論文の内容の要旨

論文題目 Analysis of HIV-1 co-receptor usage by cell fusion based assay using cloned full-length envelope

(HIV-1全長envelopeクローンを用いた細胞融合アッセイによるco-receptor usageの解析）

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Human immunodeficiency virus type 1 (HIV-1) uses two receptors upon infection: Cell surface CD4 molecule as a primary receptor and either CXCR4 or CCR5 as a co-receptor. Based on the co-receptor it uses, HIV-1 is categorized as an X4 virus, an R5 virus, or an R5X4 virus.

Co-receptor usage can be estimated by a phenotypic assay based on virus-to-cell infection (virus-cell fusion assay) utilizing pseudoviruses. Trofile^{TM} (Monogram Biosciences Inc., California, USA) is an example. The virus-cell fusion assay mimics the natural infection most accurately, but it has shortcomings such as biosafety considerations and a long turnaround time. Genotypic co-receptor assay utilizing amino acid sequences of the viral envelope has also been developed. While a genotypic assay such as the Geno2pheno assay is safe, rapid and universally accessible, its calculation depends on short amino acid sequences in the V3 region of the envelope. Calculation depends also on historical data with known genotype and phenotype. Therefore, a simple, safe and rapid phenotypic assay is in need. Our laboratory has reported a new rapid and safe co-receptor assay based on cell-cell fusion (DSP-Pheno). In an effort to compare the validity of the three assays, I first applied the envelope glycoprotein gene amplified directly from the plasma of six patients to an in-house virus-cell fusion assay, Geno2pheno assay, and cell-cell fusion assay. 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. Patients' plasma viruses are composed of quasispecies, therefore I isolated 25 clones of the whole envelope gene from the 6 patients 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. These results showed that envelope regions outside V3 should be considered in predicting the co-receptor usage. 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.

In the cell-cell fusion assay, 20/25 clones were predicted as R5X4 virus while only 5 clones were predicted as R5 virus. 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, it is likely 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. However, the cell-cell fusion assay may be useful for the research on cell-to-cell infection of HIV-1.