ImmunoTools *special* Award 2015

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Elucidation of Myr(+)Ank^{GAG}1D4 Interference in HIV-infected Colon Cell Line

More than 9 million people worldwide are in HIV treatment which are typically includes the use of combined antiretroviral drugs known as highly active antiretroviral drugs therapy (HAART) (UNAIDS, 2015). The primary goals of HAART are to reduce HIV-related illness, prolong survival, enhance quality of life, bring back immunological response and also prevent HIV transmission. Nevertheless, the emergence of multi-drug resistant mutants and the undesirable side effects of HAART are the major obstacle for the adequate management of HIV infection (1). The development of a safe and effective HIV-1 vaccine would definitely be the best solution for the ultimate control of the worldwide AIDS pandemic, unfortunately HIV-1 vaccine development efforts have not yet been proven successful (2,3). Consequently, the infancy of HIV-1 vaccine gives rise to the alternative therapy, such as protein-based and gene therapy.

Gene therapy is prevailingly under development and certain strategies have been proved to be effective such as RNA interference, aptamer, designed zinc-finger protein (4), designed ankyrin repeat protein (5) and bone marrow transplantation (6) to block various steps of HIV life cycle. Designed Ankyrin Repeat Proteins (DARPins) are protein blocks built up with varying numbers of structural motifs stack to form the repeat modules. According to the ability to be selected against the desired targets, DARPins have been raised as alternative to antibodies with the disulphide bond-independent property and lower production cost (7,8). Ank^{GAG}1D4 served as the best candidate from phage-displayed library against HIV-1 Gag polyprotein. The intracellular inhibitory effect as in the negative interference of HIV-1 Gag assembly and budding machinery was seen. These results Ank^{GAG}1D4 strongly contributed the use of this molecule as a novel therapeutic agent (5).

Further study of the myristoylated version of Ank^{GAG}1D4 (Myr(+)Ank^{GAG}1D4) was introduced in the combination with a novel anti-HIV integration protein scaffold; 2LTRZFP protein, using third generation lentiviral vector. These anti-HIV genes block both viral integration and Gag assembly. The promising results were seen in absolute

resistance to HIV-1 infection in SupT1 cell line. This resistant phenomenon was also observed in HIV-1, SIVmac, or SHIV infected human primary $CD4^+$ T cells stably expressing Myr(+)Ank^{GAG}1D4 alone (9).

Gastrointestinal tract mucosa is the initial route for HIV transmission via mother-to-child transmission (MTCT) and sexual intercourse. Moreover, the colo-rectal mucosa plays an important role of HIV transmission in men who have sex with men and also heterosexual transmission (10). Besides the virus' primary target cells, $CD4^+$ T cells and dendritic cells which span from oropharynx to rectum, the mucosa epithelial cells are reported to be infected. Although these epithelial cells do not express CD4, the virus attachment is successfully performed via galactosylceramide (GalCer) (11-13). Further study revealed the positive viral production from the infected epithelial cells and being infectious to $CD4^+$ T cells, HeLa-CD4 and CEM T lymphocytes. In addition, the virus entry using gp120 is minimal in some cell lines. These results suggested possible HIV crossing over the epithelial cell layer by infecting the mucosa epithelial cells via gp120-independent mechanism (14).

As the mucosa epithelial cells are targeted for HIV infection, these cell lines will be used in this study. Instead of using lentiviral vector system, the Myr(+)Ank^{GAG}1D4 gene will be transferred using chimeric adenovirus. Adenovirus leads to transient expression of this scaffold protein. The HIV-infected colon cell line will be transduced with chimeric adenovirus harboring Myr(+)Ank^{GAG}1D4. We hope to observe the interference of viral production as seen in the previous study.

With the large selection of antibodies and cytokines provided by ImmunoTools, it is very beneficial for us to use these antibodies to identify cell surface markers essential for characterizing cell type and adenovirus transduction using flow cytometry.

In summary, we contribute an innovative approach for HIV therapy. The usage of Myr(+)Ank^{GAG}1D4 will not be only limited to control viral production from the newly infected cells but also from chronic and latent infection.

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