Galectin-3 Promotes HIV-1 Cell-to-cell Transmission in T Cells through Up-regulating GM1 Ganglioside in Lipid Raft

Sheng-Fan Wang[1*], Chun-Sheng Yeh[1], Ching-Han Tsao[2], Daniel Hsu[3], Yi-Ming Chen[4], Huan-Yuan Chen[2], Fu-Tong Liu[2]

[1.] Department of Laboratory Sciences and Biotechnology, Kaohsiung Medcial University, [2.] Institute of Biomedical Sciences, Academia Sinica, Taiwan, [3.] Department of Dermatology, University of California Davis, CA, [4.] Center for Infectious Disease and Cancer Research, Kaohsiung Medical University, Kaohsiung, Taiwan

Background/Objective
We previously reported that galectin-3 is dependent on Alix to promote HIV-1 budding. We also found that endogenous galectin-3 localizes in membrane lipid rafts. Lipid raft integrity is essential for HIV-1 virological synapse (VS) formation. We hypothesized that galectin-3 plays a role in HIV-1 cell-to-cell transmission.

Method
Lipid raft extraction and immunoblotting were used to know galectin-3 expression level in membrane lipid raft. Cell-to-cell transmission assay was conducted to evaluate the effect of galectin-3 on HIV-1 cell-to-cell transmission. Immunofluorescence staining and live imaging observation were used to trace galectin-3 dissemination via VS and galectin-3 colocalization with Alix, Gag and GM1 on VS. FACS was used to address the effect of galectin-3 knockdown or overexpression on GM1 expressing level in CD4+ T cells.

Result
Immunoblotting results indicate that galectin-3 expresses in membrane lipid rafts in T cells. Results from immunofluorescence staining showed that galectin-3 and Alix coloclaized with Gag in lipid rafts of VS between HIV-1 infected and uninfected CD4+ T cells. Live video-microscope imaging observation showed that EGFP-galectin-3 or EGFP-Alix co-transmission with iCherry-Gag from HIV-1 effector cells to the target cells. Cell-to-cell transmission assay found that the efficiency of HIV-1 transmission is significantly increased by galectin-3 overexpression but attenuated in galectin-3 knockdown CD4+ T cells. Similar results were found in Alix knockdown CD4+ T cells. Moreover, galectin-3-promoted HIV-1 cell-to-cell transmission efficacy is positively correlated with the expression level of galectin-3 in the effector cells. Cholera toxin B(CTB) staining results showed that GM1 ganglioside expression was regulated by galetcin-3 and Alix. We found that galectin-3 compensated the effect of Alix-knockdown on HIV-1 cell-to-cell transmission via up-regulating GM1 ganglioside expression. We also observed that ectopic expression of galectin-3 N-terminal domain inhibit HIV-1 cell-to-cell transmission in CD4+ T cells.

Conclusion
We conclude that endogenous galectin-3 facilitates HIV-1 cell-to-cell transmission by up-regulating GM1 ganglioside expression.