新生老鼠大腦神經分化之研究

Studies of Neuronal Cell Differentiation in Neonatal Rat Brain.

中文摘要

腦神經細胞在出生之後即停止生長並分化為各種突觸細胞，最近的研究顯示細胞的生長與細胞週期調控蛋白及細胞週期的進程有關，但腦部神經分化的分子機轉大部分仍不清楚。在此實驗，我們使用兩種研究模式: 初代培養神經細胞(於胚胎期第 18 天解剖)，屬 In vitro 模式；出生前後不同時期大腦皮質細胞取樣，屬於 In vivo 模式。在兩模式不同的時間點偵測細胞週期調控蛋白與α-tubulin (microtubule 的組成物)的表現，來了解大白鼠出生後神經細胞生長的控制。除此以外，我們以一項新的技術-差異顯示法，來顯示 DIV 3 (培養皿培養 3 天) 與 DIV 14 兩者之間表現有差異的基因。只於 DIV 3 而非 DIV 14 天表現的有 8 個基因片段，只於 DIV 14 而非 DIV 3 表現的基因則找到了 7 個。它們被選殖進入 TA 質體與 pBS 質體。以 PCR 放大的基因片段做的引子，將用來驗證這些基因是否也會在 In vivo 模式中不同的時間點表現。我們在 In vitro 與 In vivo 兩者偵測 Rb、cyclin D3、cdk4、p16 與 p21 的表現，結果發現它們在這些時間點都沒有什麼顯著變化。細胞週期抑制蛋白: p16、p21 與 p27 在初代培養與出生前後的神經細胞都只表現少量，可能是為了抑制神經細胞在胚胎期第 18 天之後的細胞週期進行。剛出生幾天的老鼠，其大腦蛋白-α-tubulin 在 SDS-PAGE 上可看出表現為兩種形式。此較小分子量的形式在出生後開始出現到第 16 天消失，而在出生前或出生 16 天之後，只有高分子一種型式存在。在初代培養的細胞中，也只有高分子一種型式出現。由免疫沉澱法與 Potato acid phosphatase 的處理，可知此蛋白之酪氨酸未被磷酸化。為了試圖了解如何引發此現象的產生，我們將 A23187、PMA、EGTA、KCl、NMDA、安非他命、NGF 與 CRH 加入初代培養 5 天之神經細胞，但沒有一種能改變α-tubulin 的表現形式。此項結果顯示α-tubulin 在出生後二週會以低分子量的形式存在，究竟此低分子量係去酪氨酸的α-tubulin 或另一種變異型式，及引發此型態出現的原因和影響，均有待進一步的研究證實。

英文摘要

Studies of Neuronal Cell Differentiation in Neonatal Rat Brain

Neuronal cells cease proliferating before postnatal stage. It is knownthat cell cycle regulatory proteins control the progression of cell cycle, but the molecular mechanisms involved in neuronal differentiation in brain remain unclear. In the presence study, we use primary cultured neuronal cells (in vitro model,

dissected on embryonic day 18) and pre- and postnatal rat brain (in vivo model) to examine the expression of cell cycle regulatory proteins and a-tubulin, the component of microtubule. By the new technique " Differential Display ", we have identified the genes that express differently between DIV 3 (days in vitro) and DIV 14. Eight gene fragments were found expressing on DIV 3, but not DIV 14. Seven gene fragments were found expressing on DIV 14, but not DIV 3. They were cloned into TA vectors and pBS vectors. PCR amplified gene fragments will be use as probes to verify the mRNA expression of the genes on different days in prenatal and postnatal rat brain. We screened the expression of Rb, cyclin D3, cdk4, p16 and p21 both in vitro and in vivo. No significant expression of these proteins were found during these time points. Cell cycle inhibitory proteins, p16, p21 and p27 were found expressing at low level in both primary cultured cells and pre- and postnatal neuronal cells, presumably inhibiting the cell cycle progress of neuronsafter embryonic day 18. a-Tubulin, migrated as a doublet in SDS-PAGE at early postnatal rat brain. This low molecular weight form of a-tubulin was neither expressed in the primary culture cell system, nor were they expressed before birth or 16 days after birth in vivo. This protein was not tyrosine-phosphorylated demonstrated by immunoprecipitation and potato acid phosphatase treatment. To investigate the cause of these phenomenom, A23187, PMA, EGTA, KCl, NMDA, amphetamine, NGF and CRH were added to DIV 5 cultured neurons. None was found to change the expression form of a-tubulin. Further characterization of the genes found in differential display will help us understand the neuronal differentiation in neonatal rat brain.