論文の内容の要旨

論文題目　A Novel HIV-1 Phenotypic Tropism Assay Using Dual Split Protein (DSP)-Mediated Quick Membrane Fusion Detection System

和訳　細胞膜融合と二重分割タンパク質（Dual Split Protein, DSP）を用いた新規 HIV-1 表現型細胞指向性試験の開発

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Background: Coreceptor usage is one of the most fundamental steps in HIV-1 replication. Since the development of CCR5 inhibitors for clinical use, the viral tropism is a matter of great importance in the antiretroviral therapy. Co receptor tropism assay (CTA) is a prerequisite whenever the use of CCR5 inhibitor is being considered. Moreover, several studies have also indicated its correlation with disease progression. Therefore, viral tropism determination is clinically imperative. Both phenotypic (PTA) and genotypic tropism assays (GTA) are available for viral tropism determination. GTAs offer rapid and simple tropism determination. However, only V3 region sequence of HIV-1 gp120 was used in determination algorithm. Several studies reported a number of mutations outside V3 (V1, V2 or in gp41) influenced the viral tropism. Accurate prediction of viral tropism by GTAs requires further algorithm development. Currently, PTA is a gold standard. However, it requires well-trained personnel, sophisticated biosafety facilities and also is time-consuming. Here, I present the first HIV-1 PTA which does not use pseudovirus in tropism determination.

Method: Dual split protein (DSP) composed of split green fluorescent protein (GFP) and split *Renilla* luciferase (RL) was employed as a marker for cell fusion phenomenon. 70% of GFP and 70% of RL (DSP1) was stably expressed in NP2 cells expressing CD4/CXCR4 (N4X4-DSP1) or CD4/CCR5 (N4R5-DSP1). HIV-1 envelope gene from cloned reference strains or patients' plasma was ligated to an expression vector containing 30% of GFP and 30% of RL (DSP2), so called pRE11-env. pRE11-env was transfected to 293FT cells. Two days post-transfection, pRE11-env-transfected 293FT cells were overlaid to N4X4-DSP1 or N4R5-DSP1. After 6 hours of co-cultivation, the tropism could be determined by detection of either GFP signal or luciferase activity resulted from re-association of DSP1 and DSP2 among fused cells. The results were compared to in-house pseudoviral tropism assay. In Cell Analyzer was utilized for image capture. Enduren was utilized for luciferase activity measurement in fused cells. Statistical analysis was performed using one-side *t*-test to compare mean relative light unit (RLU) of luciferase activity of sample to that of negative (no Env expression) control. P value of less than 0.05 was considered statistically significant. This study has been approved by institutional review board of the Institute of Medical Science, the University of Tokyo (IMSUT: 20-31-1120).

Results: Using reference strains (BaL, HXB2, LAI, NL4-3, SF2) HIV-1 envelopes for assay validation, the tropisms were precisely determined. Fluorescent signals were proportionate with luciferase signals and completely concordant. Minor variant detection limit was evaluated by both reference strains (NL4-3/BaL) and clinical isolate clones mixtures. Results revealed 0.5% and 10% for detection of X4 and R5 minor population, respectively. Twenty clinical isolates clones were examined. The results were partly consistent with in-house pseudoviral tropism assay. Clonal analysis was performed on discordant samples and revealed higher sensitivity of this assay than in-house pseudoviral tropism assay as one possible cause of discrepancy.

Conclusion: This is the first phenotypic HIV-1 tropism assay without pseudovirus production, denominated as DuPheno. The assay offers following advantages: rapid determination (turn-around time within 5 days), simple manipulation and biosafety (no viral production). DSP offers fast and convenient tropism determination by two-way result confirmation. The assay can be used for basic research, epidemiological study, diagnostic test, drug development, and more, in both resource-rich and -limited settings.