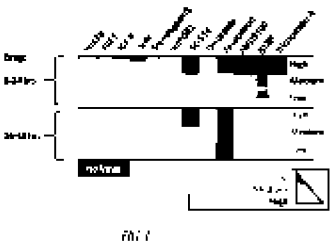
Abstract:



(EN) The present invention provides methods and compositions for improving the efficacy of viral transduction of cells. More particularly, the present invention provides methods and materials useful for safely and reliably improving the efficiency of methods for transducing cells, such as human hematopoietic stem cells (HSC), with viruses and/or viral vectors. The compositions and methods are useful for therapeutic indications amenable to treatment with hematopoietic stem cell gene therapies.

CLAIMS:

1. A method for increasing the transduction efficiency of cells cultured with a retrovirus comprising: culturing the cells and the retrovirus in a culture medium that comprises a compound that increases prostaglandin EP receptor signaling.

2. The method of claim 1, wherein the cells are stem or progenitor cells.

3. The method of claim 2, wherein the stem or progenitor cells are selected from the group consisting of: embryonic stem cells and induced pluripotent stem cells.

4. The method of claim 2, wherein the stem or progenitor cells are selected from the group consisting of: mesenchymal stem cells, hematopoietic stem cells, neuronal stem cells, retinal stem cells, cardiac muscle stem cells, skeletal muscle stem cells, adipose tissue derived stem cells, chondrogenic stem cells, liver stem cells, kidney stem cells, and pancreatic stem cells.

5. The method of claim 2, wherein the stem or progenitor cells are hematopoietic stem or progenitor cells.

6. The method of claim 1, wherein the cells are selected from the group consisting of: osteoblasts, chondrocytes, adipocytes, skeletal muscle, cardiac muscle, neurons, astrocytes, oligodendrocytes, Schwann cells, retinal cells, corneal cells, skin cells, monocytes, macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes, dendritic cells, T-lymphocytes, B-lymphocytes, NK-cells, gastric cells, intestinal cells, smooth muscle cells, vascular cells, bladder cells, pancreatic alpha cells, pancreatic beta cells, pancreatic delta cells, hepatocytes, renal cells, adrenal cells, and lung cells.

7. The method of claim 1, wherein the cells are hematopoietic stem or hematopoietic progenitor cells.

8. The method of claim 7, wherein at least about 50% of the hematopoietic stem or progenitor cells are transduced.

9. The method of claim 7, wherein at least about 75% of the hematopoietic stem or progenitor cells are transduced.

10. The method of claim 7, wherein at least about 90% of the hematopoietic stem or progenitor cells are transduced.

11. The method of any one of claims 1 to 10, wherein the compound that increases prostaglandin EP receptor signaling is selected from the group consisting of: PGA_2; PGB_2; PGD_2; PGE_1; PGE_2; PGF_2; PGI_2; PGH_2; PGJ_2; and precursors, metabolites, derivatives and analogues thereof.

12. The method of any one of claims 1 to 10, wherein the compound that increases prostaglandin EP receptor signaling is selected from the group consisting of: 15d-PGJ_2; delta12-PGJ_2; 2-hydroxyheptadecatrienoic acid (HHT); Thromboxane A2; Thromboxane B2; Iloprost; Treprostinil; Travoprost; Carboprost tromethamine; Tafluprost; Latanoprost; Bimatoprost; Unoprostone isopropyl; Cloprostenol; Oestrophan; Superphan; Misoprostol; Butaprost; Linoleic Acid; 13(s)-HODE; LY171883; Mead Acid; Eicosatrienoic Acid; Epoxyeicosatrienoic Acid; ONO-259; Cay1039; a PGE_2 receptor agonist; 16,16-dimethyl PGE_2; 19(R)-hydroxy PGE_2; 16,16-dimethyl PGE_2 p-(p-acetamidobenzamido) phenyl ester; 11-deoxy-16,16-dimethyl PGE_2; 9-deoxy-9-methylene-16,16-dimethyl PGE_2; 9-deoxy-9-methylene PGE_2; Sulprostone; PGE_2 serinol amide; PGE_2 methyl ester; 16-phenyl tetranor PGE_2; 15(S)-15-methyl PGE_2; 15(R)-15-methyl PGE_2; BIO; 8-bromo-cAMP; Forskolin; Bapta-AM; Fendiline; Nicardipine; Nifedipine; Pimozide; Strophanthidin; Lanatoside; L-Arg; Sodium Nitroprusside; Sodium Vanadate; Bradykinin; Mebeverine; Flurandrenolide;

