罩丸鋅指蛋白基因 TZF-376 之分子特性探討

Anemonin inhibits the tyrosinase activity and expression of tyrosinase-related proteins in human epidermal melanocytes

中文摘要

目前約有六分之一的夫婦可能面臨不孕症的困擾，而其中有一半問題是出自於男性。主要造成男性不孕的原因，又以在精子熟成（spermatogenesis）過程中發生障礙所造成的原因爲最主要。已知有許多鋅指蛋白（zinc finger proteins）會在精子的發育過程表現，可能影響精子發育及其功能。鋅指蛋白是一種很常見的 DNA 結合蛋白，一般是以扮演轉錄因子（transcription factor）的角色，調節許多其它基因之表現。爲了尋找精子熟成過程中新的鋅指蛋白，我們利用網路上的基因庫進行人類表現序列標示（expressing sequence tags）（簡稱 EST）clones 的搜尋，輸入關鍵字 testis 及 zinc finger，找到一些表現在睪丸的鋅指蛋白類 EST clones，並針對這些 EST clones 之序列來設計 PCR 引子，起初進行分析的 EST clones 有 AI376558、AI003931、AI014681、AA417107 四個，進行各個組織（例如：腦、肝、腎等組織）的反轉錄 PCR（RT-PCR）實驗，以便瞭解此基因在各組織的表現，並製備 riboprobes。結果發現 AI376558 在許多組織皆有表現，但是以睪丸表現量最高，此外在睪丸有兩個不同之 PCR 產物，我們猜測其有 alternative splicing 的情形，因此我們目前較專注於 AI376558 之進一步分析，並將其命名爲 TZF376。由定序結果發現這是一個具有四個 C2H2 鋅指區的鋅指蛋白，基因位在人類第一號染色體上。另外我們亦定序幾個相關的 EST clones，結果顯示這個鋅指蛋白之 alternative splicing 相當地複雜，至少有四種不同的 alternative splicing，其中有一個 clone BE551217 缺少一個 20 bp 之核酸片段，結果造成三個鋅指區之缺損，這個蛋白質有可能對具鋅指區之蛋白產生負調節作用。此外，我們根據第一號染色體上之基因組序列設計三組橫跨 TZF376 exons 之 primers，以檢測 180 例不孕症之男性是否有 TZF376 基因之缺損情形。而更進一步原位雜交分析將有助於瞭解這個鋅指蛋白在睪丸中精細胞（germ cells）及體細胞（Sertoli and Leydig cells）的表現情形，如此可對基因之功能作一初步預測。

英文摘要

About one-sixth of the couples have the problem with infertility nowadays, and half of the problems come from male. Most of male infertility results from the impairment of spermatogenesis. We searched EST Genebank and identified several testis zinc finger protein genes. We would like to know whether these genes could influence the development and function of testis. Zinc finger is an extremely common protein domain of DNA binding proteins, and zinc finger proteins usually regulate other gene expression via their roles as transcription factors. The sequences of these zinc finger EST clones was used to design the PCR primers. RT-PCR analysis indicated that one of the EST clones, AI376558 might have some alternative splicing processing. We now focus on this EST clone and name this gene TZF376. Sequence analysis of several related EST clones showed that the alternative splicing of TZF376 is complicated. We have found at least 5 different alternative spliced EST clones, BE551217, with a 20 nt deletion encodes a truncated protein without zinc finger domain. In situ hybridization analysis was performed to characterize the expression pattern of TZF376. The localization in germ cells, Sertoli or Leydig cells of testis will imply the function of the genes. Intensive PCR screening of the TZF376 coding region on human chromosome 1 were performed to see if any deletion occur in cellular DNA of 180 infertile patients.These results will imply the correlation between TZF376 gene infertility.