行政院國家科學委員會補助專題研究計畫成果報告

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選殖及定性分析調控 CCR5 表達之激發性傳譯因子

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計畫編號：NSC89－2320－B－040－021－

執行期間：88 年 8 月 1 日至 89 年 7 月 31 日

計畫主持人：謝碧琴

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執行單位：中山醫學院 生化科

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行政院國家科學委員會專題研究計畫
成果報告

國科會專題研究計畫成果報告

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中文摘要

關鍵詞：人類免疫不全病毒第一型（愛滋病毒）；愛滋病毒的受體及副受體；愛滋病毒的嗜性；激發性傳錄因子基因；啟動子；多樣性

HIV-1 要進入 CD4 細胞必須透過與細胞表面之主要受體（Receptor）CD4 及其中之一個副受體（Co-receptor）CXCR4 或 CCR5 的結合之機制。已知 CXCR4 蛋白質是做為嗜 T 淋巴球 HIV-1 株 (T-tropic strains) 的副受體，而 CCR5 是嗜巨噬細胞 HIV-1 株 (M-tropic strain) 的副受體。在臨床上，嗜巨噬細胞 HIV-1 株是早期病毒感染的主力，到了後期（愛滋病階段），絕大部份的病毒變為嗜 T 淋巴球 HIV-1 株並使用 CXCR4 為副受體。由於 HIV-1 在不同的疾病階段，使用不同蛋白質做為感染不同細胞的副受體，所以許多研究就針對這兩個蛋白質的表達做深入之探討。最近之實驗發現 Monocyte（嗜巨噬細胞的母細胞）其實也會表達 CXCR4，並且可以輕易的被嗜 T 淋巴球 HIV-1 株所感染，但是一但分化成為嗜巨噬細胞（受 Retinoic Acid; RA 刺激），則變為可被巨噬細胞 HIV-1 株感染但抗拒嗜 T 淋巴球 HIV-1 株，這樣之變化導源於副受體 CXCR4 及 CCR5 在分化時表達的消長。有許多臨床實驗也證實細胞表面上的 CCR5 分子數目的多寡，會決定或影響該個體是否會被 HIV-1 感染，或感染後 AIDS 疾病發展之速度 (disease progression)。最近更有報告指出 CCR5 基因啟動子的多樣性 (Polymorphism: 例如 pCCR5-59029A/G、pCCR5-59653T/C 、pCCR5p1 等）也會影響愛滋病的疾病發展速度，因此，了解是何種蛋白因子在調控 CCR5 蛋白之表達是非常重要的。

英文摘要

Keywords：Human immunodeficiency virus type 1, CCR5 Promoter Polymorphism, Retinoic Acid, Inducible Cellular Factor,

Human immunodeficiency virus type 1 (HIV-1) is the etiologic agent of AIDS (acquired immunodeficiency syndrome) that causes the worst global epidemic known to human history. Cellular entry of HIV-1 requires binding to both CD4 (the primary receptor) and one of the seven transmembrane G-protein-coupled chemokine recpeptors, CXCR4 or CCR5, as the major co-receptors. CXCR4 has been shown to mediate entry of T-cell line adapted (T-tropic) HIV-1 strains into peripheral blood lymphocytes (PBL) and T-cell, whereas CCR5 on the surface of PBL and monocytes/macrophages mediates the infection of macrophage-tropic (M-tropic) viral strains. The importance of chemokine receptors to the HIV-1 entry and AIDS pathogenesis has therefore promoted intensive investigation on the co-receptor usage during the viral transmission. Recent study has demonstrated that monocytic cell line U937

does expressed CXCR4 on the cell surface that is more susceptible for T-tropic HIV-1 infection. However, upon differentiation to macrophage or more mature phenotype by retinoic acid, certain U937 clones become highly susceptible to M-tropic but resist to T-tropic HIV-1 infections. This dichotomous effects in susceptibility of viral transmission is governed by the differential expression of CXCR4 and CCR5.

