• 系統編號	RN9701-3400
• 計畫中文名稱	人體組織前驅幹原細胞對於血管再生修復作用之研究
• 計畫英文名稱	A Study on the Role of Human Tissue Stem/Progenitor Cells in Neovasculogenesis
• 主管機關	行政院國家科學委員會
• 計畫編號	NSC95-2745-B038-006
• 執行機構	台北醫學大學細胞及分子生物研究所
• 本期期間	9508 ~ 9607
• 報告頁數	19 頁
• 使用語言	中文
• 研究人員	施子弼; 徐國基 Shih, Tzu-Bi, Daniel; Shyu, Kou-Gi
• 中文關鍵字	內皮前驅細胞; 造血前驅母細胞; 間質幹細胞; 血管修復再生; 組織修復; 分子機制
• 英文關鍵字	Endothelial progenitor cell, Erythroblast, Mesenchymal stem cell, Neovasculogenesis, Tissue repair, Molecular mechanism
• 中文摘要	胚胎幹細胞及成體組織幹細胞都具有應用於組織修復及血管新生的臨床價值。因此探討幹細胞於缺血或缺氧造成的損傷對於血管新生的分子調控機制格外重要。目前已知損傷的組織會吸引血流中的血管內皮前驅細胞取代壞死的組織，局部組織內含的間質幹細胞亦在週遭血管新生因子活化下，分化爲血管內皮細胞協助組織重建。血液中存在的內皮前驅細胞結合組織間質幹細胞或紅血球母細胞後，可主導組織修復及再生過程。許多研究指出分離及體外培養內皮前驅細胞的可行性，這些細胞大多來自血液中造血幹細胞及單核細胞。除了血球系細胞外，近年來非血球系的間質幹細胞也被發現於體外實驗中具血管內皮細胞分化之潛能。因此於本計畫利用基因質體學及蛋白質體學研究，比對探討及篩選內皮前驅細胞及間質幹細胞往內皮細胞分化及血管形成相關功能之重要基因。本計畫執行之主要目標包括(1)於不同氧壓的共同培養系統中，探討骨髓間質幹細胞及紅血球母細胞對周邊血內皮前驅細胞分化及血管再生所扮演之角色。(2)評估脂肪間質幹細胞作爲血管修復試劑的效益。(3)以活體外及活體試驗，鑑定出影響內皮前驅細胞及間質幹細胞分化爲內皮細胞的重要因子。在本年度研究我們建立了缺氧狀態紅血球母細胞影響間質幹細胞及內皮前驅細胞生成血管之培養系統，藉以分析間質幹細胞及紅血球母細胞於血管修復所扮演之角色。此外亦應用蛋白及基因微晶片試驗，集中探討紅血球母細胞在缺氧微環境變異影響間質幹細胞轉化血管性內邊細胞之基因及蛋白分子之表達(例如 ICAM-1, CXCR4, c-met ,CD44H and MMPs)、integrin 黏附分子(例如 integrin subunits a4, a5, b1, integrins avb3 andavb5)、血管新生性受體(例如 Flt-1, KDR, and Tie receptors)、生長因子(例如 HGF, NGF, IGF,G-SCF, GM-CSF and IL-3, 6, 8)、血管特異性轉錄因子(例如 Runx-1, Sca-1)於調控周邊血內皮前驅細胞及幹細胞衍生性血管內皮細胞之影響。藉由比對各種組織幹細胞所衍生之內皮前驅及內皮成熟細胞的功能差異性。本研究顯示紅血球母細胞在缺氧狀態下會有系統的表現細胞激素(Flt3-L, IL-6, OSM)，調節因子(IGFBP-2, IL-1ra)，造血因子(angiogenin, uPAR, VEGF)，以及化學激素主要因子(其中包括 eotaxin, ENA-78, GRO-？？, MCP-1, -3, -4, MIP-1？？, -3？？, PARC)。此外，我們觀察到當紅血球母細胞分泌各種 CC 以及 CXC 化學激素時，其作用在於發炎時的驅化反應以及活化炎性細胞驅化到受

