Highly Efficient Gene Expression in B Lymphocytes Mediating by Lentivirus Vector

Akira Tempaku [1,2]*

[1.] *Department of Neurosurgery, Hokuto Hospital, 7-5, Inadacho-Kisen, Obihiro, Hokkaido, 080-0833, Japan*

[2.] *School of Medicine, Faculty of Medical Sciences, University of Fukui, 23-3, Matsuoka-Shimoaizuki, Eiheiji-cho, Yoshida-gun, Fukui 910-1193, Japan*

*To whom correspondence; Phone: +08-155-48-8000, E-mail: tenpaku@hokuto7.or.jp

Abstract

Gene transduction and expression efficiencies among several type cell lines were compared by using vesicular stomatitis virus-glycoprotein (VSV-G) pseudotyped human immunodeficiency virus type-1 (HIV-1) based lentivirus vector. Large discrepancies of the efficiencies were shown among them. B lymphocytes showed high susceptibility of the gene transduction and expression, while other cell lines marked lower potential. Variable gene transduction strategies have been assessed to apply immunological therapies. This study showed that B lymphocytes had facilities enough to support the gene transduction and expression by lentivirus vector. Our result suggested that the lentivirus vector would be a powerful tool to express exogenous genes in B lymphocytes.

Key words: lentivirus vector, gene expression efficiency, B lymphocytes

Running title: Efficient gene expression

Introduction

Lentivirus based vector has much potential for being applied to a gene delivery vehicle. Different from other types of vectors, lentivirus vector can deliver exogenous genes into not only dividing cells but also non-dividing cells (1). The expression from the delivered genes can be further maintained stably with little gene silencing. Since exogenous genes are integrated into the host genome, proliferating cells can keep those genes permanently. The expression of the exogenous genes introduced by lentivirus vector needs several cellular factors, those support transcription of messenger RNA from long terminal repeat (LTR) region on provirus DNA. The capacity of supporting the expression of exogenous genes is various among cell types. It is important to clear why some cells have high susceptibility to lentivirus infection while others mark less potential, in order to characterize the biological interaction of lentivirus and target cell. That would become the essential knowledge to obtain desirable outcome in gene therapy using lentivirus vector. The comparisons of gene transduction and expression efficiencies among several types of cell lines have been hardly focused. This study demonstrates that several B lymphocytes-originated cells have high susceptibility to the gene transduction and expression by lentivirus vector while other types of cells have low capacity. Lentivirus vector would be applicable to treat B lymphocyte associated disorders.

Materials and Methods

Cells and Pseudotyped Virus: MT-4, MOLT-4#8, Jurkat#E6-1, HUT78, H9, PM-1, CEM, CEMx174#T-1, Ramos, BJA-B, Daudi, P3HR-1#G, Namalwa, BCBL-1, U937 and K562 cells were maintained in RPMI-1640 medium (ICN, Costa Mesa, CA) supplied with 10% of heat inactivated fetal calf serum (PAA, Linz, Austria), 100 mg/ml of streptomycin (Meiji, Tokyo, Japan) and 100 IU/ml of penicillin (Banyu, Tokyo, Japan). Luciferase reporter gene carrying pseudotyped virus was made by transfection of pNL4-3-luc plasmid DNA (2) with vesicular stomatitis virus glycoprotein (VSV-G) expression vector into 293T cell by calcium phosphate precipitation method. Three days after transfection, pseudotyped virus containing supernatant was harvested and passed through 0.45 μm filter (Millipore).

Luciferase Assay: VSV-G pseudotyped virus (2 ng) infected 1 x 10^6 cells. One hour later, cell-free virus was removed. Then, cell was cultured in 24-wells plate in 1 ml medium. Seventy-two hours post virus infection, cell was washed with magnesium and calcium ions free phosphate buffered saline twice and lysed with 100 μl of 1 x lysis buffer (Promega, Madison, WI). Luciferase activity was measured after mixing 10 μl of lysate with 50 μl of luciferase substrate solution (Promega) by counting the light intensity with luminometer (Lumat LB 9501/16; EG&G Berthold, Bad Wildbad, Germany).

