The Direct Addition of RetroNectin® Solutions in the Liquid Phase Improves the Efficiency of Lentiviral Gene Transduction.

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Abstract

RetroNectin® (a recombinant human fibronectin fragment CH-296) significantly enhances the efficiency of viral-mediated gene transfer, and is widely used in various clinical applications involving gene-engineered cells, including T cells and hematopoietic cells.

However, there are several challenges in manufacturing clinical-grade gene-engineered T-cell products that must be addressed in order to standardize manufacturing methods and achieve consistent results. To simplify and streamline the gene transduction process, it is feasible to incorporate RetroNectin as a transduction enhancer directly into the liquid. This modification would reduce the number of steps involved and can be easily implemented in automated cell processing systems.

In this study, we evaluated the efficiency of enhancing gene transduction by directly adding RetroNectin into the liquid phase during the transduction process of T cells using lentiviral vectors. The results showed that the direct addition of RetroNectin and lentiviruses significantly improved transduction efficiency in T cells, with a 1.5 to more than 3-fold increase compared to viral infection without RetroNectin. Importantly, the addition of RetroNectin did not have any adverse effects on cell viability, cell growth, or cell phenotype.

The Structure of RetroNectin® and the mechanism of RetroNectin-mediated transduction

RetroNectin®

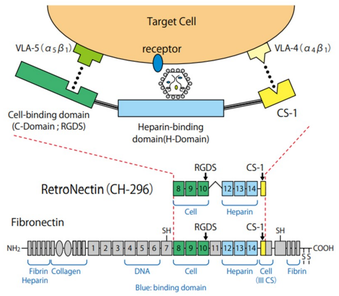
Recombinant human fibronectin fragment CH-296

functional domains:

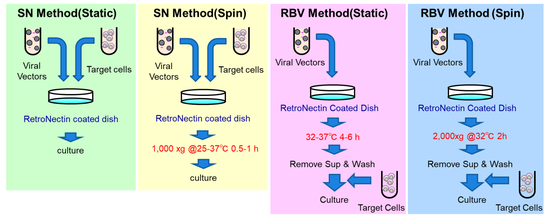
- Heparin-binding domain(H-domain) ⇒bind to viral particles
- Cell-binding domain (C-domain) ⇒bind to VLA-5*
- CS-1 sequence ⇒bind to VLA-4*

*VLA-4&5= Integrin (Fibronectin Receptors)

Enhance lentiviral- and retroviral-mediated gene transduction by facilitating the colocalization of target cells and viral particles.



The standard protocols for RetroNectin mediated transduction

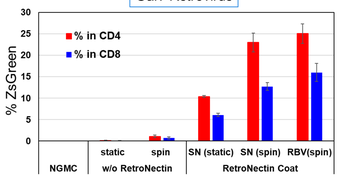


T cell transduction using various protocols

Transduction : 48-well plate (2x10^5 cells/0.3mL/well)

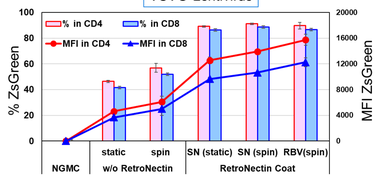
Day 0 PBMC stimulation (αCD3/CD28 beads)
Day 3 RV Td (Galv-RV-ZsGreen1:MOI=0.01)
add medium (x3 volume) 4h after Td
Day 6 FC Analysis

Galv-Retrovirus



Day 0 PBMC stimulation (αCD3/CD28 beads)
Day 1 LV Td (VSVG-LV-ZsGreen1:MOI=0.7)
Day 2 Expansion Culture 1:5
Day 6 FC Analysis

VSVG-Lentivirus

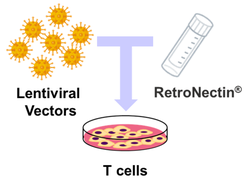


NGMC: non-gene modified cell

RBV(spin) > SN (spin) > SN (static) >> spin w/o RN > static w/o RN

Results: Direct addition of RetroNectin® solution in T cell transduction using lentiviral vector

