生長休止基因基因體分析，載體構築及篩選剔除生長休止基因的老鼠

Gas8 genomic mapping, construction of Gas8 targeting vector and Gas7 knockout mice screening

中文摘要

生長休止基因 7 和 8，(簡稱 Gas7 and Gas8)，是利用基因捕捉法從進入生長休止狀態的小鼠纖維母細胞中篩選出來的。Gas7，已有報告指出會表現在大腦皮層，海馬迴，和小腦。在小鼠中發現兩種不同的 mRNA 存在，分別是 Gas7 及 Gas7 isoform ：Gas7-cb。Gas7 產生的蛋白包含 421 個月安 基酸，分子量約 48kDa；Gas7-cb 產生的蛋白包含 320 個月安 基酸，分子量約 38kDa，分別表現在大腦和小腦。初步的實驗已證實 Gas7 蛋白可與 F-actin 直接作用，而且大量表現 Gas7 蛋白可以促進神經母細胞的 neurite-like 生長情形。爲了解 Gas7 蛋白在活體內的功能，我們發展出其基因剔除鼠的模式。我亦藉由南方吸漬墨點法篩選到 Gas7 的基因剔除鼠。並整理出此老鼠的譜系，及目前的代數。接著將 Gas7 基因剔除鼠和野生型的 B6 老鼠做交配，以期得到純品系的 Gas7 基因剔除鼠。希望能夠更明顯的看到老鼠的表型。

本文的另一重點 Gas8 蛋白含有 489 個月安基酸，分子量約 57kDa 主要表現在睪丸和輸卵管。在經過免疫化學染色法後發現 spermatid 和 spermatozoa 的尾部以及肺支氣管、輸卵管上皮纖毛都有大量 Gas8 表現(Yeh et al., manuscript in preparation)。暗示著 Gas8 的功能可能細胞分化有關。同樣地，爲更進一步了解 Gas8 基因在活體內的功能，我同時針對 Gas8 作分析及建構，以期發展出 Gas8 的基因剔除鼠，除了確認 Gas8 的基因體結構，及限制酉每 地圖。同時，也完成 Gas8 基因的次片段和胚胎幹細胞、老鼠 genomic DNA 篩選用的探針建構。整體上，從 Gas7 到 Gas8 的實驗是一個有系統性的學習如何完成基因剔除鼠的基因分析，建構和老鼠飼養及活體分析。未來的工作將會針對 Gas7 基因剔除鼠的神經組織培養，動物行爲作研究，並完成 Gas8 基因剔除鼠。

英文摘要

Growth arrest-specific gene 7 and 8 were originally identified by using a gene promoter trapping strategy from growth arrested NIH3T3 cell. Gas7 was reported expression in vivo selectively in neuronal cells of the mature cerebral cortex, hippocampus, and cerebellum. There are two kinds mRNA transcription, one is Gas7 and the other is Gas7 isoform: Gas7-cb. Gas7 its open reading frame is 421 amino acid encoded 48-kDa protein and it expresses in cerebrum; Gas7-cb are 320 amino acid encoded 38-kDa protein and it expresses in cerebellum. The preliminary results are that Gas7 interact with F-actin and Gas7 over-expression in undifferentiated neuroblastoma cell cultures dramatically promotes neurite-like outgrowth. In order to

understand Gas7 in vivo function, we developed Gas7 knockout mice model. Here I screened and obtained Gas7 knockout mice by performing Southern blotting analysis. The pedigree shows how many mice generations now. After repeat 10 generations backcross to get pure B6-strain background Gas7 knockout mice. At that time we will investigate Gas7 knockout mice phenotype obviously.

The second, Gas8 is 489 amino acid and encodes 57kD protein that is highly expressed in testis and adnexa. Immunohistochemical analyses were shown that Gas8 is specifically expressed in mature spermatid and highly localized in the cilia of epithelial cells from pulmonary bronchi and fallopian tubes (Yeh et al., manuscript in preparation). To understand the in vivo function of Gas8 gene, we have used gene targeting approach to develop Gas8 knockout mice model. For this purpose, genomic structure of mouse Gas8 gene has been characterized. I have determined exon-intron junction of the Gas8 gene and also the restriction enzyme map. In order to construct the Gas8 targeting vector, I have subcloned two fragments included targeted region and probe specific for Gas8 knockout ES cell and mice screening. This thesis let me systematically learn all knockout mice strategy from genomic analysis to mice breeding. Future work we will focus on Gas7 knockout mice neuron primary culture, the mice behavior and Gas8 knockout mice model development.