行政院國家科學委員會補助專題研究計畫成果報告

愛滋病毒蛋白 VPR 之功能研究及其與 HAX-1 和脊椎肌肉萎縮基因之作用

計畫類別：☑個別型計畫　□整合型計畫

計畫編號：NSC 89 －2314－B－002－384－

執行期間： 89 年 8 月 1 日至 90 年 7 月 31 日

計畫主持人：黃立民教授

本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份

□赴大陸地區出差或研習心得報告一份

□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

執行單位：台大醫學院小兒部

中　華　民　國 90 年 10 月 30 日

行政院國家科學委員會專題研究計畫成果報告

愛滋病毒蛋白 VPR 之功能研究及其與 HAX-1 和脊椎肌肉萎縮基因之作用

Preparation of NSC Project Reports

計畫編號：NSC 89-2314-B-002-384

執行期限：89 年 8 月 1 日至 90 年 7 月 31 日

主持人：黃立民　　台大醫學院小兒部

一、中文摘要

Vpr 為愛滋病毒附屬基因蛋白由 96 個氨基酸所組成。病毒生殖週期中 Vpr 執行的多效性功能包括核換位，細胞週期阻斷，異位活化，增進病毒複製，與細胞凋亡。酵母菌雙混種系統是鑑定蛋白與蛋白作用之利器，本實驗室已成功地利用此系統尋找到一些會與 Vpr 蛋白作用之細胞蛋白，包括訊息傳遞蛋白 HAX-1，脊椎肌肉萎縮基因蛋白 SMN1(survival of motor neuron 1, human spinal muscular atrophy disease gene)。藉一系列 Vpr 刪除突變蛋白(deleted muntants)已鑑定出那些次元是與 HAX-1 或 SMN1 相互作用的關鍵點。本研究計畫業已證明在酵母菌雙混種系統中當 HAX-1 融合上 DNA 接合次元具有轉錄活化的效應，在 T 淋巴細胞株內 Vpr 可增加 HAX-1 轉錄活化的作用。關於 Vpr 與 SMN1 相互作用的功能意義較難釐清是基於神經蛋白 SMN1 如何誘導運動神經失常(degeneration of motor neuron)造成肌肉萎縮(muscular atrophy)仍是待解的謎題。相關研究指出 Vpr 會造成神經細胞的凋亡(apoptosis)，部份研究亦證明 SMN1 與抗細胞凋亡的蛋白 NAIP(neuronal apoptosis inhibitory protein)及 Bcl-2 都有關係，我們未來的研究將進一步探究 SMN1 與 Vpr 之間功能聯結的關係。

關鍵詞： 愛滋病毒，酵母菌雙混種系統，Vpr, HAX-1

Abstract

HIV-1 Vpr, a 96-amino-acid 14-kDa protein, has several important and interesting functions, including nuclear translocation of the pre-integration complex, cell cycle arrest at the G2/M phase, transactivation, enhancement of virus replication, and apoptosis. To understand the role of Vpr in HIV-1 life cycle and pathogenesis, yeast two-hybrid system had been used and cDNA encoding HAX-1 or SMN1 protein was identified. Interaction between Vpr and HAX-1 (or SMN1) was characterized by in vitro protein-protein binding assay. With the aid of a panel of Vpr deletion and point mutants, we have determined the domains of Vpr involved in the interaction with HAX-1 (.or SMN1). HAX-1 displayed a significant transcriptional effect in yeast when it was fused to a DNA binding domain. Vpr could enhance this transcription effect of HAX-1 in Jurkat cells.

It is difficult to evaluate the interaction of Vpr and SMN1 since little is known about SMN1 how to cause the degeneration of motor neuron the developing to spinal muscular atrophy disease. Newly studies find that Vpr induces apoptosis in neuronal cells, and the correlations between SMN1 and antiapoptosis protein, NAIP (neuronal apoptosis inhibitory protein) and Bcl-2, has been proved. In coming project we will try to verify the possible functional mechanism.

This article provides guidance for report writing under the Grant of National Science Council beginning from fiscal year 1998.

Keywords.: HIV-1, Vpr, yeast two-hybrid system

二、緣由與目的

HIV-1.has six auxiliary genes; 2 of them are essential (tat and rev) and 4 are accessory

(vif, vpr, vpu and nef) (Subbramanian and Cohen, 1994). These 4 accessory genes have attracted most intense research interest in recent years as we come to know their close relationship with in vivo pathogenicity. This project is aimed to understand the operating mechanism of HIV-1 vpr gene by identification of Vpr-interacting cellular proteins. Yeast two-hybrid system is a powerful tool in disclosing protein-protein interaction (Chien et al, 1991; Durfee et al, 1993; Gyuris et al, 1993). Successfully identification of SMN1 and HAX-1suggests that Vpr may be involved in spinal muscular atrophy, signal transduction, and apoptosis by using two-hybrid system. Up to now, several functions have been attributed to HIV-1 Vpr including moderating viral replication, nuclear transport of preintegration complexes, transactivation function, cell cycle arrest at G2/M phase, and apoptosis effects on the host cells (for review see Huang and Jeang, 1995; He et al., 1995; Stewart et al., 1997). Identification of Vpr-interacting cellular proteins can not only understand the working mechanisms of Vpr but reveal possible novel function of Vpr-associated proteins.