Atenolol; Pindolol; Gaboxadol; Kynurenic Acid; Hydralazine; Thiabendazole; Bicuclline; Vesamicol; Peruvoside; Imipramine; Chlorpropamide; 1,5-Pentamethylenetetrazole; 4-Aminopyridine; Diazoxide; Benfotiamine; 12-Methoxydodecenoic acid; N-Formyl-Met-Leu-Phe; Gallamine; IAA 94; and Chlorotrianisene.

13. The method of any one of claims 1 to 10, wherein the compound that increases prostaglandin EP receptor signaling is selected from the group consisting of: prostaglandin E2(PGE_2), or 16,16-dimethyl PGE_2.

14. The method of any one of claims 1 to 13, further comprising culturing the cells and retrovirus in the presence of a histone deacetylase (HDAC) inhibitor.

15. The method of claim 14, wherein the HDAC inhibitor is selected from the group consisting of: Trichostatin A (TSA), valproic acid (VPA), sodium butyrate, suberoylanilide hydroxamic acid (SAHA), sodium phenylbutyrate, depsipeptide, trapoxin (TPX), cyclic hydroxamic acid-containing peptide 1 (CHAPl), MS-275, LBH589, and PXD-101.

16. The method of any one of claims 1 to 15, wherein the retrovirus is a lentivirus.

17. The method of any one of claims 1 to 16, wherein the retrovirus is a Human immunodeficiency virus (HIV) virus.

18. The method of any one of claims 1 to 17, wherein the retrovirus is pseudotyped with a vesicular stomatitis virus G-protein (VSV-G) envelope protein.

19. The method of any one of claims 1 to 18, wherein the cells are cultured in the presence of the compound that increases prostaglandin EP receptor signaling prior to transduction.

20. The method of claim 19, wherein the cells are cultured with the compound that increases prostaglandin EP receptor signaling for at least about 2 hours.

21. The method of claim 19, wherein the cells are cultured with the compound that increases prostaglandin EP receptor signaling for at least about 4 hours.

22. The method of any one of claims 1 to 19, wherein the cells are cultured in the presence of the compound that increases prostaglandin EP receptor signaling during transduction.

23. The method of claim 22, wherein the cells are cultured in the presence of the compound that increases prostaglandin EP receptor signaling for at least about twenty-four hours.

24. The method of any one of claims 21 to 23, wherein the cells are cultured in the presence of the compound that increases prostaglandin EP receptor signaling during the first twenty-four hours of transduction.

25. The method of any one of claims 21 to 24, wherein the cells are cultured in the presence of the compound that increases prostaglandin EP receptor signaling during the first forty-eight hours of transduction.

26. The method of any one of claims 1 to 25, wherein the retrovirus comprises a vector comprising:

a) a left (5') retroviral LTR;

b) an expression control sequence operably linked to a gene of interest; and

c) a right (3') retroviral LTR.

27. The method of any one of claims 8 to 26, wherein the retrovirus comprises a vector comprising:

a) a left (5') HIV-1 LTR;

b) a Psi packaging sequence (Ψ+);

c) an HIV-1 central polypurine tract/DNA flap (cPPT/FLAP);

d) a rev response element (RRE);

e) a β-globin promoter and a β-globin locus control region (LCR) operably linked to a gene of interest; and

f) a right (3') retroviral LTR that comprises:

i) one or more insulator elements, or

ii) a rabbit β-globin polyA sequence (rβgpA).

