老鼠生長休止基因蛋白八與其交互作用蛋白之生物功能探討

Characterization of Growth Arrest Specific Gene 8 and its Binding Protein Gas8-BP3

中文摘要

生長休止基因 8 (Gas8) 是由處於細胞靜止期的小鼠纖維母細胞株-NIH3T3 中以基因捕捉法被選殖出來的，Gas8 主要表現在老鼠的睪丸中，而且其表現在後減數分裂時期是高度被調控的。此外，Gas8 會積聚在 round spermatids 的細胞質還有精子的尾巴中。然而，Gas8 在生理上的功能仍然不清楚，因此此論文的目標在於藉由分離和研究 Gas8 的結合蛋白(binding proteins)來探討 Gas8 的功能。我們藉由 yeast two-hybrid 的系統，利用 Gas8 當作餌(bait)、mouse testis cDNA library 當作魚(fish)來調出 Gas8 的結合蛋白。總共有三個 Gas8 的結合蛋白被分離出來，Gas8 結合蛋白一(Gas8-BP1)稱作 Axin，Gas8 結合蛋白二(Gas8-BP2)稱作 Meiosis Nuclear structural protein 1，Gas8 結合蛋白三(Gas8-BP3)為一個新的蛋白稱作 Mus musculus hypothetical protein 4921501M07。在這裡，我們描繪了 Gas8 結合蛋白三(Gas8-BP3)這個新的蛋白。結果顯示，在 GST pull-down assay 還有 co-immunoprecipitation 的實驗中我們證明 Gas8 能和 Gas8-BP3 有交互作用，而且 Gas8-BP3 的 C 端部分可能和 Gas8 作結合。

利用老鼠多種組織的北方點墨法(Northern blot)分析，我們證實了 Gas8-BP3 會高度表現在成年的老鼠睪丸中，而且它的表現在發育的睪丸中是被調控的。為了進一步了解 Gas8-BP3 在老鼠睪丸中表現的型態，我們自己製造了 Gas8-BP3 的抗體(antibody)並且利用此抗體作了免疫組織化學染色(immunohistochemical staining)的實驗。結果顯示 Gas8-BP3 的蛋白主要表現在 round spermatids，spermatocytes 而且也表現在 spermatogonia 中。此外，我們發現 Gas8 也能和微質管(microtubule)有物理上的交互作用。總結來說，Gas8 和 Gas8-BP3 兩者都會表現在老鼠的睪丸中並且在睪丸的發育中都是被調控的，顯示出它們兩者在睪丸發育的過程中扮演一些角色。

英文摘要

Gas8, originally identified in growth arrested NIH3T3 cells by Molony Murine leukemia virus gene-trapping is predominantly expressed in mouse testis, with its expression highly regulated during post meiotic sperm development. In addition, Gas8 accumulates in the tails of sperm, the cytoplasm of round spermatids and also in sperm flagella. However, Gas8 function remains unclear. This thesis investigated Gas8 function in mice by isolating and studying its binding proteins. We isolated Gas8 binding proteins by yeast two-hybrid assays using Gas8 as bait and a mouse testis cDNA library as the fish. Three Gas8 binding proteins were isolated. Gas8-BP1

is Axin, Gas8-BP2 is Meiosis Nuclear structural protein1, and Gas8-BP3 is Mus musculus hypothetical protein 4921501M07. In this study, the novel protein--Gas8-BP3 was further characterized. The results showed that Gas8 could interact with Gas8-BP3 in GST pull-down assays as well as in co-immunoprecipitation experiments, while the C-terminal portion of Gas8-BP3 may serve as the Gas8 interacting region.

Using multi-tissue Northern blot analysis I confirmed that Gas8-BP3 was highly expressed in adult mouse testis and this expression was regulated developmentally. In order to understand the expression pattern of Gas8-BP3 in mouse testis, we generated Gas8-BP3 antibody and preformed the mouse testis immunohistochemical staining. The result suggested that Gas8-BP3 protein was predominantly expressed in round spermatids and spermatocytes but also expressed in spermatogonia. In addition, Gas8 physically interacted with microtubules. In summary, Gas8 and Gas8-BP3 are both expressed in testes and regulated developmentally, suggesting that they may play roles in spermatogenesis.