損組織。再者，紅血球母細胞所分泌的各種細胞激素調節因子在缺氧狀態下刺激成人周邊血間質幹細胞時，展現了多元化的功能像是間質造血、肌細胞再生、神經細胞再生以及骨生成作用。這些結果顯示，紅血球母細胞除了化學驅化性以及造血性調節以外，在間質細胞的恆定上扮演重要角色且促進自體內分泌和旁體內分泌的平衡以及受損組織的修復。因此，血管新生不但要依賴內皮前趨細胞還要加上細胞的微環境去提供可溶性因子、細胞間質以及細胞間交互作用才能調控幹細胞、前趨細胞在受傷血管中進行修補以及再生。這些實驗結果提供我們研究整個系統的平台，缺氧誘發紅血球母細胞所導致內皮前驅細胞及間質幹細胞在血管修復再生作用中扮演角色之探討。

- 英文摘要

Recent studies show that both embryonic and adult stem cells have considerable potential for the repair and regeneration of damaged tissue. Understanding the regulatory mechanism of stem／progenitor cell mediated neo-vasculogenesis is essential for tissue repair after stroke or myocardial infraction. Several studies have shown angioblasts and the endothelial progenitor cells (EPCs) were derived from circulating blood. Injured tissues endothelial progenitor cells (EPCs) were recruited to replace necrotic cells. Mesenchymal stem cells (MSCs) also can be induced toward the endothelia trans-differentiation. EPCs and MSCs or erythroblasts are concerted to promote the neo-vasculogenic repairing of the ischemic damaged tissue. In this study we focused to gain better understanding of the vasculogenesis-related gene expression involvements of EPCs and MSCs. Specific aims of this study include: (1) To define the role of MSCs and erythroblasts in PB-EPC maturation and neo-vasculogenesis under the oxygen stress culture conditions, (2) To evaluate the efficiency of the MSCs as vascular regenerative agents, and (3) To identify factors that influent the vascular formation potential of EPCs and MSCs in in vitro and in vivo studies. In this budget year, we have established EPCs-MSC and EPCs-erythroblast co-culture systems that allowing us to analyze the roles of MSCs and erythroblasts in neovasculogenesis. We have also identified the stress influences of the microenvironment (such as ICAM-1, CXCR4, c-met, CD44H and MMPs), integrin adhesive molecules (such as integrin subunits alpha4, alpha5, beta1, integrins alphavbeta3 and alphavbeta5), angiogenic receptors (Flt-1, KDR, and Tie receptors), growth factors (HGF, NGF, IGF, G-SCF, GM-CSF and IL-3, 6, 8), and vascular specific transcription factors (Runx-1, Sca-1) which are known involved in the regulation of circulating EPCs and stem cell derived vascular endothelial cells. In this study we showed erythroblasts under the hypoxia stress, there were various hierarchically expressed cytokines (Flt3-L, IL-6, OSM), regulatory factors (IGFBP-2, IL-1ra), angiogenic factors (angiogenin, uPAR, ..VEGF), and followed by the other prevailing factors belong to chemokines family (including eotaxin, ENA-78, GRO-alpha, MCP-1, -3, -4, MIP-1, -3, PARC). Furthermore, we observed various CC and CXC chemokines secreted by erythroblasts are known as inflammatory and／or angiogenic factors, which can induce inflammation by chemoattracting and activating inflammatory cells to damaged tissue. Moreover, many cytokine／regulatory factors secreted by erythroblasts are shown to exert pleiotropic functions on the mesenchymal angiogenesis, myogenesis, neurogenesis and osteogenesis as demonstrated by stimulating the Ad-MSCs under the hypoxia stress (Table II). These results imply besides chemotaxis and hematopoietic regulation, erythroblasts in concerting mesenchymal cells display important roles on homeostasis and promotion of the tissue wound healing by a well balanced autocrine／paracrine net working mechanism. Therefore, vasculogenesis depends not only on the EPCs, but the cellular microenvironments, to provide the soluble factors, extracellular matrix and cell-cell interactions to modulate the fates of stem／progenitor cells in vascular injury repair and regeneration. Results obtained from this study provide us a better insight into system biological events in ischemic induced erythroidal mediated roles of EPCs and MSCs in neo-vasculogenesis.