28. The method of any one of claims 8 to 26, wherein the hematopoietic stem or progenitor cells are administered to a patient suffering from a hemoglobinopathy.

29. The method of claim 28, wherein the hemoglobinopathy is β-thalassemia or sickle cell disease.

30. The method of any one of claims 8 to 26, wherein the retrovirus comprise a vector comprising:

(a) a left (5') HIV-1 LTR;

(b) a Psi (Ψ) packaging signal;

(c) a cPPT/FLAP;

(d) an RRE;

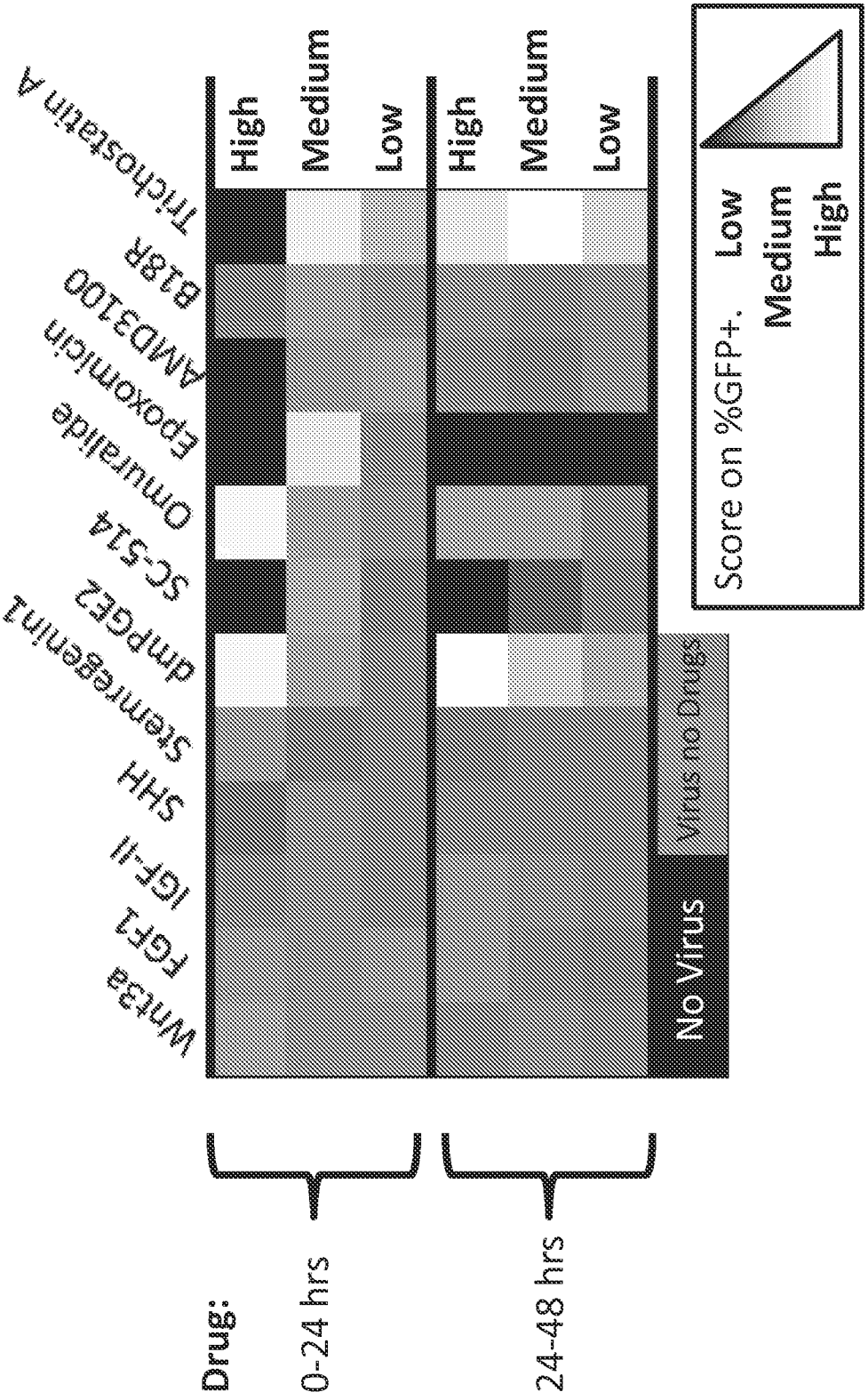
(e) a MND promoter, operably linked to a polynucleotide encoding a human ABCD1 polypeptide;

