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• 計畫英文名稱	The regulation of HTm4 gene expression		
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• 研究人員	柯順龍 Ko, Shun-Long		
• 中文關鍵字	血液細胞; 抗 HTm4 多株抗體; 酵母菌雙向雜交技術		
• 英文關鍵字	Hematopoietic cell; Anti-HTm4 polyclonal antibody; Yeast two-hybrid assay; KAP; CDK2		
• 中文摘要	HTm4 調控細胞週期的行程是經由它本身和 CDK-associated phosphatase (KAP)之間的合作來達成的。我們最近發現在同步生長的細胞中、外加生產(ectopicexpression)的 HTm4 會造成它們的細胞循環停止在 G0 的階段。此 G0 細胞循環中止之主因可能是 CDK2 被 KAP 去磷化(dephosphorylation)而造成、而 KAP 的去磷化之功能的增強更因它和 HTm4 結合而來的。由於體內 HTm4 蛋白質的表現主要是局限於血液細胞、因而 HTm4 應該被視為血液細胞特有的細胞循環調控者。目前我們對於 HTm4 調控機制尚未了解、於是這個研究計劃是針對這個方向來進行。本計劃著重於用老鼠系統來研究 HTm4 蛋白質之功能和調控機制、其包括體內(in vivo)和體外(in vitro)的研究。我已經開始生產兔子抗 HTm4 多株抗體(在此稱 mN25)。mN25 可偵測到老鼠脾臟細胞內所產生的 HTm4 蛋白質、這正反應出 mN25 抗體的專一性、其將適合用於研究 HTm4 蛋白質之組織分佈和其與其它體內蛋白質的互相對應關係。在尋求和 HTm4 有互動反應的蛋白質(interacting proteins)方面、我們利用酵母菌雙向雜交技術(yeast two hybrid techque)。Interacting proteins 的來源是老鼠第 17 天胚胎的互補 DNA(cDNA) library。目前初步篩選出 156 cDNA clones。進一步仔細的分析實驗正在進行當中。		
• 英文摘要	HTm4 modulates cell cycle progression through its interaction with a CDK-associated phosphatase (KAP). We have demonstrated that the ectopic expression of HTm4 brought about G0 cell cycle arrest in the synchronized host cell (Ko, etal, J. Clin. Invest. 2002, 109:51-58), and the occurrence of this cell cycle arrest is likely through the dephosphorylation of CDK2 by KAP, a function promoted by its binding to HTm4 protein. In addition, HTm4 is expressed predominately in cells of hematopoietic origin in both human and		

murine, which qualifying HTm4 as a hematopoietic cell cycle regulator. Thus far the mechanism by which the regulation of HTm4 gene expression might be monitored is still unknown. We set out to study the function of HTm4 in murine system, which can facilitate the goals at hands with both in-vitro and in-vivo experimental approaches. We have started producing rabbit anti-HTm4 polyclonal antibody, which was shown to have interacted with HTm4 protein in murine spleen cells. In addition, HTm4-Yeast two- hybrid hybridization was performed to identify the interacting proteins in a library derived from day 17 fetal cDNA library. We have isolated 156 yeast colonies that met the criteria of positive reaction for possible HTm4-target protein interaction. Work is currently under way in the analysis of every isolated clones and authentication of true interaction between HTm4 and its true target proteins.