ImmunoTools *special* Award 2015

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Development of novel scaffold proteins for HIV gene therapy

Human immunodeficiency virus (HIV) is the virus that causes acquired immunodeficiency syndrome by destroying $CD4^+$ T cells. It is more than 30 years that HIV was first recognized but there is no effective vaccine for preventing infection. The current standard of care of HIV-infected patients is highly active antiretroviral therapy (HAART) which can reduce viral load and improve immune system. However, the evidence of multidrug resistant HIV-1 mutants and the side effects of the drugs have been reported (*von Laer D, 2006*).

Currently, gene therapy or intracellular immunization is one of the several strategies which are developing for HIV alternative treatment. This approach is composed of two categories, RNA-based agents and protein-based agents (*Kitchen SG, 2011*). Several scaffold proteins have been designed to inhibit HIV replication. In our team, we have recently established the novel two scaffold proteins. $Ankyrin^{GAG}1D4$ selected from ankyrin library can target capsid domain of HIV-1 gag polyprotein. It represented good inhibitory effects on viral replication (*Nangola S, 2012*). Another one is 2LTRZFP, the designed zinc-finger protein which can block HIV-1 integration by binding to the HIV-1 integrase recognition sequence at the 2-LTR-circle junction of HIV-1 DNA before integration into host chromosome (*Sakkhachornphop S, 2009*). Since those scaffolds can interact with viral protein and DNA at critical steps of HIV life cycle and act as intracellular inhibitors, these lead to an idea to develop stem cell HIV gene therapy based on our antiviral molecules.

Combined scaffolds can stably express in SupT1 cells using a third generation of lentiviral vector system. The cells that expressed combined scaffold proteins

demonstrated that they can inhibit HIV replication after HIV challenging with a high MOI. To generate T lymphocytes and macrophages resistant to HIV infection, we are going to transfer these two genes into $CD34^+$ cells using lentiviral transduction. Since $CD34^+$ cells can differentiate into target cells of HIV including $CD4^+$ T lymphocytes and macrophages. The immune cells expressed antiviral proteins can prevent the viral replication and can be long-lasting HIV protection. Moreover, induced pluripotent stem cells (iPSCs) are a type of pluripotent stem cells that can be generated directly from adult cells and have a lot of advantages, for example it has the capacity of self-renewal and can be made in a patient-matched manner. The human fibroblasts will be transduced with lentivirus expressing 2LTRZFP/Ankyrin^{GAG}1D4 and reprogrammed into human induced pluripotent stem cells (iPSCs) by over expression of human transcription factors. Then, human iPSCs will be differentiated into $CD34^+$ hematopoietic progenitor cells and $CD4^+$ T lymphocytes or macrophages subsequently to observe the inhibitory effect for HIV replication. According to this plan, the stem cell and hematopoietic markers will be performed by flow cytometry. For this, ImmunoTools antibodies and cytokines detailed below would be a huge benefit for our study.

In summary, we provide an innovative approach for the treatment of HIV. The novel two scaffold proteins developed in our project could be considered as an alternative treatment for inhibiting HIV-1 infection by applying in hematopoietic stem cell which can be a one-time treatment in HIV-infected patients who resist to HAART treatment.

ImmunoTools *special* AWARD for **Koollawat Chupradit** includes 25 reagents

FITC - conjugated anti-human CD4, CD11b, CD14, CD33, CD45, CD56, HLA-DR, Control-IgG1, Control-IgG2a, Control-IgG2b,

PE - conjugated anti-human CD34, Control-IgG1,

APC - conjugated anti-human CD3, CD4, CD8, CD11b, CD11c, CD14, CD15, CD19, CD38, Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V

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