小鼠精原幹細胞的培養與分化

Cultivation and Differentiation of Neonatal Mouse Germ Line Stem Cells

中文摘要

精原幹細胞是成體幹細胞的一種，在雄性生物體中負責持續產生成熟精子。未開始分化的精原幹細胞大量存在於新生小鼠的睪丸內。其胚胎時期的演變是從原始生殖細胞（primordial germ cells）分化爲生殖母細胞（gonocytes），待出生後分化爲精原幹細胞（spermatogonial stem cells/生物體內 或稱 germ line stem cells/體外培養）。精原幹細胞的培養，目前有含血清和不含血清的培養系統，在含有血清的培養系統下，精原幹細胞表現轉錄因子 Oct-4 和鹼性磷酸酶活性，但也會有 c-kit 表現。c-kit 表現在已分化的精原幹細胞。本實驗室已建立一套不含血清的精原幹細胞體外培養系統，在此培養系統下，精原幹細胞表現胚胎幹細胞或精原幹細胞特有基因 Oct-4, Mvh, Fragilis, Stella, Nanog, Piwi-like 2, Sox2, Zfp145，以及強烈的鹼性磷酸酶活性，表現微弱的精細胞基因 Daz1 和 Tex14，但不表現 c-kit。這顯示了此培養系統維持了精原幹細胞保持在未分化的狀態。另外，利用血清、維他命 A 酸和其他生長因子，可以誘導精原幹細胞進行體外分化形成表現 c-kit 的細胞、神經細胞以及不成熟的肝臟細胞。此外，也利用帶有 GFP 綠色螢光基因的精原幹細胞進行體內分化的研究。以囊胚注射的方式，將體外培養的精原幹細胞送入囊胚，囊胚植入假受孕母鼠體內，觀察帶有 GFP 的精原幹細胞在胚胎發育過程中的分佈。穩定、能維持精原幹細胞特性的培養系統，有助於日後精原幹細胞在成體發育過程的研究，以及細胞株的建立。

英文摘要

Germ line stem cells (GSCs) are the cells which are able to self-renew and differentiate into sperm. It includes primordial germ cells (OCT4+c-KIT+), gonocytes (OCT4+c-KIT-), and spermatogonial stem cells (SSCs; OCT4+c-KIT- for Asingle to Apair early SSCs, and OCT4+c-KIT+ for Aaligment late SSCs). Many approaches have been shown to successfully cultivate putative GSCs in serum-containing medium in vitro. However, most of these cells are belonging to OCT4+c-KIT+ late developmental stage of GSCs; and the cultivation of early-undifferentiated GSCs (OCT4+c-KIT-) in serum free culture system has not been well defined. For this, we recently got some embryonic body-like colonies (EB-like) from testis of neonatal ICR mice under selective serum-free culture condition. These colonies showed in strong alkaline phosphatase activity, OCT-4 protein expression, and strong expression of GSC-specific genes, including Oct-4, Mvh, Fragilis, Stella, Piwi-like 2, Nanog, Sox2, Zfp145; very weak expression of differentiated germ cell-specific genes like Daz1 and

Tex14, but not c-kit (a stem cell factor receptor) and Gcnf (the Oct-4 repressor). The Oct-4 expression level of the GSCs is around 70 % of that in ES cells. Immunocytochemical study showed that these colonies expressed OCT-4, CD29, CD49f and SSEA-1; but not CD117 (c-kit). These OCT4+c-KIT- cells are able to differentiate into c-KIT+ germ cells, neuron-like cell lineage, and unmature hepatocytes under selective induction system in vitro. Further observation by utilizing EGFP-GSCs cells and blastocyst microinjection, we found the early OCT4+c-KIT- cells are capable of contributing to the three embryonic germ layers of chimeras' offspring. Together with these observations, in this thesis, we present a successful proliferation of early-stage (OCT4+C-KIT-) germ stem cells under a serum free culture system, and suggest the pluripotent potential of these early OCT4+C-KIT- GSCs colonies