行政院國家科學委員會補助專題研究計畫 x 成果報告 □期中進度報告

探討單核細胞分化的分子機制及 BNIP3 在 all-trans retinoic acid 誘導 U937 細胞分化中所扮演之角色

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執行單位：中山醫學大學生化學科

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（一）計畫中文摘要。（五百字以內）

單核細胞分化、急性骨髓細胞白血病、急性前骨髓細胞白血病、All-*trans* retinoic acid、cDNA microarray hybridization、RT-PCR、1.25-dihydroxyvitamin D3、PMA、BNip3

人體血液中循環的單核細胞與組織中的巨噬細胞組成了單核細胞吞噬系統。在骨髓造血過程中，單核細胞會循環到組織而分化成巨噬細胞，當發炎反應產生時，巨噬細胞會被活化以進行個體免疫防禦作用，故單核血球的分化是innate 免疫系統中很重要的生理現象。除此之外，當血球分化不正常時也會造成嚴重疾病，例如急性骨髓細胞白血病（Acute Myeloid Leukemia；簡稱 AML）為一全球性的血癌疾病，臨床上病患體內存有大量未分化的骨髓細胞，而其中又以急性前骨髓細胞白血病（Acute Promyelocytic Leukemia；簡稱 APL）佔多數，APL 患者多數具有染色體重組 t(15;17)現象，因此產生 promyelocyte-retinoic receptor α（*PML-RAR* α）融合基因，治療上利用 All-*trans* retinoic acid（ATRA）可以有效的促進單核血球分化為巨噬細胞因而減輕疾病的症狀。所以單核血球的分化在病理上及治療上均佔有極重要的影響，本計畫之一將探討單核血球/巨噬細胞分化的分子機轉。

由於人類基因體計畫的完成，對於疾病醫學的研究有很大的幫助，cDNA microarray hybridization 技術的應用更是使我們對於基因功能的研究快速的推進。本實驗室先前曾利用 ATRA 來處理人類 U937（myelomonocytic cell line）細胞，誘導其分化成巨噬細胞，在不同的刺激時間點下收集細胞的 mRNA，做 cDNA 晶片（1200 點，sequence varified）雜交試驗，以探討單核細胞分化成巨噬細胞時可能參與調控之基因的表達變化。由晶片雜交實驗結果我們得到 45 個基因（BNip3、PI3K γ 及 HSP90 等）表達有明顯的變化，初步 RT-PCR 的結果也證實晶片雜交實驗之正確性，並且 antisense BNip3 oligonucleotide 處理 U937 細胞可以抑制 ATRA 所誘導的分化，因此 BNip3 基因的表現對單核細胞分化可能具有重要的影響，故本計畫之二將探討 BNip3 在 ATRA 誘導 U937 細胞分化過程中所扮演的功能。

綜合之，血球分化是非常複雜但又極重要的生理機制，目前對於 monocyte/macrophage 分化的調控過程仍然不清楚。由於先前使小數目的 cDNA 晶片，已經可以發現少數重要基因(如 BNip3)的表達與分化有關，故在此計畫書中；（1）我們想用 1 萬點以上的 cDNA chip 全面（genome-wide）探討 U937 細胞在不同試劑如 1.25-dihydroxyvitamin D3、ATRA、TPA 誘導分化下其基因表達的變化，此實驗結果應有助於了解 monocyte/macrophage 分化的分子調控機制，同時對於 AML 疾病的產生及治療更能掌握。（2）BNip3 基因在 ATRA 誘導 U937 細胞分化過程中會大量表達，而 antisense BNip3 oligonucleotides 處理

U937細胞可以有效抑制ATRA所誘導的分化。因此，我們也想進一步確認BNip3在ATRA誘導U937細胞分化過程中，所扮演的角色為何？

(二)計畫英文摘要。(五百字以內)Acute Myeloid Leukemia、Acute Promyelocytic Leukemia、All-*trans* retinoic acid、cDNA microarray hybridization、RT-PCR、1.25-dihydroxyvitamin D3、PMA、BNip3

Myeloid progenitors in the bone marrow differentiate into promonocytes and then into circulating monocytes which migrate through the blood vessel walls into the various organs to become macrophages. This is an important phenomenon in the immune system. Besides, when the cell differentiation were blockade for example, acute myeloid leukemia (AML) is a worldwide disease, the pathologic feature of AML is the excessive accumulation of immature nonlymphatic bone marrow precursor cells in the marrow itself and in peripheral blood. One of the sub-group acute promyelocytic leukemia (APL), is characterized by the arrest of granulopoiesis at the promyelocytic stage and is generally associated with a t(15；17) translocation that fuses the *PML* gene to the *RAR* α gene. All-*trans* retinoic acid (ATRA) induced differentiation therapy has been successful as a treatment for APL. However, ATRA has had limited success as a single agent in the treatment of other hematopoietic malignancies. Therefore to understand the molecular mechanisms that control the myelocytic cell differentiation will further improve the differentiation therapy for leukemia. In this proposal one of the subject is to study the molecular mechanisms that control of monocyte/macrophage differentiation.

Gene expression microarrays are a powerful new tool in molecular biology. We have applied cDNA microarray technology to study the gene expression patterns of ATRA- induced U937 cell differentiation. For this, the cells were treated with ATRA at different time points, followed by cDNA-chips (1200, MillenniaChip) hybridization. We thus identified 45 cellular genes (BNip3、PI3K γ and HSP90 etc.) whose expressions were induced or repressed during U937 cell differentiation and their expression patterns were later confirmed by RT-PCR. Antisense BNip3 oligonucleotides treatment can block the ATRA-induced U937 differentiation, which implicated the expression of BNip3 may be play an important role in the ATRA-induced U937 differentiation. Therefore, in this proposal the second subject is to characterize the function of BNip3 in ATRA-induced U937 cell differentiation.

