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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※ 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 · ※

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計畫主持人：黃立民教授

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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

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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)。進一步的研究發現在 HeLa 細胞株同時表達 HAX-1 與 Vpr-GFP 螢光標定蛋白時，N 端與全長的 HAX-1 蛋白均有阻斷 Vpr 蛋白核換位(nuclear transport)的能力；而 C 端則不具此阻斷能力(blocking function)。我們假定 HAX-1 停滯病毒蛋白於細胞質之功能(cytoplasmic retention)必定造成宿主細胞功能的改變繼而影響病毒感染之致病機轉。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

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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. Furthermore, over-expressed HAX-1 or N-terminal of HAX-1 in HeLa cells blocks the nuclear transport of a Vpr-GFP fusion protein while C-terminal of HAX-1 fail to block Vpr-GFP nuclear transport. We hypothesize that cytoplasmic retention of HIV-1 regulatory protein Vpr by protein-protein interaction with human cytoplasmic protein HAX-1 may cause functional changes in the host cell that affect HIV-1 pathogenesis.

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.

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

二、緣由與目的

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 (for review see Huang and Jeang, 1995; He et al., 1995; Stewart et al., 1997). 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. Successfully identification of SMN1 and HAX-1suggests that Vpr may be involved in spinal muscular atrophy, signal transduction, and apoptosis. Notably, in addition to HIV-1 Vpr, HAX-1 is also targeted by EBNA-LP of Epstein-Barr virus and K15 protein of Kaposis's sarcoma-associated herpesvirus (KSHV)(or HHV 8, human herpesvirus 8)(Tyson V.S et al., 2002). 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

Table1. Subcellular localization of GFP-Vpr, GFP-HAX-1, GFP-Hax N, GFP-Hax C

To examine the subcellular localization of HAX-1, HAX-1deleted mutants (Hax N or Hax C), and Vpr proteins in HeLa cells, all the proteins were fused to GFP to generate easily monitored fluorescence chimera proteins by using fluorescence microscopy assay.

GFP -Vpr	**nucleus**
GFP -HAX-1	**cytoplasmic**
GFP -Hax N	**cytoplasmic**
GFP -Hax C	**cytoplasmic**

Table2. HAX-1 excludes Vpr from nucleus, and SMN1 disrupts the nucleart of a Vpr-GFP fusion protein

To examine whether HAX-1 alters the nuclear transport of a Vpr, we used fluorescence microscopy to monitor the localization of Vpr-GFP fluorescent protein in HeLa cells cotransfected with Vpr and HAX-1 (or Hax N, Hax C truncate form) expressing plasmids, as well as SMN1.

Vpr-GFP + vector	**nuclear**
Vpr-GFP + HAX-1	**cytoplasma**
Vpr-GFP + Hax N	**cytoplasma**
Vpr-GFP + Hax C	**nuclear**
Vpr-GFP + SMN1	**Spot pattern both in cytoplasmic and nucleus**

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 excludes Vpr from the nucleus, and SMN1 disrupts the nuclear localization of a Vpr-GFP fusion protein. We hypothesize that cytoplasmic retention of HIV-1 regulatory protein Vpr by protein-protein interaction with human cytoplasmic protein HAX-1 may cause functional changes in the host cell that affect HIV-1 pathogenesis.

In addition to HIV-1 Vpr protein, HAX-1 is also targeted by EBNA-LP of EBV and K15 protein of KSHV (Yasushi K et al., 2000 ;Tyson V.S et al., 2002). In second year of this three-year project, we will try to verify the possible functional mechanism of Vpr/HAX-1 association as well as Vpr/SMN1 association. 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.

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