Polymerase chain reaction (PCR): Twenty-four hours post psuedotyped virus inoculation on 1 x 10^6 cells, the amount of provirus DNA was quantified by PCR. Briefly, cell was lysed with urea lysis buffer (4.7 M urea, 1.3% SDS, 0.23 M NaCl, 0.67 mM EDTA, 6.7 mM Tris pH 8.0). DNA

was isolated by phenol chloroform extraction method followed by ethanol precipitation. PCR was performed in 1 x PCR buffer (20 mM Tris pH 8.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each dNTPs and 0.05 U/μl recombinant Taq DNA polymerase) with each 0.5 mM of M667 (5'-GGCTAACTAGGGAACCCACTG-3') and M661 (5'-CCTGCGTCGAGAGAGCTCTGGTTT-3') primers to amplify provirus DNA (R/gag region), or glo-A (5'-ACACAACTGTGTTCACTAGC-3') and glo-B (5'-CAACTTCATCCACGTTCACC-3') primers to amplify β- globin coding DNA (3). PCR reaction was programmed as first denature for 5 min at 94˚C, followed with 30 cycles of reaction as denature for 1 min at 94˚C, as annealing for 1 min at 65˚C (R/gag) or at 55˚C (β- globin), as extension for 2 min at 72˚C, and finally extension for 10 min at 72˚C. All PCR was done by Takara Thermal Cycler MP (Takara, Kyoto, Japan).

Results and discussion

Gene transduction and expression efficiencies among several types of cell lines mediating by VSV-G pseudotyped HIV-1 based lentivirus vector were compared. MT-4 cell, which was transformed by human T cell leukemia/lymphoma virus type-I (HTLV-I), and all tested B lymphocytes showed high susceptibility to the gene transduction by lentivirus vector. In contrast, T lymphocytes except MT-4 and other types (monocyte and myeloma) of cells showed low susceptibility (Fig. 1A). One logarithmic scale or more gaps of luciferase activity counts were

observed among B lymphocytes and other types of cells. CEMx174 cell (complex of T and B lymphocytes) showed middle level of susceptibility to lentivirus vector mediated gene transduction and expression.

Provirus DNA formation capacities were compared among all tested cell lines by PCR method. There were no remarkable discrepancies of provirus DNA formation among all tested cells (Fig. 1B). These results indicated that the potential of integrated gene expression varied among cell types. Especially, B lymphocytes had higher capacity to help provirus DNA transcription than other type cells. Relative gene expression amounts calibrated by cellular DNA amounts were aligned in Figure 1C. It cleared the large varieties of gene expression levels were due to some cellular factors. Herpes virus negative B lymphocytes (Ramos and BJA-B) showed high susceptibility to lentivirus vector mediating gene transduction and expression. It suggested that high expression level of reporter gene was not due to any factors, which derived from herpes viruses (Epstein-Barr virus; Daudi, P3HR-1 and Namalwa, or Kaposi sarcoma associated herpes virus; BCBL-1).

All used T lymphocytes released some factors into culture supernatant. Those enhanced the infection of HIV-1 to cells. In contrast, B lymphocytes except BJA-B did not produce any factors (4). However, no remarkable discrepancies about the quantities of synthesized provirus DNA were observed among each type of cells (Fig. 1B). It suggested that the virus entry and provirus DNA synthesis occurred equally in all tested cells. This result denied that soluble factors in culture

supernatant enhanced the virus entry and provirus DNA formation efficiencies. These discrepancies might be derived from the difference of target cells characters. Because this study used flowing cells since previous study (4) used adherent cells to calculate the virus infection efficiency. Previous study showed culture supernatant of T lymphocytes contained some cellular factors, which accelerated the progression of HIV-1 infection to CD-4 positive Hela cell. Undetermined factors were presumed to prompt virus entry. Different from the previous report, this study was performed with lymphocytes as virus target cells instead of Hela cell. These differences of study designs might bring the opposite results. Some modulation of virus replication efficiency at post integration steps might bring the gap of the gene transduction and expression efficiencies among each cell. Many research showed that the expression of provirus DNA was influenced by various factors. DNA methylation, change of chromatin structure and association of histone protein induced gene silencing (5-8). Several cellular proteins regulated provirus DNA silencing (9-14). Many molecules were identified as transcriptional modulators. These cellular machineries influenced the amounts of messenger RNA transcription from provirus LTR. In addition, integration of provirus into host genome occurred to restricted local hotspot dominantly (15). Further, the transcription activity was controlled by integration site dependent manner (16). Provirus, inserted in lamin-associated domains (LADs), had lower activity of LTR transcription. These evidences were obtained by studies used by single type of cell, respectively. However, the comparisons of the gene silencing intensity among different types of cell were little in our

knowledge. It is unclear whether provirus prefers to integrate in LADs in T lymphocytes or B lymphocytes carry much more transcription supporting factors than T lymphocytes. Results of our study would open the new research field to clear the cell type dependent regulation of exogenous genes transduced by lentivirus vector.

