經由Hemin、HMBA及TPA誘導人類血球K562細胞內CSF-1R分析

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摘 要

幹細胞 (stem cell) 為形成血球組織及器官最原始之細胞，具 有分裂增殖及分化特性。細胞離開細胞週期後，因外來訊號刺激 後，經磷酸化酵素在其中伴演傳遞訊息之角色，形成似瀑布狀訊 息傳遞，因而導致分化進行。K562 細胞，為人類慢性骨髓性白血 病細胞，主要為血球細胞之前驅細胞 (progenitor) 。此細胞存在 的狀態，是進入了血球的分化路徑中，並具有部分幹細胞特性， 故若是添加不同誘導劑則會使K562 細胞分化成不同型態之細胞。 本論文以K562 細胞株為試驗細胞株，探討酪胺酸激.與絲胺 酸/酥胺酸激.參與K562 細胞株之紅血球分化途徑中的影響。添 加三種不同誘導劑hemin、HMBA (hexamethylene bisacetamide) 及 TPA (12-O-tetraphorbol 13-acetate) 於K562 細胞培養液時，令這些 外來刺激物使K562 細胞走向分化成紅血球或單核球細胞株之途 徑。當K562 細胞經由HMBA 及hemin 誘導後走向紅血球系細胞 時，同時會提高及　型血紅素的累積；而經由TPA 誘導物的添 加，會改變K562 細胞的構型，由原本的球狀表面變形成不規則 狀，而分化為單核球細胞。爾後將帶有放射線　32P-ATP 之退化性 引子 (degenerate primer) 放大酪胺酸激. (tyrosine kinase) 以及 絲胺酸/酥胺酸激. (serine/threonine kinase) 之片段。利用限制酵 素剪切，而後選殖出差異性之片段。試驗中發現到K562 細胞進行紅血球分化途徑時，蛋白質激.C-　(PKC-　) 與macrophage colony-stimulating factor receptor (MCSF-1R) 皆參與其中分化過 程。構築CSF-1R 基因之啟動子與加強子於pGL-3 載體，當轉染 K562 細胞後，添加TPA 於細胞培養液中，造成大量細胞凋亡，　其原因值得進一步探討。

關鍵詞 : 幹細胞、分化作用、HEMIN、HMBA、TPA

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