活性氧分子在神經生長因子誘導 PC12 細胞分化所扮演角色之探討

Studies on the Roles of ROS during NGF-induced Differentiation of PC12 Cells

中文摘要

氧化還原狀態(redox status)與許多細胞生理過程息息相關，包括細胞增生、分化及凋亡。本論文以大鼠腎上腺的嗜鉻細胞瘤細胞(PC12)爲細胞模式進行系列研究，探討神經生長因子(nerve growth factor，NGF)誘導 PC12 細胞分化的過程中，活性氧分子(reactive oxygen species，ROS)與抗氧化酵素(antioxidant enzymes)所扮演的角色。

以神經突觸外生(neurite outgrowth)及神經細胞特有的指標蛋白包括：tyrosine hydroxylase (TH)與 neurofilament-L (NF-L)的表現，確定 NGF 可誘導 PC12 分化。爲瞭解此過程中是否有 ROS 的參與，因此利用多種抗氧化物或抗氧化酵素處理細胞。結果發現，NAC 可有效抑制 NGF 誘導 PC12 分化，但・OH 的特異性清除劑 mannitol 以及・O2-的特異性清除劑 tiron 則無明顯地抑制作用。利用流式細胞儀的技術，以 2’,7’-dichlorodihydrofluorescein diacetate (DCFH-DA)及 dihydroethidium (HEt)分別檢測 H2O2 及.O2-，發現在 NGF 處理 1 分鐘時胞內 H2O2 約提升 3 倍左右，但.O2-並無明顯變化情形。由上述結果顯示，H2O2 可能參與 NGF 誘導 PC12 的細胞分化過程並具備要能。此外，由於 H2O2 的變化可能改變胞內抗氧化酵素的活性，因此本論文進一步分析在 PC12 分化過程中，抗氧化酵素包括 catalase (CAT)、glutathione reductase (GRx)、copper-zinc superoxide dismutase (Cu/ZnSOD)及 manganese superoxide dismutase (MnSOD)活性的變化，發現僅有 CAT 的活性在分化中期有明顯增加 1.5 倍的現象($p<0.01$)。

Mitogen-activated protein kinase (MAPKs)爲胞內重要的訊息傳遞分子之一，其中 extracellular signal-regulated protein kinase (ERK)、c-Jun N-terminal protein kinase (JNK)與 p38 之活性，易受到 ROS 之調控。本論文分析在 NGF 誘導 PC12 細胞分化過程中，這些 ROS 敏感性 MAPKs 的表現情形，及其活性是否受到 ROS 的調節。結果發現，ERK 在經過 NGF 處理 5 分鐘時即有 8.7 倍的活化，並能保持 2~3 倍的活化程度至 3 小時後；JNK 則在 10 分鐘時有短暫地活化 14.2 倍，而 40~60 分鐘時有第二次較弱的活化，之後便回復至基礎值(basal level)；相反的，p38 在 20 分鐘時活性會降低 80 %，之後會漸漸恢復至基礎值左右。在經過 NAC 的前處理後，NGF 誘導 ERK 的活化現象，幾乎可被完全抑制，表示 ERK 的活化可能經由 NGF 誘導產生的 H2O2 所調控。綜合上述結果顯示，NGF 可能藉由提升胞內 H2O2 濃度，並透過 ROS 敏感性 MAPKs 的作用，最後導致 PC12 神經細胞的分化。但其詳細機轉，仍有待進一步確認。

英文摘要

The redox signaling has been shown to correlate with various biological processes including cell proliferation, differentiation and apoptosis. In our research, the rat adrenal pheochromocytoma (PC12) cells was used as a cell model to investigate the roles of reactive oxygen species (ROS) and antioxidant enzymes in nerve growth factor (NGF)-induced differentiation. The neuronal specific differentiation markers, tyrosine hydroxylase (TH) and neurofilament (NF)-L, were detected by immuno- blot to confirm the differentiation stage of PC12 cells. Among numerous antioxidant compounds or enzymes, only N-acetylcysteine (NAC) could suppress the NGF-induced PC12 differentiation. However, tiron (.O2- specific scavenger) and mannitol (.OH specific scavenger) exerted only minor effects. By flowcytometry method, minor but significant generation of cellular hydrogen peroxide (H2O2), but not superoxide anion (.O2-), was detected after treatment with NGF. These results indicated that H2O2 might play a pivotal role during PC12 differentiation. The antioxidant enzymes might be responded to redox homeostasis during PC12 differentiation. By enzyme kinetic analysis, the activities of catalase (CAT)、glutathione reductase (GRx)、copper-zinc superoxide dismutase (Cu/ZnSOD) and manganese superoxide dismutase (MnSOD) were examined. Only the activity of CAT was elevated slightly but significantly (1.5 folds) after NGF-induced differentiation of PC12 cells.

ROS has been suggested as a signaling molecule to regulate the so-called ROS-sensitive MAPKs, such as extracellular signal-regulated protein kinase (ERK), c-Jun N-terminal protein kinase (JNK) and p38. Therefore, the activities of ROS-sensitive MAPKs were analyzed by immuno-blot. ERK and JNK were activated dramatically during the initial stage of NGF-induced PC12 differentiation as prolonged and transient profiles, respectively. Nevertheless, p38 activity was decreased (~80 %) at 20 minutes time-point and then recovered to basal level. After pre-treatment with NAC, the NGF-induced activation of ERK was suppressed, which suggested that ERK might be a down-stream molecule of H2O2 production mediated by NGF. Based on these results, ROS, especially H2O2, might play a pivotal role during NGF-induced differentiation of PC12 cells.