Several cellular transcription factors prompted gene transcription from LTR (17-21). Previous reports and Figure 1A suggested that differences of transcription factors' expression pattern among cells made a large gap of susceptibilities to lentivirus vector mediating gene transduction and expression. B lymphocytes might carry enough amounts of factors which support transcription from LTR than other type cells did. In contrast, high susceptibility of MT-4 cell to lentivirus vector mediating gene transduction and expression were due to the enhancement of transcription level by HTLV-I derived Tax protein (22).

Clinical application of lentivirus vector to a vehicle of exogenous gene has fronted some difficulties that should be conquered. The concentration of vector virus and the length of vector virus exposure time are critical to get enough outcomes (23). Gene transduction and expression efficiencies depend on the concentration and the exposure time of vector virus. However, virion would be inactive within one-day incubation at 37°C. It makes the limitation of gene transduction and expression efficiencies. Genetic modifications of lentivirus vector are essential to sophisticate gene transduction strategy (24-27). Expression level of the exogenous genes from integrated provirus has an important role to success gene therapies. Gene transduction into B lymphocytes by

several available methods has still some difficulties in clinical application, including low efficiency of gene transfer into cells. To conquer the difficulty, several groups have presented some strategies (1, 28, 29). This study showed that HIV-1 based lentivirus vector possessed the potential enough to introduce exogenous genes into B lymphocytes. Furthermore, lentivirus vector could maintain high expression level from the delivered genes in B lymphocytes (Fig. 1A). We demonstrate that lentivirus based vector would be a powerful tool for exogenous genes delivery into lymphocytes. Using lentivirus vector would be applicable to many fields that need high gene transduction and expression efficiencies.

References

1. Frecha C, Levy C, Cosset FL et al: Advances in the field of lentivector-based transduction of T and B lymphocytes for gene therapy. Mol Ther 2010; 18: 1748-57.

2. Connor RI, Chen BK, Choe S et al: Vpr is required for efficient replication of human immunodeficiency virus type-1 in mononuclear phagocytes. Virology 1995; 206: 935-44.

3. Zack JA, Arrigo SJ, Weitsman SR et al: HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. Cell 1990; 61: 213–22.

4. Tempaku A, Maeda Y, Song W et al: Human T-cell leukemia virus type-I (HTLV-I) tax is not the only one factor to enhance human immunodeficiency virus type-1 (HIV-1) infection in culture-supernatants. Virus Genes 2001; 23: 77-80.

5. Lewinski MK, Bisgrove D, Shinn P et al: Genome-wide analysis of chromosomal features repressing human immunodeficiency virus transcription. J Virol 2005; 79: 6610-9.

6. Yoder JA, Walsh CP, Bestor TH: Cytosine methylation and the ecology of intragenomic parasites. Trends Gene 1997; 13: 335-40.

7. Mizutani T, Ito T, Nishina M et al: Maintenance of integrated proviral gene expression requires Brm, a catalytic subunit of SWI/SNF complex. J Biol Chem 2002; 277: 15859-64. Epub.

8. Martens JA, Winston F: Recent advances in understanding chromatin remodeling by Swi/Snf complexes. Curr Opin Genet Dev 2003; 13: 136-42.

9. Poleshko A, Palagin I, Zhang R et al: Identification of cellular proteins that maintain retroviral epigenetic silencing: evidence for an antiviral response. J Virol 2008; 82: 2313-23.

10. Svoboda J, Hejnar J, Geryk J et al: Retroviruses in foreign species and the problem of provirus silencing. Gene 2000; 261: 181-8.

11. Swindle CS, Klug CA: Mechanisms that regulate silencing of gene expression from retroviral vector. J Hematother Stem Cell Res 2002; 11: 449-56.

