4.05 %降至 40.42 ± 3.83 % ($p<0.001$)。另一方面，細胞加入 ROS (活性氧分子)清除劑，NAC，autophagy 之比例亦由 51.68 ± 0.85 %降至 34.92 ± 2.64 % ($p<0.001$)。實驗結果顯示，ROS 與 GSK-3β之訊息傳遞路徑可能參與 ceramide 所誘導之 autophagy。

英文摘要

Autophagy, an evolutionarily conserved mechanism for degradating and recycling of long-lived proteins, is considered as a type II programmed cell death (type II PCD). Aggregation of amyloid-beta (Aβ) induces brain cells death is one of the main causes of Alzheimer's disease (AD). Ceramide, a ubiquitous constituent of eukaryotic membranes, is a downstream metabolite of Aβ, which was often exploited to mimic

the toxicity of Aβ. In this study, using C6 glioma cells as a cell model, we investigated the cytotoxity of ceramide. As revealed by flow cytometry staining with acridine orange, we observed that the percentage of autophagy and apoptosis reached a plateau of 47.24 ± 2.73 % ($p<0.001$) and 27.3 ± 5.5 % ($p<0.01$) after 24-h and 36-h exposure of the C6 glioma cells to 30 μM ceramide. Using immunoblot assay, we detected the processing of microtubule-associated light chain 3 (LC-3) and the expression of Beclin 1 in a time-dependent manner, which have been found to promote autophagy. Moreover, transmission electron microscopy (TEM) analysis demonstrated that the formation of double-layer autophagosomes were observed in the cytosol, indicating that ceramide is able to induce autophagy in C6 glioma cells. In addition, using vacuolar-ATPase inhibitor, bafilomycin A1 (BafA1), to suppress the ceramide-induced autophagy, we found that the percentage of apoptosis was increased from 5.35 ± 0.72 % to 23.21 ± 6.89 % ($p<0.05$), suggesting that autophagy might play a protective role in C6 glioma cells treated with ceramide. In the study of glycogen synthase kinase-3β (GSK-3β), a serine/threonine kinase, we demonstrated that ceramide induced a decrease of the phosphorylated level of Ser9 of GSK-3β which implied that the activation of GSK-3β. Besides, GSK-3β inhibitor, SB216763, and a reactive oxygen species (ROS) scavenger, N-acetyl-L-cysteine (NAC), was able to reduce the percentage of autophagy from 56.51 ± 4.05 % to 40.42 ± 3.83 % ($p<0.001$) and 51.68 ± 0.85 % to 34.92 ± 2.64 %, respectively. It suggests that GSK-3β and ROS are involved, at least partially, in autophagy. We conclude that ceramide-induced autophagy was regulated by ROS and GSK-3β signal transduction pathway.