The development of pluripotent hematopoietic stem cell in the bone marrow to generate a peripheral tissue macrophages requires a series finely controlled, the molecular mechanisms of this event remain unclear. From our previous study, we are

able to gain some promising results with a small cDNA chip hybridization, therefore, in this proposal, we propose to:

（1）genome- wide identify of monocyte/macrophage differentiation associated genes with different differentiation inducer (such as 1.25-dihydroxyvitamin D3、ATRA、TPA) by cDNA microarrays. （2）Since BNip3 gene expression is important for ATRA-induced U937 cell differentiation, it would be interesting to study the function of BNip3 in ATRA-induced U937 cell differentiation.

（三）計畫結果

由於本計畫只得到一年經費補助，故實驗無法完全執行，謹將現有之結果呈報。

Fig. 1 全反維生素 A 酸會抑制 U937 細胞的生長

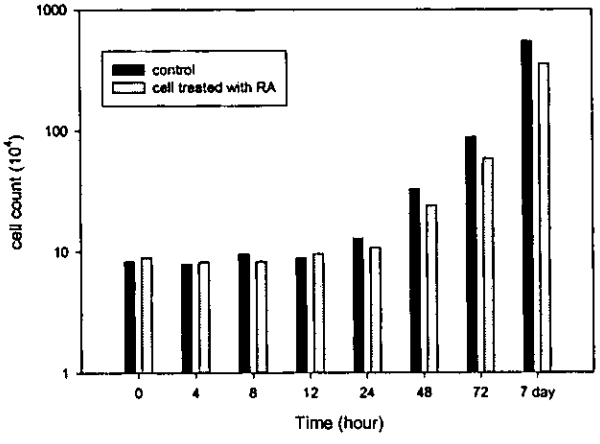


Fig. 2 全反維生素 A 酸的處理會增加 U937 細胞 CD11b 的表現

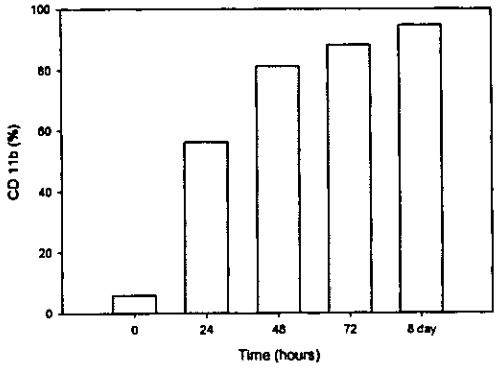
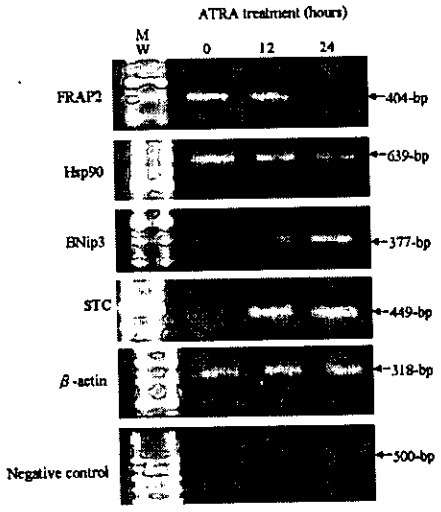


Fig. 3 mRNA from the U937 cells treated with ATRA for 0, 12 and 24h, were subjected to RT-PCR. The amplified β-actin gene fragment served as a control for normalizing the amounts of mRNA samples used in this study. The negative control denotes that the RT-PCR reaction was performed without reverse transcriptase, this control is for detection genomic DNA contamination during RNA preparations.



In figure 3, the expression of FRAP2 and Hsp90 were down regulated and the expression of BNip3 and STC were up regulated in the ATRA-induced U937 cell differentiation respectively. The results of the RT-PCR experiment, therefore, agreed with the data derived from the previous cDNA microaray hybridization experiments (figure 1).

Fig. 4 全反維生素 A 酸會增加 U937 細胞 BNip3 蛋白的表現

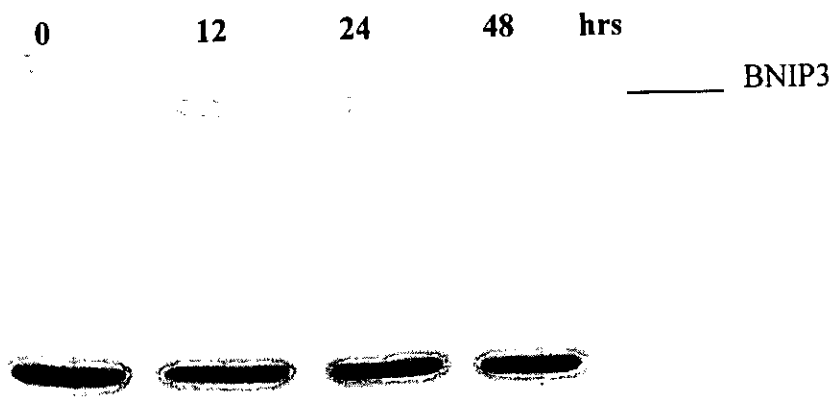


Fig. 5 BNip3 antisense oliginucleotide 會抑制 U937 細胞 BNIP3 的蛋白表現

Anti-sense(uM)	0	0	0.5	10
RA (24 hours)	-	+	+	+