# 人類抗單純泡疹病毒第一型抗體片段的產生及其特性分析

# Generation and Characterization of Human Anti-Herpes Simplex Virus Type-1 Fab Fragments


## 中文摘要

單純泡疹病毒疾病(Herpes Simplex Viral Diseases) 是一種常見的疾病。單純泡疹病
毒(Herpes Simplex Virus; HSV)主要分成第一型(Type I)及第二型(Type II)，其中以第
一型的感染佔較大的比例及致死率。人類單株抗體(Human Monoclonal Antibodies)在病
毒疾病的預防及治療上有很重要的潛在性。聯合基因組庫技術(Combinatorial Library T
echnique)是一種利用噬菌體表現系統(Phage Display System)來大量製造並篩選對特定
抗原特異性之抗體的技術，目前這種技術也被利用來製造一些單株抗體。我們由帶有抗 HS
V-1 抗體的健康人的單核球，分離出 RNAs 並反轉錄成 cDNAs，再以聚合酵素鏈鎖反應(Polym
erase Chain Reaction)放大輕鏈(包含 κ 和 λ chain)及重鏈(Heavy chain)的基因片段，
並選殖入 pComb3 載體中，組成四個基因組庫。我們由 λ 輕鏈擊重鏈所建構的噬菌體基因組
庫中，篩選(Biopanning)出帶有抗 HSV type I(HSV-1)抗體基因的噬菌體，經由富化(Enri
chment)之後隨機挑選 50 個菌株，以膠體電泳分析來確認其質體 DNA 的大小，其中有 28 個菌
株是正確的。再由這 28 個菌株中挑選 20 個利用 IPTG 來誘導抗體蛋白片段(Fab)的表現，並
以西方點墨法(Western Blotting Assay)，酵素結合免疫吸附分析法(Enzyme-linked Imm
unoabsorbent Assay; ELISA)，及免疫螢光法(Immunofluorescent Assay)來分析 Fab 的特
性。由結果得知，我們有將 Fab 片段由大腸桿菌中誘導出來且爲人類的抗體，且這些 Fab 片
段和 HSV-1 Ag 有結合能力。以上述的結果，我們得知可由大腸桿菌中製造出具有和 HSV-1

Ag 結合的人類抗體蛋白片段。

## 英文摘要

Enzymatic deficiency of 21-hydroxylase causes the congenital adrenal hyperplasia (CAH), an autosomal recessive disorder. There are two copies of human P450c21-hydroxylase gene, the active CYP21B gene which encodes the active enzyme, and the pseudogene, CYP21A. It's known that the -167/-64 region of CTP21 gene determined its basal transcription activity. Based on its higher transcription activity and nuclear protein binding affinity of CYP21B gene, the authors therefore hypothesized that differences on the promoter strength of CYP21A and CYP21B genes may be due to their different protein binding affinity. Comparing the sequences within that region of the CYP21A and CYP21B genes, there are only four nucleotides differences. In this study, we analyzed the role of individual gene-specific nucleotides within that region for the transcription activity and protein binding ability. Our results showed that both protein binding ability and transcription activity of CYP21B gene were decreased when G residue at -104 position changed to the CYP21A sequence, A. We also found that multiple nuclear proteins present in adrenal cells could interact with the -123/-88 region of CYP21 gene, but -104G→A did not affect the species of proteins which could bind to the -123/-88 region of CYP21 gene. In summary, our results indicated that the G residue at -104 position of the CYP21B gene is important for both of the DNA-protein interaction and basal transcription activity of CYP21B gene.