Many correlation studies have also suggested that the levels of CCR5 on the $CD4^+$ cells surface determine susceptibility for the infection of M-tropic strains and the rate of the disease progression in human. Other studies have also confirmed that the polymorphism of the CCR5 promoter has closely related to the disease progression to AIDS, pCCR5-59029G/G individuals progressed to AIDS on average 3.8 years more slowly than pCCR5-59029A/A individuals, therefore, it is important to study the factors that regulate the CCR5 expression.

緣起及目的

It is estimated that 40 millions people will be infected with Human immunodeficiency virus type 1 (HIV-1) in year 2000 worldwide. 3
Epidemic of HIV-1 is no doubt the worst catastrophe in human history. HIV-1, a lentivirus, belongs to a genus of the Retroviridae family, and causes acquired immunodeficiency syndrome (AIDS) in human (1). Depending on the physical conditions of the host, HIV-1 can establish a clinical latent stage in the infected hosts in excess of 15 years (2). One of the mechanisms whereby HIV-1 escapes host immune surveillance, leading thereby to the virus' latent period, is the constant altering of its immunogenic surface antigens.

Human immunodeficiency virus uses $CD4^+$ as the primary receptor and chemokine co-receptors to enter target cells (3). Chemokine receptors belong to the superfamily of G protein-coupled receptors that have seven transmembrane domains. They can be divided in two groups: the α (CXC) and the β (CC) subfamilies, which have or does not have a single amino acid, respectively, inserted between the first and the second cysteine residues of the proteins. The binding of chemokines to their receptors induces a rapid calcium influx and inflammatory responses in the receptor-bearing cells (4). However, recent studies have demonstrated that CCR5 and CXCR4 mutants defective in G-protein signaling are still active in mediating HIV-1 infection (5,6).

The chemokine receptors CXCR4 and CCR5 are the major co-receptors for HIV-1 entry into $CD4^+$ cells. The importance of chemokine receptors for HIV-1 entry and AIDS pathogenesis has promoted intensive investigations (7,8). It is found that during primary infection, most HIV-1 isolates are macrophage-tropic (M-tropic) and the viral isolates tend to use CCR5 for cell entry (9). While at the later stage, M-tropic viruses tend to become less prominent and are generally replaced by T- cell tropic (T-tropic) viruses (10,11), which use CXCR4 for viral entry. This correlation of the disease progression and HIV-1 tropism is believed to be the result of altering co-receptor usage driven by the selection of new target cells for infection (12).

For studying the infection of M-tropic HIV-1, promonocytic cell line, such as U937, has been frequently used as a cell line model (13). However, recent studies demonstrated that certain U937 cell clones are relatively

resistant to the infection of M-tropic HIV-1 isolates, but susceptible to T-tropic viruses (14). Upon differentiation of these U937 clones to macrophage or mature phenotype by retionic acid, U937 cells become highly susceptible to M-tropic but resist to T-cell tropic HIV-1 infections. The change in susceptibility of viral transmission is governed by the differential expression of CXCR4 and CCR5 (15). Recently, Kostrikis L. G. et al. also reported a nucleotide transition from C to T at position 59653 in the promoter (pCCR5-59653T) region was in complete linkage disequilibrium with the codon 64 missense mutation of CCR2, suggesting that the action of CCR2-V64I allele in delaying HIV-1 disease progression is through the pCCR5-59653 mutation that directs less CCR5 expression (16).

Since the differential expression of CCR5 in cells surface is likely to control the HIV-1 infection and the disease development, it is important to understand how the cellular factor(s) regulate the CCR5 protein expression. Based on the previous studies in our lab, in this proposal we intends to **(A)** Cloning and characterization of the gene(s) for the inducible cellular factors that bind to the promoter of the CCR5 during the monocytic cells differentiated by retinoic acid treatment and further to study the CCR5 protein expression by the promoter activity assay. **(B)** DNase 1 Footprinting analysis of the polymorphic CCR5 promoter with the nuclear extracts prepared from the RA treated or untrated U937 cell. Further to clone those DNA binding proteins by screening the subtractive cDNA library of U937 with specific motifs (which were identified during the footprinting analysis) on the polymorphic CCR5 promoter sequences. Determined CCR5 genotype from 30-50 individual, by PCR *in-situ* hybridization and FACScan analysis to detect the mRNA、cell surface CCR5 protein expression whether associate with the polymorphic CCR5.

