人體周邊血促進血管生成之類內皮前驅細胞的分離培養及鑑定

Isolation and characterization of human peripheral blood derived endothelial progenitor-like cells for neo-vasculogenesis

中文摘要

內皮前驅細胞能夠修復受傷的血管，促進血管的再生，本實驗分離健康成人之週邊血單核細胞，利用特殊的培養方法，促使貼附性單核細胞分化爲類內皮前驅細胞，並鑑定其特徵及功能。自體外培養七天後得到的類內皮前驅細胞，具有攝取 DiI-Ac LDL 以及結合 ULEX-1 的功能，此特徵與前人所報導的內皮前驅細胞相像，並且藉著反轉錄聚合?連鎖反應以及免疫螢光染色，此細胞也表現內皮細胞之特有基因：VE-cadherin、vWF、eNOS，以及內皮細胞之特有蛋白：vWF。在細胞的功能性分析方面，得知類內皮前驅細胞在體外的細胞間質萃取物 (Matrigel) 上可以促使類似血管的生成；藉由流式細胞儀偵測培養天數不同的細胞表面分子表現：血源性細胞標的：CD105 會隨著培養天數大幅降低，另外，早期內皮細胞的內皮血管生長因子接受器 1 & 2 (Flt-1 & KDR) 隨著天數表現則逐漸升高，顯示血源性單核細胞轉分化 (trans-differentiation) 的趨勢。此外，在形成類內皮前驅細胞的過程，可以偵測到代表早期細胞的標記基因：Oct-4、Rex-1、Sox-2 的表現逐漸增高，並且細胞端粒?活性也增強，顯示成人細胞在經由培養之後會表現早期細胞的特性。進一步了解內皮前驅細胞分化過程中分泌了哪些激素，收集其第一天至第四天以及第四天至第七天，類內皮前驅細胞形成過程的培養液，以人類細胞激素蛋白表達微陣列之比對分析，可以得知養成類內皮前驅細胞第四天至第七天，細胞會大量表現：Hemangiogenic factors/Pro-angiogenic factors (b-FGF、G-CSF and GM-CSF)，Chemokines/Pro-inflammatory factors/Pro-angiogenic factors (GRO/CXCL-2、IL-8/CXCL-8、 I-309/CCL-1、I-TAC/CXCL-11、MCP-1/CCL-2、RANTES/CCL-5) 以及 Cytokine/Pro-inflammatory factors (IL-13) 這些細胞激素，可能經由 paracrine 或 autocrine 的機制調控其本身的分化，促使血管的生成及持久性和穩定性，或者扮演其他生理性功能。

經由以上的實驗結果證明在體外 (in vitro) 的情況下可以使成人週邊血中的血源系細胞轉分化或去分化成爲類內皮前驅細胞，並且可能經由細胞激素的刺激促進血管的再生，進一步將利用動物實驗證實。

英文摘要

This study describes the generation and characterization of human adult peripheral blood mononuclear-derived endothelial progenitor-like cells (EPLCs) as a resource may potentially useful for neo-vasculogenesis.

Peripheral blood mononuclear cell derived EPLCs are characterized by their phonotypical expression of CD34–, CD105+, CD14++, CD31++, CD45+++, and VEGF receptors positive (Flt-1++ and KDR++). These cells up-taking AC-LDL, binding ULEX-1 functions and exhibit tube formation in the Matrigel culture system in resemble to the CD34+, KDR++, AC133+ endothelial progenitor cells (EPCs). EPLCs express endothelial-specific genes such as VE-cadherin, vWF, and eNOS as functioning endothelial cells (ECs). During the course of EPLCs formation culture, increased early genes (Oct-4, Rex-1, and Sox-2) expression and enhanced telomerase activity were detected as their positive cell surface marker CD105 was switched off and at mean time turned on the angiogenic receptors Flt-1, and KDR. In contrast to the previously finding of angiogenic factors G-CSF, VEGF, HGF secreted by the EPCs18, the current study reveals that EPLCs secrete additional hemangiogenic factors/pro-angiogenic factors (b-FGF、GM-CSF), chemokines/pro-inflammatory factors/pro-angiogenic factors (GRO/CXCL-2、IL-8/CXCL-8、 I-309/CCL-1、I-TAC/CXCL-11、MCP-1/CCL-2、RANTES/CCL-5), and cytokine/pro-inflammatory factors (IL-13).

Taken together, the present study demonstrates that EPLCs can be derived from transforming human adult hematopoietic mononuclear cells into functioning endothelial progenitor like cells phenotype via the process of trans-dedifferentiation in vitro that may benefit for the vascular wound healing and regeneration in vivo. Animal model studies of the EPLCs are undergoing.