精原幹細胞的培養與鑑定研究

Cultivation and Characterization of Mouse Germ Line Stem Cells

中文摘要

幹原細胞精原幹細胞(germ line stem cells)爲一群具多功能(pluripotent)分化特性的早期幹原細胞，其可以自體新生(self-renew)，若分化爲精細胞，攜帶遺傳相關的基因，產生子代，進行世代交替。在小鼠系統中精原幹細胞包括三個部份：原生殖細胞 (primordial germ cells)，生殖細胞 (gonocytes)，及未分化的 A 型精原母細胞 (type A spermatogonia / spermatogonial stem cells)。

我們利用特別的培養系統，將新生小鼠體內的睪丸取出，讓其內部的細胞釋出，進行體外培養實驗，數天後觀察細胞形成群落(clones)，其型態類似早期培養胚胎幹細胞時會出現的胚體 (embryoid body; EB)，利用染色鑑定得知此群細胞呈現很強的鹼性磷酸酶 (alkaline phosphatase; AP) 活性表現。從 RT-PCR 與細胞免疫染色的結果得知其表現 Oct-4 的基因與蛋白質，其細胞表面抗原的表現則爲 SSEA1+，α6-integerin+，β1-integerin+，c-Kit-，CD31-，CD34-。除此之外檢測在培養皿中的細胞，則有 Oct-4+ Mvh+ Stella+ Fragilis+ Daz+ Piwi+ Tex14+ Gcnf+ 的基因表現。

將此群落的細胞打散，培養在含 condition medium 的 Methylcellulose 培養基，可以看到細胞 recolonization 的現象。我們進一步利用添加維他命 A 酸 (retinoic acid; RA)，來刺激精原幹細胞進行增生。初步的結果顯示在 0.5-2 μM 的 RA 處理下可促進細胞增生最高至 175 %。

英文摘要

Germ line stem cells are unique in stem cell biology and transmit genetics to next generation. Mouse germ line stem cells includes primordial germ cells (PGCs), gonocytes, and spermatogonial stem cells (SSCs). SSCs are responsible for maintaining spermatogenesis throughout life in the male by continuous production of daughter cells that differentiate into spermatozoa.

We successfully got some germ cell clones from culture neonatal ICR mice testis cells at specific condition in vitro. These clones look like embryoid body in morphology and show strong alkaline phosphatase activity, suggesting its primordial cells potential. In this report, we identify this clone by detection several genes by RT-PCR including Oct-4pos Mvhpos Stellapos Fragilispos Dazpos Piwipos Tex14pos Gcnfneg . In addition, we characterized the Oct-4 protein and other cell surface antigen expression by immunocytochemistry. This clone shows Oct-4pos, SSEA1pos, α6-integerinpos, β1-integerinpos, c-Kitlow, CD31neg, and CD34neg ,

like PGC or SSC origin.

As retinoic acid (RA) acts to stimulate proliferation of PGCs, we also test the cell proliferation ability of these clones induced by RA in vitro. Our preliminary result shows that these clones could be recolonized and the cell number increased to 175% at 2 μM RA concentration in condition - methylcellulose culture medium.