行政院國家科學委員會專題研究計畫　期中進度報告

愛滋病毒 Vpr 蛋白之功能研究及其與 HAX-1 和脊椎肌肉萎縮基因之作用(2/3)

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計畫類別：■個別型計畫　　□整合型計畫

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計畫主持人：黃立民教授

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□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 92 年 5 月 31 日

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Preparation of NSC Project Reports

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一、中文摘要

Vpr 為愛滋病毒附屬基因產物，由 96 個氨基酸組成 14kDa 蛋白。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。先前的研究中本實驗室利用酵母菌雙混交系統從「骨髓基因庫」找到一些與 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)。SMN 蛋白廣泛存在各種組織、細胞中，此蛋白突變時會導致「神經細胞凋亡」。HeLa 細胞表現 SMN 蛋白會造成 Vpr 分佈位置變化，令我們推測 SMN 會影響 Vpr 的生物功能。由細胞週期實驗顯示 SMN 似乎會影響 Vpr 導致的細胞凋亡作用。關於這兩個蛋白生物功能相關性需更進一步實驗系統來釐清，未來研究重點將放在這兩個蛋白參與在「細胞凋亡」的訊息傳遞路徑上。

關鍵詞： 愛滋病毒, 酵母菌雙混種系統, Vpr, HAX-1

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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. Over expressed SMN alter the localization of GFP-Vpr fusion protein within HeLa cells, indicating SMN play unknown role in Vpr biological function. Previously studies show that SMN modulate neuron cell apoptosis, but little is known about SMN1 how to cause the degeneration of motor neuron the developing to spinal muscular atrophy disease (SMA). Other 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 correlation between Vpr and SMN that involved in apoptosis pathway.

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

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.

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三、結果與討論

SMN1 disrupts the nuclear localization of a Vpr-GFP fusion protein

To examine whether SMN alters the nuclear localization of a Vpr, we used fluorescence microscopy to monitor the localization of Vpr-GFP fluorescent protein in HeLa cells cotransfected with Vpr and SMN expressing plasmids.

Vpr-GFP + vector	**nucleus**
Vpr-GFP + SMN	**cytoplasma nuclear**
SMN-GFP + vector	**cytoplasma nuclear**

The interaction of Vpr deletion mutant and SMN1 in 2-hybrid system

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

Vpr deleted mutant	β-gal activity
Wt (1-96 a.a.)	＋＋＋＋
Vp I (1-42 a.a.)	－
Vp II (43-82 a.a.)	－
VpIII (77-96 a.a.)	－
VpI+II (1-82 a.a.)	＋＋＋
VpII+III(77-96a.a.)	－

四、計畫成果自評

In this study, we have successfully identified the new Vpr interacting proteins, HAX-1 and SMN1, through yeast two-hybrid screen. In this study we successfully identified a new Vpr interacting protein, survival motor neuron (SMN1) protein, through yeast two-hybrid screen. Here, we first report that SMN1 disrupts the nuclear localization of a Vpr-GFP fusion protein 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 SMN1 in AIDS neuropathogenesis through further addressed study.

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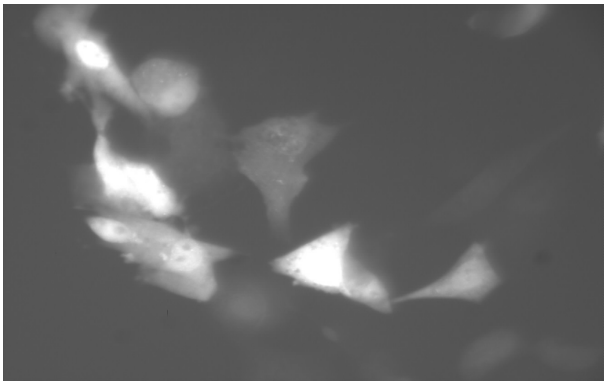
Vpr-GFP+vector



Vpr-GFP + SMN1