12. Yao S, Sukonnik T, Kean T et al: Retrovirus silencing, variegation, extinction, and memory are controlled by a dynamic interplay of multiple epigenetic modifications. Mol Ther 2004; 10: 27-36.

13. Ellis J: Silencing and variegation of gammaretrovirus and lentivirus vectors. Hum Gene Ther 2005; 16: 1241-6.

14. Ellis J, Yao S: Retrovirus silencing and vector design: relevance to normal and cancer stem cells? Curr Gene Ther 2005; 5: 367-73.

15. Schroder ARW, Shinn P, Chen H et al: HIV-1 integration in the human genome favors active genes and local hotspots. Cell 2002; 110: 521-9.

16. Marcello A, Dhir S, Dieudonne M: Nuclear positional control of HIV transcription in 4D. Nucleus 2010; 1: 8-11.

17. Pereira LA, Bentley K, Peeters A et al: A complication of cellular transcription factor interactions with the HIV-1 LTR promoter. Nucl Acid Res 2000; 28: 663-8.

18. Rohr O, Marban C, Aunis D et al: Regulation of HIV-1 gene transcription: from lymphocytes to microglial cells. J. Leukoc Biol 2003; 74: 736-49.

19. Lee MH, Padmashali R, Andreadis ST: JNK1 is required for lentivirus entry and gene transfer.

J Virol 2011; 85: 2657-65.

20. Leyva FJ, Anzinger JJ, McCoy JP et al: Evaluation of transduction efficiency in macrophage colony-stimulating factor differentiated human macrophages using HIV-1 based lentiviral vectors. BMC Biothech 2011; 11: 13

21. Rawat P, Mitra D Cellular heat shock factor 1 positively regulates human immunodeficient virus-1 gene expression and replication by two distinct pathways. Nucl Acid Res 2011; 39: 1-14.

22. Cheng H, Tarnok J, Parks WP: Human immunodeficiency virus type 1 genome activation induced by human T-cell leukemia virus type 1 Tax protein is through cooperation of NF-kappaB and Tat. J Virol 1998; 72: 6911-6.

23. Tempaku A: Random Brownian motion regulates the quantity of human immunodeficiency virus type-1 (HIV-1) attachment and infection to target cell. J Health Sci 2005; 51: 237-41.

24. Chinnasamy D, Chinnasamy N, Enriquez MJ et al: Lentiviral-mediated gene transfer into human lymphocytes: role of HIV-1 accessory proteins. Blood 2000; 96: 1309-16.

25. Dardalhon V, Herpers B, Noraz N et al: Lentivirus-mediated gene transfer in primary T cells is enhanced by a central DNA flap. Gene Ther 2001; 8: 190-8.

26. Dropulic B: Genetic modification of hematopoietic cells using retroviral and lentiviral vectors: safety considerations for vector design and delivery into target cells. Curr Hematol Rep 2005; 4: 300-4.

27. Funke S, Maisner A, Muhlebach MD et al: Target cell entry of lentiviral vectors. Mol Ther

2008; 16: 1427-36.

28. Bovia F, Salmon P, Matthes T et al: Efficient transduction of primary human B lymphocytes and nondividing myeloma B cells with HIV-1-derived lentiviral vectors. Blood 2003; 101: 1727-33.

29. Janssens W, Chuah MK, Naldini L et al: Efficiency of onco-retroviral and lentiviral gene transfer into primary mouse and human B-lymphocytes is pseudotype dependent. Hum Gene Ther 2003; 14: 263-76.

