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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探討本土愛滋病毒抗藥性突變-病毒亞型與突變位置之相關性以及已知突變位置以外之新抗藥性突變的研究
Study of drug resistance mutations in HIV-1 infection - the impact of local circulating HIV-1 subtypes on mutation pattern and the search of other possible drug mutation sites

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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

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□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

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一、 中文摘要

愛滋病藥物治療常因服藥不規則而失敗,此時要選擇有效藥物以達再度控制病毒必須仰賴抗藥性檢驗.我們從事病毒抗藥性基因的突變,首先建立國內愛滋病毒反轉錄酶以及分解酶基因之共同核酸列序,分別 E 亞型與 B 亞型之差異. 我們從治療失敗患者的病毒中發現多處胺基酸突變,但其中只有少數是已知會造成抗藥性的首要突變. 其餘變化位置有可能是補償因突變造成病毒生長不良之次要突變或是病毒在治療前本來就有的異動. 我們比較治療前後的基因,發現兩者均有,且數目相當. 我們證實突變位置會隨時間而越積越多,並因而使抗藥性加強. 因此計算突變數目可約略得知抗藥性之程度. 我們建議在治療前先取得酵素基因之列序,據此設計使用何種藥物治療,並作爲一但治療失敗時, 以病毒突變位置之數目判定抗藥性之程度.

關鍵詞：愛滋病　抗藥性突變

Abstract

The treatment of HIV infection with HAART may fail due to poor adherence. The choice of salvage therapy needs the guidance of drug resistance tests. In Taiwan, we use genotyping to search the drug resistance mutations. We first determined the consensus sequence of RT and protease gene specific to Taiwan isolates. Primary mutations are known to confer drug resistance, but secondary mutations are needed to improve the fitness of mutant viruses. We find that it is necessary to compare the genetic sequence of viruses obtained before treatment and after treatment failure in order to differentiate between secondary mutation and polymorphism. We found that many non-primary mutations noted in drug resistant viruses are actually polymorphism. However, there is no golden rule to interpret the secondary resistance mutations. According to our results, we note that more and more mutations accumulate during the development of drug resistance. Therefore, we hypothesize that the number of mutations may be a good indicator of drug resistance.

Keywords　antiretroviral therapy　drug resistance mutation　genotype

二、 Background:

The highly active antiretroviral therapy (HAART) can suppress the replication of human immunodeficiency virus (HIV-1) to a very low level, however, does not eliminate them [1]. The initial successful HAART may fail during the continuing treatment required to refrain the virus from rebound. After the initiation of HAART for four years, the failure rate is around 40% (personal observation). Most patients who failed the regimens had poor adherence. The accumulation of drug resistance mutations results in treatment failure. To control the progression of HIV disease again, potent new drugs to which the mutant viruses are susceptible must be used to replace the old regimen. Recent guidelines have recommended the routine use of HIV-1 drug resistance testing in this particular clinical setting [2,3].

To study the pattern of drug resistance mutations occurred in Taiwan, we confronted two unanswered questions. The first problem is the interpretation of genotypic resistance. Most authors used the key (primary) drug resistance mutations in the reverse transcriptase and protease genes to guide their interpretation [2,4]. However, many changes in genetic codon are not associated with known drug resistance, and their significance remains unknown. The second question is that whether different subtypes share the same resistance profile? According to our previous study, about one fifth of HIV-1 infected patients in Taiwan harbored subtype E [5]. Since most of resistance studies in the Western world were performed on patients infected withy subtype B, the mutations found in patients infected with subtype E and who failed the antiretroviral treatment should be analyzed.

It has been shown that mutant virus is less fit tan wild type [6]. Therefore, wild type virus predominated again when HAART was discontinued because of treatment failure. We hypothesized that during development of drug resistant mutants, other mutations besides key resistance mutations must accumulate in order to achieve better fitness. If this is true, then mutant who has more mutation sites will be the one that has been under the pressure of HAART for a longer time and will be more resistant. The cross-resistance will occur among drugs in the same category and multi-drug resistant strains have been reported [7]. However, it may be difficult to differentiate between viral polymorphism and true secondary mutations. Therefore, we compare the viral strains isolated before treatment and during treatment failure. The number of substitution in amino acid is counted in RT and protease genes. The mutations in RT gene at position after amino acid 220 are not studies in most genotype analysis. We are interested in this area and make the same approach to find out the impact of this area on drug resistance.

