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• 計畫中文名稱	進行性肌陣攣性癲癇相關之 Cystatin B 基因的表現 對於神經細胞凋亡之影響
• 計畫英文名稱	--
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• 中文關鍵字	細胞凋亡; 癲癇; PC12; 硫氫蛋白胺抑制劑 B; 老鼠腎上腺髓質腫瘤細胞
• 英文關鍵字	Apoptosis; Epilepsy; Cystatin B; PC12 cell
• 中文摘要	Cystatin B 是一種廣泛存在於各種組織的蛋白質，其含有 98 個氨基酸，會抑制多種硫胱氨酸蛋白酵素的作用，包 括 Cathepsins L、H、B、S 及木瓜蛋白酵素。Cystatin B 基因的突變導致人類第一型進行性肌陣攣性癲癇，而缺少 Cystatin B 的基因轉殖鼠也發展出類似症狀，且小腦顆粒細胞的的凋亡增加。缺乏 Cystatin B 表現對於中樞神經細胞凋亡促進的原因尚不清楚。由於其它研究顯示 Cathepsin B、D 及 L 與神經細胞的凋亡有關，有人認爲 Cystatin B 含量的降低會導致 Cathepsin 活性不正常提高，而活化了細胞凋亡所必需的 Caspases。爲了進一步了解 Cystatin B 在神經細胞凋亡中所扮演的角色，本計畫中將 Cystatin B 的基因的 cDNA 以正向或反向轉殖入表現載體中，並將這些殖體轉染到腎上腺親鉻母細胞瘤細胞株 PC12 中，挑選 Stable trasfected cell clones，藉以提高或是抑制 Cystatin B 的表現。由於 PC12 細胞在神經生長激素 NGF 的刺激後會分化成神經細胞，這些 Cystatin B 表現量不同的 PC12 細胞株將可用來研究該蛋白對於未分化 PC12，以及及受 NGF 刺激分化之 PC12 細胞之細胞凋亡的影響。未來還可進一步用來研究 Cystatin B 蛋白與細胞凋亡相關的機制。
• 英文摘要	Cystatin B is a ubiquitously distributed small protein 98 amino acid in size, which binds and inhibits the cystein proteases including cathepsins L, H, B, S and plant cysteine protease papain. Mutations in cystatin B gene caused progressive myoclonic epilepsy (EPM1) in human, and cystatin B deficient mice represent similar symptom, with increased apoptosis in cerebellar granule cells. The mechanism by which lacking of cystatin B expression promotes apoptosis in CNS is still not clear. However, other studies showed that cathepsin B, D and L are involved in the apoptosis of serum deprived PC12 cells and hippocampus CA 1 pyramidal neuron after ischemia, and cathepsin B is involved in the processing of several procaspases. It was proposed that reduction in cystatin B might increase apoptosis by inappropriate activation of cathepsins and thereby increase

the activation of caspases that are required for the apoptosis to occur. To further investigate the function of cystatin B protein in apoptosis of neuronal cells, we cloned the cDNA of cystatin B gene in either sense or antisense orientation into the pCDNA3 expression vector, transfect these plasmid into the rat adrenal pheochromocytoma PC12 cells, and select for stable transfected clones. The cell clones expressing higher (sense) or lower (antisense) level of cystatin B protein were selected for our experiments. Since the PC12 cell can be induced to neuronal differentiation by NGF, these stable trasfected cell clones can be used to study the effect of cystatin B protein on the apoptosis of undifferentiated and NGF- induced neuronal- differentiated PC12. In the future, these can also be used to study the detail mechanism of how cystatin B involves in apoptosis.