Figure legends

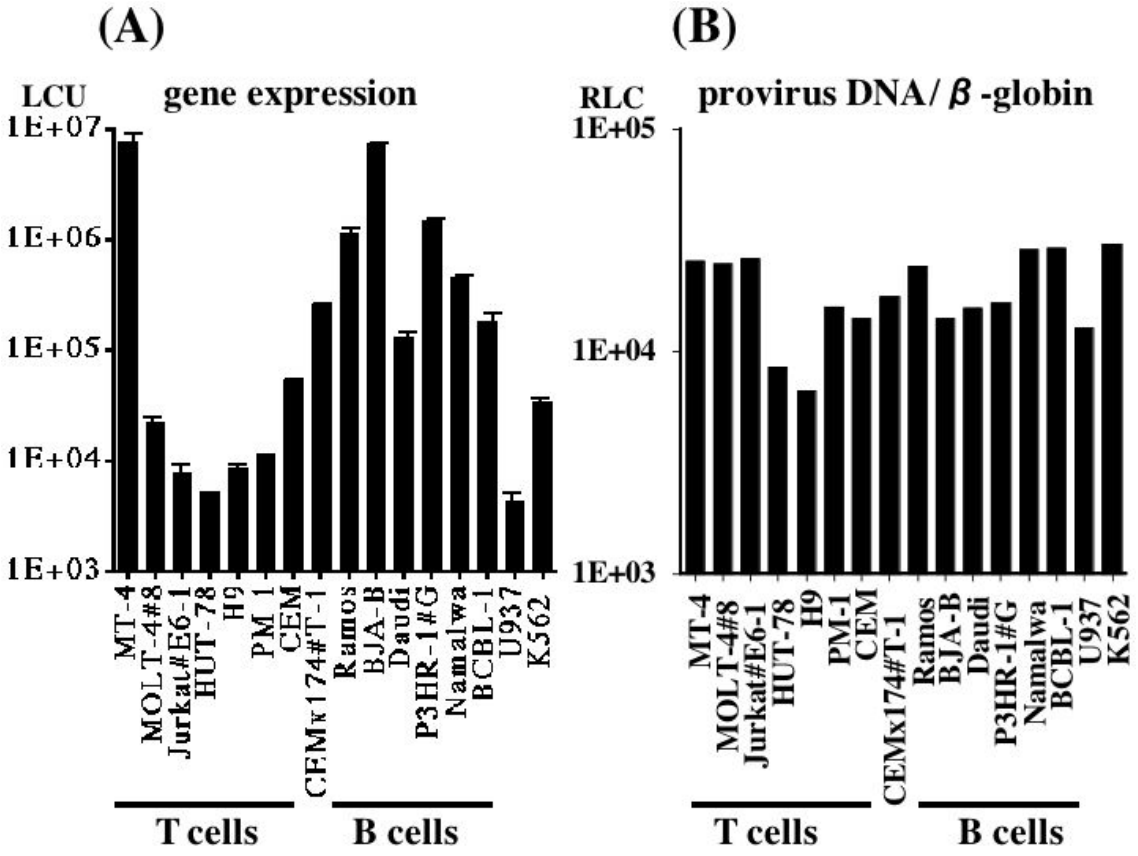
Figure 1

(A) Comparison of gene transduction and expression efficiencies by VSV-G enveloped lentivirus vector. T lymphocytes (MT-4, MOLT-4#8, Jurkat#E6-1, HUT-78, H9, PM-1, CEM), T and B complex cell (CEMx174#T-1), B lymphocytes (Ramos, BJA-B, Daudi, P3HR-1#G, Namalwa, BCBL-1), monocyte cell (U937) and myeloid cell (K562) were used as target cells. Infectivity was measured at three days post infection by luciferase assay. Vertical axis meant the light counts of luciferase activities. Representative result of three times experiments, which were performed with using different times cultured cells, was aligned. Average counts were showed by black bars. All experiments were performed as triplicate assay (n=3). Every result of absolute count in same type of cell was within 1% discrepancy.

(B) Quantification of synthesized provirus DNA amount by PCR. Two-ng of vector virus infected 1 x 10^6 cells. One day after infection, total DNA was extracted and subjected to the PCR analysis. PCR products were separated by electrophoresis in 2% agarose gel. The quantities of PCR products were measured by densidometer. Vertical axis meant the light intensity of provirus DNA product band calibrated by ß-globin DNA amount (light intensity of provirus DNA/ß-globin DNA). Representative result of two times experiments, which were performed with using different times cultured cells, was aligned. Every experiment was done as single assay (n=1).

(C) Relative gene expression levels were compared. Vertical axis meant the gene expression amount, which was calibrated by the provirus DNA amount. The equation of calibration was that; light count of luciferase activities/protein amount/ light intensity of provirus DNA/ light intensity of β-globin DNA. Representative result of two times experiments, which were performed with using different times cultured cells, was aligned. Every experiment was done as single assay (n=1). Every calibration results showed similar tendency.

Figure 1



(C)