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• 計畫英文名稱	Molecular and Functional Characterization of the Testis-Specific Homeobox Gene TSX1 (III)		
• 主管機關	行政院國家科學委員會	• 計畫編號	NSC91-2320-B038-009
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• 中文關鍵字	睪丸; 精子熟成; 同源箱基因; 不孕症		
• 英文關鍵字	Testis; Spermatogenesis; Homeobox gene; Infertility		
• 中文摘要	除了 Y 染色體上之某些片段確知與男性不孕有關外，是否有其他染色體上的基因牽涉這類病變，並不是很清楚，同時為探討同源箱基因(homeobox gene)在睪丸與精子發育的過程所扮演的角色，我們利用 PCR 的方法找到一個新的人類同源箱基因 (Homeobox gene)，在組織表現檢測結果中發現其在睪丸中有相當高的專一性表現，我們將之命名為 TSX1 基因 (testis specific homeobox gene)，我們利用反轉錄 PCR(RT-PCR)及原位雜交 (in situ hybridization)等技術，推知 TSX1 基因是表現在睪丸的精細胞上，我們推測 TSX1 基因與精子發育的過程可能有很大的關聯性。由於 TSX1 基因在睪丸有高專一性的表現，因此我們相當好奇是否有些男性不孕是由於 TSX1 基因的突變所造成。所以我們以 4 組的 PCR 引子檢測 250 位睪丸起因不孕症男性之染色體區域，但並未發現有不正常的 PCR 產物出現。我們進一步以 SSC 方式檢測是否有點突變或小片段基因被刪除，也未發現有不正常的片段出現。我們已經證明了 TSX1 基因並不存在於人類以外的其他物種。在過去，我們用人類的 TSX1 基因做為探針 (probe)，對數個小鼠睪丸 cDNA libraries 進行 TSX1 小鼠基因之篩選，也密切注意 GenBank 是否有小鼠 Tsx1 基因之發現。然而一直徒勞無功，我們於是進行了 Zoo blot，果然證實 TSX1 只存在於 human 的 genome 中，而未存在於其他物種中。為了能更進一步探討 TSX1 在活體中之功能，於是我們建立了 TSX1 基因轉殖鼠。經由 PCR 檢測，我們在 70 隻小鼠中共找到 8 隻帶有 TSX1 基因的 founder 小鼠，將這些小鼠與 wild type 小鼠交配後所生的小鼠經過 western blot 分析，我們發現有一個 line 母鼠的卵巢及三個 lines 的睪丸有 TSX1 之蛋白表現，但在組織及生育功能上並未發現有進一步之性狀。由這些基因轉殖鼠的結果看來，我們所使用的 pEF 啟動子，雖然是可以到處都表現的啟動子，但是在絕大多數組織中，並未發現有 TSX1 之表現，可能是受到「position effect」的影響，而在三個睪丸表現 TSX1 基因之轉殖鼠中缺乏性狀的結果，顯示睪丸可能存在某些調控機制壓抑了 TSX1 過量表現可能造成之傷害。		

- 英文摘要

There is not much genetic information about male infertility other than some segments on Y chromosome. To uncover more genetic information related to spermatogenesis and identify the function of homeobox genes in the testis, degenerated oligonucleotide PCR screening of testis cDNA libraries was performed to isolated more testis specific genes. One novel human testis-specific homeobox gene was identified and named TSX1 (testis-specific homeobox). RT PCR and Northern analysis confirmed that TSX1 is expressed in the adult testis and in situ hybridization identified that the expression is in the germ cells. These results suggest that TSX1 may play an important role during germ cell development. To determine whether mutations in TSX1 are related to impaired spermatogenesis, an intensive PCR screening was performed on 250 infertile man DNA samples with 4 sets of primers across the TSX1 gene locus. There was no unexpected PCR products identified in any of the 250 DNA samples. Further SSCP analyses were performed and no aberrant PCR fragment were identified. We have tried intensively and failed to identify the mouse *Tsx1* ortholog through frequently GenBank searching and many times mouse testis cDNA libraries screening. We then proved that the TSX1 is only present in the human but not other species' genome by Zoo blot analysis. This data implied that TSX1 is a gene appeared late during evolution and may have a more specific function to human. To understand the in vivo function of TSX1, we generated TSX1-overexpression transgenic mice with a ubiquitous promoter pEF. We obtained 8 transgenic founder lines from 70 mice. After western blot analysis, we identified 1 transgenic line with TSX1 expression in the ovary and 3 lines with expression in the testis. However, no significant phenotype in fertility or abnormal histology in reproduction tracts was observed after analysis for 3-4 generations. From the results of transgenic mice, we concluded that the pEF promoter was suppressed in most of the mouse tissues although it was assumed as a ubiquitous promoter, and the pEF promoter was also under the regulation of mechanisms which repressed the damage might arise from the overexpression of TSX1.