

Construction of *tat*-and *nef*-defective HIV-1 and screening of natural extracts with anti-HIV-1 activity

Ann Hwee Lee, Man ki Song, Young Ah Suh, and Young chul Sung^o
Department of Life Science, Pohang University of Science and
Technology, Pohang 790-784, Kyung-puk, Korea

Human immunodeficiency virus type 1 (HIV-1) contains several nonstructural genes which are required for the viral replication and disease pathogenesis. Among them, *tat* and *nef* genes encode an essential transactivator of HIV-1 LTR and a pluripotent protein which seems to be essential for the in vivo but not in vitro viral replication, respectively. We constructed two *tat* and *nef* defective HIV-1 and tested for their ability to replicate in several T cells. The defective viruses did not replicate in CD4⁺ T cells, but rescued in the recombinant Jurkat-*tat* cell which also contains *tat* gene. The replication of *tat* and *nef* defective HIV-1 which expresses chloramphenicol acetyltransferase(CAT) gene was easily detected by a sensitive CAT assay. No revertant was identified during the passages of the mutant viruses for more than two months in Jurkat-*tat* cells. *tat* and *nef* defective HIV-1 could be used instead of wild type virus for several purposes such as inhibitor screening and development of attenuated AIDS vaccine.

Natural products, total number of 268, were screened to test for their effect on the replication of human immunodeficiency virus type 1(HIV-1). Five of them, such as *Eriobotrya japonica*, *Eugenia caryophyllata*, *Cuscuta chinensis*, *Glycyrrhiza uralensis*, and *Coptis chinensis* were shown to be effective in inhibiting the replication of HIV-1 in tissue culture and their selectivity indexes were 42, 40, 14, 18 and 65, respectively. To further fractionate *Coptis chinensis*, which is shown to be highest anti-HIV-1 activity, methanol extracts of *Coptis chinensis* were fractionated into methylene chloride at pH3, pH10 and water residue. The selectivity indexes of CH₂Cl₂(pH3), CH₂Cl₂(pH10) and water residue were 50, 22 and 98 respectively. Our results show that the water residue of *Coptis chinensis* was the most effective for anti-HIV-1 activity.