• 系統編號	RN9310-0548
• 計畫中文名稱	原始造血幹原細胞之體外維護及增殖研究(核心計畫)(III)
• 計畫英文名稱	In Vitro Maintenance and Expansion of Primitive Haemopoietic Stem Cells (Core Project) (III)
• 主管機關	行政院國家科學委員會
• 計畫編號	NSC91-3112-B038-004-M51
• 執行機構	台北醫學院細胞及分子生物研究所
• 本期期間	9108 ~ 9207
• 報告頁數	10 頁
• 使用語言	--
• 研究人員	施子弼 --
• 中文關鍵字	造血幹原細胞; 體外增殖; 臍帶血; 紅血球; 人類間質幹細
• 英文關鍵字	CD34+ Hematopoietic stem/progenitor cells; Umbilical cord blood; Erythroblast; Human processed lipoaspirate
• 中文摘要	近年來幹原細胞生理特性探討及臨床應用的潛能受到廣泛重視。幹原細胞主要受到外在因子及內在因子的影響共同決定細胞的命運。血球幹原細胞因爲可分化成各種不同功能的血球系，因此它具備多重複雜的分化調節機制。如何控制細胞滯留於未分化狀態，以及特定細胞種類的分化是經由調控某些特定分化基因的表現，目前尚未非常清楚。本計畫旨在有效率地分離純化造血幹原細胞並冷凍保存，提供幹原細胞臨床應用或基礎研究。我們延續對(1)造血幹細胞之分離及針對幹細胞往紅血球分化相關基因進行分子特性探討。(2)藉由二維電泳分析比對紅血球分化早中晚之蛋白圖譜得知與分化相關之蛋白種類。(3)利用免疫缺陷小鼠將組織間質幹細胞與造血幹細胞進行骨髓移植比較間質幹細胞之來源與輔助造血之功能。我們將少量早期紅血球母細胞之 mRNA 進行放大數千倍反應以供 cDNA array 分析。針對早期胚胎肝臟及新生兒臍血與成人週邊血之造血幹細胞在紅血球生長分化上之差異比較結果顯示 CD34+HSP/C 幹細胞具有發育階段特異性及功能決定性，此差異不影響單一血球系成熟的路徑。另外，爲了探討基質細胞與造血系統微環境之互動影響，我們也分離自不同組織分離人類間質幹細胞並進行體外分化，探討其多重分化潛能。初步觀察到不同來源有分化爲間質組織功能細胞的現象。具體而言，我們在本計畫年度共完成(1)利用所建立之體外紅血球生成的模式，經由基因晶片分析及二維電泳分析，探討紅血球成熟過程之早、中、晚期基因及蛋白表達之分子機制。(3)我們觀察比較得知人體組織基質細胞之非同質性、組織功能性。(2)增進造血幹細胞之增殖及體外維護效率。爲得知是否能協助造血幹原細胞，我們也分離具基質細胞潛能之人類間質幹細胞 PLA，與造血幹細胞同時給予體內免疫缺陷(NOD/SCID)之老鼠，比較骨髓及脂肪取得之間質幹細胞分化潛能差異以及不同來源提供造血微環境 (microenviroment)的能力之相關性。
• 英文摘要	In recent years, the multi-potentials of CD34+ hematopoietic stem/progenitor cells (HS/PC) on broad clinical applications are widely investigated. Characterization of HS/PCs has therefore been the key for understanding how normal hematopoiesis precisely regulated at molecular level. Because of the

multiple-lineage commitment capacity of HS/PCs is decided both by extrinsic (environmental) and intrinsic (intracellular) factors. However, due to the limited number and the lack of unique cell marker of HS/PCs have hampered the progress of many studies. In the previous budget years, we have established a solid phase mRNA amplification, single cell culture, and single cell RT-PCR technologies that allowing us to continue explore this invitro study. In this budget year, we have continue (1) to characterize the CD34+ HS/PC subpopulation, identifying structural and functional changes in lineage specific erythropoietic differentiation of CD34+ HS/PCs and (2) to examine the proteomic variation of the early- and late- erythroblasts by two dimensional electrophoretic mapping (2DE) analysis. (3) to identify the cross-talk between stromal cells and HSCs that supporting normal hematopoietic reconstitution, we have conducted a comparative analysis of MSC and PLA that influencing the CD34+ HS/PCs transfusion efficiency in NOD/SCID mice. We have focused in comparative molecular characterizations of the CD34+ HS/PC population in fetus, new born and adult in terms of identifying their characteristic differences in lineage specific erythropoietic differentiation. We have also achieved a thousand folds in amplification of mRNA of early erythroblasts for cDNA microarray and subtraction analyses. To understand the supporting role of stromal cells that supporting normal hematopoietic reconstitution and ex vivo expansion we have isolate and primary culture of several tissue mesenchymal stem/progenitor cells and characterizing their potential heterogeneity. A comparison of MSC and PLA that influencing the CD34+ HS/PCs transfusion efficiency in an in vivo NOD/SCID mice study has been carried out and confirming the tissue specified role of the somatic MSCs. In summary, we have accomplished the proposed studies in this budget year including (1) to identify the developmental stage dependent erythropoietic gene expression regulatory differences. (2) to identify the molecular and functional heterogeneity of human tissue mesenchymal stem/progenitor cells. (3) to improve the in vitro maintenance and expansion of primitive hematopoietic stem cells. To understand the cross-talk between stromal cells and HSCs that supporting normal hematopoietic reconstitution, we have isolate and primary culture of processed lipoaspirate (PLA) and characterizing their differentiation potentials. A comparison of MSC and PLA that confirming the supporting the CD34+ HS/PCs transfusion efficiency by MSCs in NOD/SCID mice has been made.