三、 Results and discussion

Subtype-specific polymorphism

To study the mutations in RT and protease genes, the first thing is to establish the consensus sequence of these genes in Taiwan. This is done by analyzing different viral isolates circulating in Taiwan. Subtype-B and E specific polymorphism was found during the process. We also noted that the prevalent viral strains in Taiwan have certain amino acid sequence in RT and protease gene

that is somewhat different from the consensus sequence found in other countries. For instance, the amino acid 63 in protease gene is pro in most viral strains isolated in Taiwan.

The primary and secondary drug resistance mutations in protease gene

We hypothesize that the number of mutations can be used to interpret the level of drug resistance. Most of the genotyping use primary mutation site to predict drug resistance. The significance of secondary mutations is unknown. It is also difficult to differentiate between polymophism and secondary mutations. The following examples demonstrate how we approach this problem.

Two patients received HAART since April 1997. Virological failure developed later due to poor adherence. Several salvage regimens were tried but failed. The mutations in protease gene are detected.

Polymorphism can be differentiated from secondary mutations by comparing the amino acid sequence before treatment and after virological failure.

Patient Lin JG The protease gene sequence, the bold letter represents primary mutation

8 May 97' K10I M36I K41R K45R Q61H K70T
16 Apr 01' K10I ~~K20R~~ M36I K41R K45R ~~I54V~~ ~~Q61N~~ K70T ~~A71I~~ ~~I72T~~ ~~G73T~~ **V82A L90M**

Clearly, amino acid 10, 36, 41, 45, 70 and 93(I93L) are polymorphism and amino acid 61 is also a polymorphism but has a subsequent secondary change. There are 6 polymorphism and 6 secondary mutations and 2 primary mutations. The patient had high level of drug resistance and failed indinavir/ritonavir 800/200mg regimen. Therefore, a total number of 8 mutations may represent high level of resistance, however, the pre-existing polymorphism must be excluded from calculation.

The accumulation of mutations over time

Patient Chen LD

1 June 98'	I13V	G17D		M36I	**M46I**		H69K		L89T
22 Apr 99'	I13V	G17D	K20K/I	M36I	**M46I**	I62V/I	H69K	A71V	L89T
11 Apr 00'	I13V	G17D	~~K20I~~	M36I	**M46I**		H69K	~~A71V~~ ~~I84V~~	L89T

The patient started HAART in April 1997, he soon experienced virological failure in June 1998. The primary resistance mutation M46I was noted very early when the patient first experienced treatment failure. Secondary mutations continue to develop under the pressure of HAART. This case shows that the number of mutations may be a useful tool to evaluate the level of drug resistance. However, we do not study the pre-treatment plasma sample, we do not know how many mutations existed in virus isolated in June 1998 are actually polymorphism.

If primary mutations are used to represent the occurrence of drug resistance, the number is too small for clear-cut comparison, since patient Lin had 2 while patient Chen had only one primary mutation. When the weight of secondary mutations is added for interpretation of genotyping, we may have better cut-off point. In clinical practice, we used Indinavir 800 mg plus ritonavir 200mg to treat both patients. As expected, Lin failed the salvage therapy and Chen had successful control of viral replication.

The mutations in RT gene beyond amino acid 220 --------- The dark side of the mountain

In most reports, the analysis of genotype drug resistance in RT gene focuses on the N terminal part of the gene. Little attention had been paid to the amino acid sequence beyond position 220. We studied the changes in amino acid sequence of the entire RT gene before HAART and after treatment failure. The accumulation of primary and secondary mutations was also noted in RT gene as the examples of protease gene described above. However, most of the mutations in C terminal half proved to be polymorphism, only a few changes appeared to be the result of drug resistance. These mutations could be secondary mutations.

四、 The future application of this study in drug resistance test

We have demonstrated that in analyzing genotypic resistance, secondary mutations should be differentiated from polymorphism. Therefore, we need to have the genotype data of every patient before HAART. The data facilitate the choice of HAART in naïve patients. The necessity of pre-treatment genotype study is justified by the evidence that many treatment naïve patients have drug resistance already. We also found that the number of primary and secondary mutations in RT and protease gene may be good indicator of level of drug resistance. We plan to use a formula to calculate the level of resistance. In the formula, more weight is put on primary mutations.

Resistance index = 2x number of primary resistance + number of secondary resistance

This formula can be tested by using salvage therapy with high Cmin/IC90 ratio, such as Lopinavir/ritonavir or Indinavir/ritonavir.

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