［課程−２］

審査の結果の要旨

氏名　魏　雅稚　Wei Yazhi

This research based on cell-cell fusion assay, a novel co-receptor assay, to discover the co-receptor usage of HIV-1 envelope gene derived from HIV-1 infected patient serum comparing with FDA approved Geno2pheno assay and virus-cell fusion assay, and to further investigate the amino acid mutation correlated with the co-receptor usage in virus-cell fusion assay, a standard co-receptor assay. And the following results were got:

1. The Geno2pheno assay predicted R5 co-receptor usage in all six patients; however, the results of the phenotypic assays using the whole envelope protein were quite different: The virus-cell fusion assay predicted 3 R5 and 3 R5X4, and the cell-cell fusion assay predicted that all 6 were R5X4 viruses.

2. Patients' plasma viruses are composed of quasispecies. 25 clones of the whole envelope gene from the 6 patients were isolated and tested their co-receptor usage. The Geno2pheno assay (using False Positive Rate <10), predicted that 17 or 8 clones could use CCR5 or CXCR4, respectively. An in-house virus-cell fusion assay, which is supposed to reflect the natural infection, predicted that all 25 clones could use CCR5 and that only 4 out of 8 clones predicted by Geno2Pheno could use CXCR4. In the cell-cell fusion assay, 20/25 clones were predicted as R5X4 virus while only 5 clones were predicted as R5 virus.

3. In patient IMS0718, the amino acid mutation correlating R5 to R5X4 change distributed in C1, C2, and V3, however the frequency of the mutation was not statistically significant. In patient IMS1000, 11 amino acid differences accumulated in C1, V2 and C3. Among them, the clustering of amino acid mutation in V2 was statistically significant.

4. By comparison between the virus-cell fusion assay and the cell-cell fusion assay, the amino acid mutation correlating the R5 to R5X4 change were scattered in C1, V1, C3, V4, and V5.

These results suggested first that the amino acid change in V2 which covers V3 before CD4 binding could influence the subsequent co-receptor binding. Second, these results support the conclusion that the cell-cell fusion assay might over-estimate the CXCR4 usage and it would be difficult to apply the cell-cell fusion assay to the clinical decision on the use of CCR5 antagonist, maraviroc. This research made efforts to improve understanding the three co-receptor assays in application of co-receptor usage of clonal envelope genes derived from patients, and clarified amino acid mutations in V2 were correlated with co-receptor usage. This research is award of a Doctor's degree.