

小鼠睪丸新同源箱基因 Tpros 之分子特性探討

Molecular Characterization of a Novel Murine Testis Expressed Homeobox Gene Tpros

中文摘要

同源箱基因(homeobox genes)能轉譯出同源區蛋白質(homeodomain proteins)在動物發育與組織器官成熟過程中常扮演轉錄調節因子(transcription factor)的角色。同源區乃一具高保留性的 60 個胺基酸功能區，能夠與目標基因上游啓動子(promoter)結合以達到調控目標基因是否表現的目的。我們利用退化性引子聚合酶鏈鎖反應(degenerated PCR)的方法在小鼠睪丸的 cDNA library 找到一新的同源箱基因 cDNA，利用該基因同源區蛋白質序列與資料庫比對發現，該基因與 prospero-like 家族(subfamily)基因有高度相似性，認為其可能屬此一家族，因此將其命名為 Tpros (testis pros-like homeobox)。我們發現 Tpros 基因有複雜的選擇性轉錄 (Alternative transcripts)，包含兩種蛋白質序列之不同的轉錄型態。經由北方點墨法和 RT-PCR 分析，得知該基因在小鼠睪丸有高度專一性表現，且約在小鼠出生後第 25 天開始表現，利用原位雜交分析發現 Tpros 專一表現在細精小管中的精細胞(spermatid)，推測其可能在精子生成過程後期的型態變化階段中扮演重要角色。我們也建構了 Tpros 的融合蛋白質，用來生產抗體，未來可利用免疫組織染色 (immunohistochemistry) 來進一步觀察 Tpros 在睪丸中表現的情形。根據之前分子特性的結果，我們推測 Tpros 基因可能在精子生成過程中扮演重要角色。爲了瞭解 Tpros 基因在活體器官內的功能，我們建構了基因剔除小鼠載體，將來藉由分析基因剔除小鼠表型來瞭解該基因的功能。

英文摘要

Homeobox genes encode homeodomain proteins which play a role as transcription factors in the developmental process. Homeodomain is a functional domain with 60 highly conserved amino acids, which can interact with the promoters of their target genes to regulate the expression of these genes. By using a degenerated-oligonucleotide-PCR strategy, we isolated a novel murine homeobox gene, and named it Tpros (testis pros-like homeobox) gene, because of its high homology to prospero subfamily genes. From the blast search, we found that Tpros has complicated alternative transcripts with at least 2 different open reading frames and 5 different transcripts. From the results of Northern blot and RT-PCR analyses, we identified that Tpros is specific expressed in mouse testis since postnatal days 25 with a message size of 1.6 Kb. In situ hybridization show that Tpros is expressed at spermatids in the seminiferous tubules. Tpros fusion proteins were also produced in

order to generate antibody so that we can further adopt immunohistochemistry to observe the Tpros protein expression in the testis. Based on the results from our preliminary molecular characterization, it's suggested that Tpros may play an important role during spermatogenesis. In order to understand the in vivo function of Tpros in the organism, we are in the processing of establishment of Tpros gene knockout mice. We will be able to know more about what Tpros plays in the mouse testis through analyzing the phenotype of Tpros deficiency mice.