脊髓小腦運動失調症小鼠及 Purkinje 細胞模式之建立

Establishment of Spinocerebellar Ataxia Mouse and Purkinje Cell Models

中文摘要

脊髓小腦運動失調症(spinocerebellar ataxia,SCA)爲一群異質性的三或五核苷擴增的神經退化性疾病，患者小腦、腦幹、脊幹及周邊神經系統等出現漸進式的退化。大部分的 SCA(如一、二、三、六、七、十七型等)源自於致病基因轉譯區內 CAG 三核苷的擴增，導致突變的蛋白沉積在細胞內，對神經細胞造成傷害。SCA8 基因表現於腦部，此 RNA 上並無開放的解讀架構(open reading frame)。SCA8 RNA 的 5'端和 KLHL1 RNA 的 5'端互補(自轉譯起始處至第一個 intron)，即兩個基因共用一段 DNA 序列只是方向相反。KLHL1 蛋白的結構和腦專一表現的 NRBP 及 KLHL2 蛋白非常相似，故其功能可能和肌動蛋白細胞骨架的組成相關。由於兩者皆表現於小腦，顯示 SCA8 RNA 可作爲一 antisense-RNA，來調節 KLHL1 基因的表現。

目前已知 SCA8/KLHL1 在人類及小鼠各組織及細胞株的表現情形，與先前的研究顯示有些許差異，我們也利用 In-situ hybridization 來確認 SCA8/KLHL1 在小腦及睪丸表現的位置。此外，由 SCA8 病人之臨床表徵判斷很可能是小腦神經細胞之退化所造成，所以我們將小腦專一性表現之啓動子攜帶綠色螢光蛋白(GFP)基因之表現質體轉入具多能性之 P19 embryo carcinoma cells，在做誘導分化之過程中，可偵測綠螢光蛋白之表現而建立小腦主要的神經細胞 Purkinje 細胞株，利用這種特定細胞株將可進一步探討 SCA8 的致病機轉。另外我們也已利用 Purkinje 專一表現之啓動子帶領 nitroreductase 基因之 DNA 片段建立誘導模式的基因轉殖鼠，nitroreductase 可藉由 prodrug CB1954 之處理而產生有毒衍生物以專一性傷害小腦，因此可模擬類似小腦萎縮的病症，利用這些轉殖鼠我們將進一步應用於治療相關疾病的基因及藥物的研究。

英文摘要

Autosomal dominant spinocerebellar ataxias (SCA) are a clinically and genetically heterogeneous group of neurodegenerative disorders primarily affecting the cerebellum. Several forms (SCA1, SCA2, SCA3, SCA6, SCA7, SCA17) share unstable CAG repeat expansions in the coding region that are translated into polyglutamine tracts. The polyglutamine tract is thought to exert its effect through a toxic gain of function of the corresponding protein. Spinocerebellar ataxia type 8 (SCA8) is reported to be caused by an unstable CTG repeat expansion in the 3' untranslated region of a novel gene, KLHL1AS, on chromosome 13q21.However, KLHL1AS dose not encode protein, and was proposed to play an anti-sense

regulatory role on the sense strand gene, KLHL1. To seek for a proper model for the study of SCA8, we first identified the expression pattern of KLHL1 and KLHL1AS in various tissues of both human and mice. In-situ analyses were also performed to further characterize the cellular level of expression in cerebellum and testes. We also constructed an expression plasmid in which an EGFP reporter gene was driven by a Purkinje-specific promoter. Purkinje cell lineage will be sorted through P19 cells transfected with this construct and differentiated in-vitro. In addition to the cell model, a transgenic mouse model with inducible nitroreductase is also under investigation. With the established cell or animal models we will be able to create an ideal environment for further studying the molecular mechanisms underlying the pathogenesis of SCA, including SCA8.