Direct addition of RetroNectin®



Materials

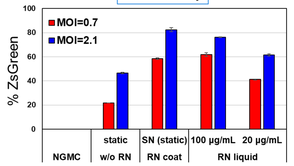
For Lentivirus preparation:
LVpro™ Packaging Mix with pLVpro series
(TakaraBio code:6195, 6962-6968)
Lenti-X™ Maxi Purification Kit
(TakaraBio code:631233, Z1233N)
For T cell culture:
T cell TransAct, human (Miltenyi Biotec)
LymphoONE™ T-Cell Expansion Xeno-Free Medium
(TakaraBio, code WK552S)
For Transduction:
RetroNectin® (TakaraBio, code T100A)
Transduction Enhancer A&B (commercially available)

Exp.1: Direct addition of RetroNectin solution

Transduction : 48-well plate (2x10^5 cells/1.0 mL/well)

Day 0 PBMC stimulation (αCD3/CD28 beads)
Day 1 LV Td (VSVG-LV-ZsGreen1) MOI=0.7, 2.1
Day 2 Expansion culture 1:2
Day 5 FC Analysis

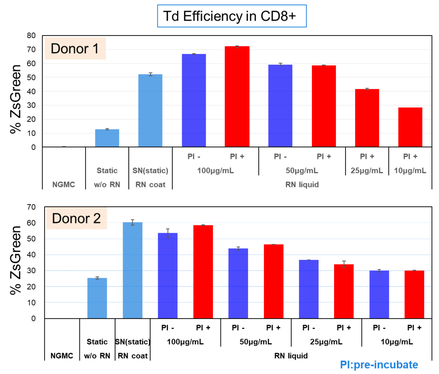
Td Efficiency



Direct addition of RetroNectin in liquid phase improved the Td efficiencies

Exp.2: Concentration of RetroNectin & Pre-incubation

Td Efficiency in CD8+



Day 0 CD4/8 selection & stimulation (αCD3/CD28 beads)
Day 1 LV Td (VSVG-LV-ZsGreen1)
48 well plate/4x10^5 cells/mL/0.5 mL/well
w/ or w/o pre-incubation of LV&RN
37℃ 1h
Day 2 Expansion culture 1:10
Day 5/6 FC Analysis

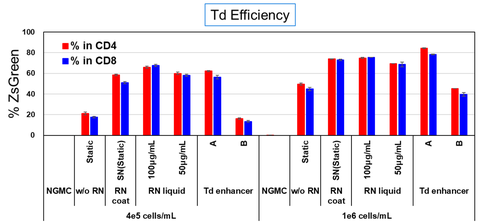
✓Pre-incubation slightly increased the Td efficiencies in some conditions, but not significant.

✓A correlation was observed between the enhancing effect and RetroNectin concentration.

Exp.3: RetroNectin vs. other Td enhancer products

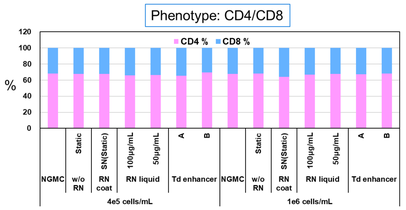
Day 0 CD4/8 selection & stimulation (αCD3/CD28 beads)
Day 1 LV Td (VSVG-LV-ZsGreen1)
48 well plate/4x10^5 or 1x10^6 cell/mL /0.5 mL/well
Day 2 Expansion culture 1:4 or 1:10
Day 6 FC Analysis

Td Efficiency

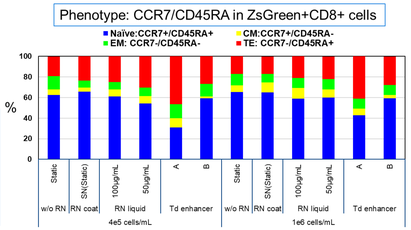


Td Efficiency:
RetroNectin ≈ Enhancer A >> Enhancer B
Phenotype:
% of CCR7$^+$/CD45RA$^+$ RetroNectin ≈ Enhancer B > Td enhancer A
Cell viability & Cell Growth:
No significant difference among all conditions (data not shown)

Phenotype: CD4/CD8



Phenotype: CCR7/CD45RA in ZsGreen+CD8+ cells



Conclusion

Direct addition of RetroNectin solution can improve the Lentiviral Td efficiencies in T cells

- **1.5 to more than 3-fold increase compared to viral infection without RetroNectin**
- **Increasing the RN concentration leads to a greater enhancement effect**
- **No adverse effects on cell viability, cell growth, or cell phenotype**

New RetroNectin protocol is simple & can be easily integrated into automated cell processing systems.

COI:
Y.O., Y.K., and S.O are employees of TakaraBio Inc.