Detection of HIV-1 minority drug-resistant and X4-tropic variants by Next-Generation Sequencing to improve the prediction of clinical antiretroviral treatment outcome

Executive summary

Aim and Objective

In Hong Kong, approximately 700 new HIV-1 cases were reported annually, resulting in around 7000 cumulative cases being reported over the past three decades. Under prolonged usage of antiretroviral (ART) treatment, these patients would have an increased risk of developing resistance against multiple ARV classes. Standard genotypic resistance testing platforms relies fundamentally on Sanger sequencing platform, but it has been proved that minor variants with a <20% prevalence that were undetected by Sanger sequencing would also be clinically important.

In this study, we aimed to establish and validate an accurate next-generation sequencing (NGS) platform with increased sensitivity in identifying drug-resistant quasispecies in order to ensure a better disease progression control in HIV-1 patients.

Project design and target population

This study aimed to focus on HIV-1 patients under prolonged ARV therapy. In collaboration with the Integrated Treatment Centre, Department of Health, a total of 181 longitudinal serum samples were retrospectively available from 37 PIs/NRTIs/NNRTIs-experienced patients. These patients were selected based on 1) the availability of treatment-naïve sample for comparison or 2) required or might have failed subsequent deep salvage therapy. Partial *pol* gene covering PR and RT genes, *int* gene and V3 loop of *env* gene was amplified by Polymerase Chain Reaction (PCR) and were sequenced by Illumina platforms and analysed by DeepChek®, with minor variant cut-offs set at 3%, 5% and 20%. The results were then compared to Sanger sequencing results.

Main achievements

Among the 181 samples, valid sequencing was reported in 166/181 (91.7%) samples with an average coverage of over 100,000X, which would be sufficient to detect drug-resistant quasispecies below 5%. When compared to Sanger sequencing, NGS platform showed optimal concordance when the mutation prevalence was at >60% for NNRTI/NRTI, and >20% for PI. More importantly, 5/37 patients were found to have early progression of NRTI/NNRTI or PI resistance as detected by NGS platform but were missed by Sanger sequencing platform. The result was further supported by INI resistance identification study. A total of 13/166 specimens (7.1%) from 7 patients were found to have mutations that could minimally reduce the susceptibility towards INI. Of these 13 specimens, Sanger sequencing can only identify 6 specimens from 3 patients with INI resistance-related mutations with a prevalence of >20%, which was consistent to previous study that Sanger sequencing can only provide accurate reports on quasispecies species only when mutation prevalence was higher than 20%.

Conclusion

In summary, NGS platform demonstrated to be a much better platform in identifying minor population. With the ability to pick up resistance-related mutations at an earlier stage at a lower prevalence, NGS platform can significantly improves treatment prognosis and prediction of resistance progression during highly active ART therapy. This allows a much better treatment guidance and disease progression control.

以次世代基因定序方法檢測 HIV-1 少數抗藥性亞種和 X4 變異體在預測抗逆轉錄病毒療法的成效

執行摘要

目的

每年香港約有 700 宗新 HIV-1 感染案例。過去三十年, 香港共有 7000 宗 HIV-1 個案。因患者需長期接受抗逆轉錄病毒(ARV)療法, 這些病人在療程期間出現多重抗藥性的風險亦會大增。現時抗藥性檢測以 Sanger 測序為準, 可是 Sanger 未能有效檢驗出變異率在 20%以下但在臨床上同等重要的抗藥性亞種。故此項研究我們希望以次世代基因定序(NGS)設立更精準的檢驗平台以提高檢驗抗藥性亞種的敏感度, 從而有效地控制 HIV-1 患者的疾病進展。

研究設計和對象

此項研究針對長時間接受 ARV 療法的患者。在衛生署綜合治療中心的合作下我們追溯了 37 位接受 PIs/NRTIs/NNRTIs 療法的患者收集了 181 個縱向血清樣本。這些患者是根據 1) 有未接受治療前的血清樣本, 和 2) 需要救援性治療 進行篩選。樣本以 PCR 培增包含 PR 和 RT 的部份 *pol*, *int* 和 *env* 中的 V3-loop 部份, 再以 Illumina 平台測序, 最後用 DeepChek®分析, 以 3%, 5%和 20%為隔斷以找出少數抗藥性亞種。檢測結果再和 Sanger 的結果作對比。

主要發現

在 181 樣本中有 166/181 成功進行排序分析, 當中平均覆蓋值為 100,000X, 足夠找出少於 5%的抗藥性亞種。當 NNRTI/NRTI 的突變發生率> 60%而 PI 為>20%時, NGS 平台和 Sanger 測序的結果顯示出最佳的一致性。更重要的是 NGS 成功找出在 5/37 患者中出現早期 NNRTI/NRTI 或 PI 的抗藥性但被 Sanger 測序遺漏。而在 INI 抗藥檢測中有 13/166 (7.1%) 出現早期 INI 抗藥性, Sanger 測序只能找出當中 6 例有突變發生率>20%的 INI 耐藥相關突變亞種, 這也和先前的研究即 Sanger 只能準確找出突變發生>20%少數抗藥性亞種的結果吻合。

結論

總括而言，NGS 平台在找出少數抗藥性亞種有更好的表現。這意味 NGS 平台能有效改善 ARV 療法預後和儘早預防抗藥性的發生，並提供更好治療指導和疾病進展的控制。