結果及討論

The result showed that three haplotypes; P1, P2, and P4 were identified in this group, and the frequencies of P1/P1, P1/P2, P1/P4 and P4/P4 were 20.65%, 1.08%, 41.30% and 36.95%, respectively. We further correlated the genotype with the levels of CCR5 expression on cell surface. There were no significant differences between the promoter genotypes and CCR5 protein levles had been detected.

Since p59029 A drives 2-fold greater CAT activity than p59029 G *in vitro*, we speculated that P1 has a stronger promoter activity. We further analyzed the correlation of the three ccr5-59653 genotypes (C/C, C/T, and T/T) with the level CCR5 expression on PBMC derived from P1 homozygote. From this FACScan data, found a significantly lower percentage of PBMC and CD4+ cells expressing CCR5 which had two 59653 T alleles (Fig.1). Finally, the results of gel retardation, show the different binding pattern factors were detected due to the polymorphism of 59029 (G/A) or p59653 (C/T) (Fig.2).

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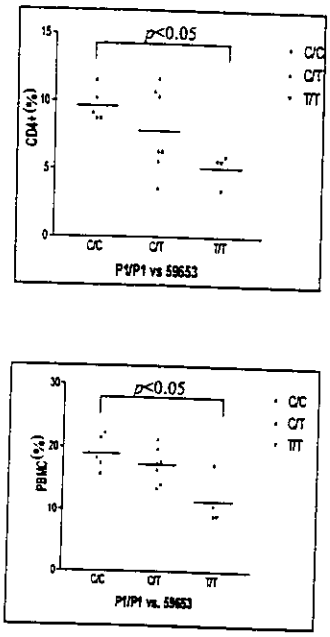

Fig.1：ccr5-p59653 基因的多形性-減少PBMC及 $CD4^+$ 細胞中具CCR5蛋白的細胞百分比數量。若p59653 爲T/T，則會使具CCR5 蛋白的PBMC與$CD4^+$細胞數量減少；p<0.05爲具統計意義。

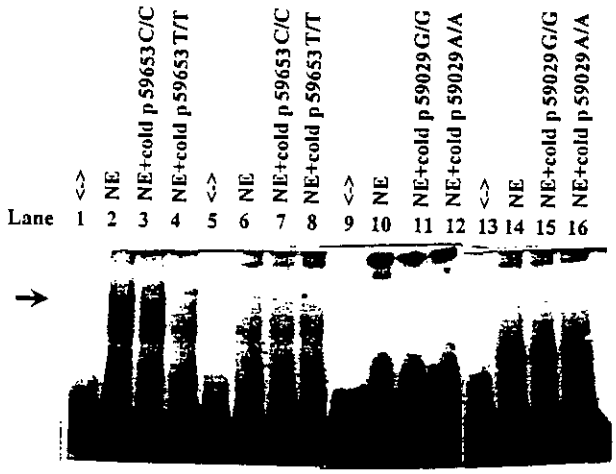

Fig.2：以gel retardation 分析啓動子多形性對未以*all-trans* retinoic acid 刺激的 U937 細胞核抽取物的結合有何不同，及不同多形性間的交互作用。lane1-lane 16 皆含標定 ^{32}P的探針(25bp)，根據探針的不同可分爲四組（1）lane 1-lane 4爲ccr5-p59029 A探針（2）lane 5-lane 8爲ccr5-p59029 G探針（3）lane 9-lane 12爲ccr5-p59653 T探針及（4）lane 13-lane 16 爲ccr5-p59653 C探針。（-）未加入細胞核抽取物。NE：加入未以RA 刺激的U937細胞核萃取物 6ug。cold表加入未標定^{32}P的探針10ng