三、結果與討論

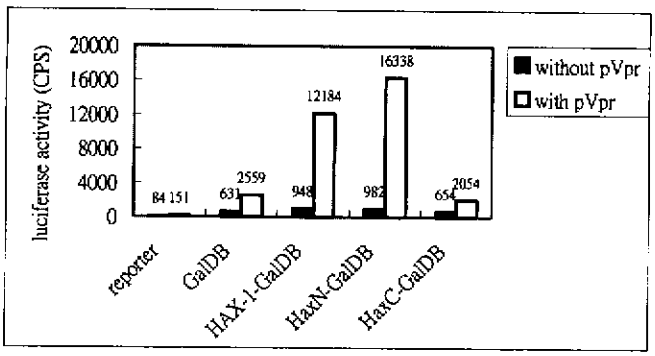
The interaction of Vpr and HAX-1

To identify new Vpr-associated proteins, we used the yeast two-hybrid screen of a human Bone Marrow cDNA library (Clontech, USA) with Vpr as bait. Here, we report the identification of a new Vpr binding partner, HAX-1, which is an HS1-associated protein. In spite of previously studies showing that HAX-1 associated with HS1, PKD2, and EBNA-LP, little is know about the biological function (Yauhiro et al.,1997; Anna et al.,2000; Yasushi et al.,2000). Our findings suggest that HAX-1 has transcriptional activation effect when it is fused to the DNA biding domain in yeast two-hybrid system, and Vpr further enhances this activation effect of HAX-1 restricted in Jurkat cells. We conclude that that HAX-1 might be involved in transcription machinery of the cell. In the presence of HIV-1 Vpr, this transcriptional function will be improved.

Table1. The activation effect of LexADB-HAX-1 hybrid protein in yeast system

LexA DB-HAX-1 deletion muntant	-Histidine + 20mM3-AT	-Histidine + 200mM 3-AT	β-gal activity
1. HAX-1 (1-279 a.a.)	++++	+	++++
2. Hax N (1-140 a.a.)	++++	++	++++
3. Hax C (141-279 a.a.)	–	–	–
4. Hax N48 (48-140 a.a.)	++++	+	++++
5. Hax N94 (94-140 a.a.)	±	–	±

Fig.1.Vpr augmentation of the activation effect of



GalDB-HAX-1 fusion protein in Jurkat cells

The interaction of Vpr and SMN1

Our data have showed the strong interaction between Vpr and SMN1 and the binding sites also have determined in yeast two-hybrid system.

GalAD / LeXADB	HAX-1	
	-His +10mm3-AT	β-gal activity
Wt(1-96 a.a.)	++++	++++
VpI(1-42 a.a.)	—	—
VpII(43-82 a.a.)	—	—
VpIII(77-96a.a.)	—	—

四、計畫成果自評

In this stydy, we have successfully identified

the new Vpr interacting proteins, HAX-1 and SMN1, through yeast two-hybrid screen. Here, we first report that HAX-1 has transcriptional activation effect when it fused to DNA binding domain in yeast, and Vpr further enhance this activation effect in mammalian cell system. Our data also showed the strong interaction between Vpr and SMN1 and the binding sites have determined in yeast two-hybrid system. We believe that our study may clarify the pathologic role played by Vpr and its newly associated proteins, HAX-1 and SMN1, in. AIDS pathogencity by future study.

五、參考文獻

1. Anna RG, Anna C, Norbert G, Stefan S. The polycystic kidney disease pretein PKD2 interacts with Hax-1, a protein associated with the actin cytoskeleton. Proc Natl Acad Sci USA 2000; 97: 4017-4022

2. Chien CT, Bartel PL, Sternglanz R, Fields S. The two-hybrid system: A method to identify and clone genes for proteins that interact with a protein of interest. Proc Natl Acad Sci USA 1991; 88: 9578-9582.

3. Durfee T, Becherer K, Chen PL, Yeh SH, Yang Y, Kilburn AE, Lee WH, Elledge SJ. The retinoblastoma protein associates with the protein of phosphatase type 1catalytic subunit. Genes & Dev 1993; 7: 555-569.

4. Gyuris J, Golemis EA, Chertkov H, Brent R. Cdi 1, a human G1- and S-phase protein phosphatase that associates with Cdk2. Cell 1993; 75: 791-803.

5. He, J., Choe, S., Walker, R., Di Marzio, P., D. Human immunodeficiency virus type I virol protein R (Vpr) arrest cells in G2 phase of cell by inhibiting p34cdc2 activity. J Virol 1995, 69. 6705-6711

6. Huang LM, Jeang KT. HIV Vpr: roles in viral replication and cellular metabolism. in Myers G et al., eds. Human Retroviruses and AIDS 1995: III-3-III-10.

7. Stewart, S.A., Poon, B., & Chen, I.,S. Human immunodeficiency virus type I Vpr apoptosis following cell cycle arrest, J Virol 1997, 71. 5579-5592

8. Subbramanian RA, Cohen EA. A, Cohen EA. Molecular biology of human immunodeficiency virus accessory proteins. J Virol 1994; 68: 6831-6835.

9. Suzuki Y, Demoliere C, Kitamura D, Takeshita H, Deuschle U, Watanabe T. HAX-1, a novel intracellular protein, localized on mitochondria, directly associates with HS1, a substrate of Src family tyrosine kinases. J Immunol 1997;158: 2736-44.

10. Yasushi K, Kaori N, Mie I, Tomoko M, Michiko T, Mikiko et al. Interaction of Epstein-Barr virus nuclear antigen leader protein with HS1-associated protein X-1: implication of cytoplasmic function of EBNA-LP. J Virol 2000; 74: 10104-10111.