(f) a right (3') HIV-1 LTR; and

(g) a rabbit β-globin polyadenylation sequence.

31. The method of any one of claims 1 to 30, wherein the retrovirus is replication defective.

32. The method of claim 30, wherein the hematopoietic stem or progenitor cells are administered to a patient suffering from an adrenoleukodystrophy or an adrenomyeloneuropathy.


Drug:
0-24 hrs
24-48 hrs
High
Medium
Low
High
Medium
Low
No Virus
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Score on %GFP+.
Low
Medium
High
Wnt3a
FGF1
IGF-II
SHH
Steemregenin1
dmPGE2
SC-514
Omuralide
Epoxomicin
AMD3100
B18R
Trichostatin A

FIG. 1

COMPOUNDS FOR IMPROVED VIRAL TRANSDUCTION

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 61/541,736, filed September 30, 2011, which is incorporated by reference herein in its entirety.

STATEMENT REGARDING SEQUENCE LISTING

The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is BLBD_006_01WO_ST25.txt. The text file is 30 KB, was created on September 27, 2012, and is being submitted electronically via EFS-Web.

BACKGROUND

Technical Field

The present invention generally relates to improving the efficacy of methods of viral transduction of cells. More particularly, the present invention provides methods and materials useful for improving the efficiency of transducing cells, such as human hematopoietic stem cells (HSC), with viruses and/or viral vectors that may be useful for therapeutic indications.

Description of the Related Art

The Food and Drug Administration (FDA) has not yet approved any human gene therapy product for sale. Current gene therapy is experimental and has not proven very successful in clinical trials. Little progress has been made since the first gene therapy clinical trial began in 1990. In 1999, gene therapy suffered a major setback with the death of 18-year-old Jesse Gelsinger. Jesse was participating in a gene therapy trial for ornithine transcarboxylase deficiency (OTCD). He died from

multiple organ failures 4 days after starting the treatment. His death is believed to have been triggered by a severe immune response to the adenovirus carrier.

Another major blow came in January 2003, when the FDA placed a temporary halt on all gene therapy trials using retroviral vectors in blood stem cells. FDA took this action after it learned that a second child treated in a French gene therapy trial had developed a leukemia-like condition. Both this child and another who had developed a similar condition in August 2002 had been successfully treated by gene therapy for X-linked severe combined immunodeficiency disease (X-SCID), also known as "bubble baby syndrome." FDA's Biological Response Modifiers Advisory Committee (BRMAC) met at the end of February 2003 to discuss possible measures that could allow a number of retroviral gene therapy trials for treatment of life-threatening diseases to proceed with appropriate safeguards. In April of 2003, the FDA eased the ban on gene therapy trials using retroviral vectors in blood stem cells.

Recently, however, several groups have led moderately successful gene therapy trials in combating several diseases. In, 2008, UK researchers from the UCL Institute of Ophthalmology and Moorfields Eye Hospital NIHR Biomedical Research Centre announced a successful gene therapy clinical trial for treatment of Leber's congenital amaurosis, a type of inherited blindness. The results showed that the experimental treatment is safe and can improve sight (Maguire *et al.*, *N Engl J Med.* 358(21):2240 (2008)).

In 2011, Neurologix, Inc. announced positive results in a Phase 2 trial of its investigational gene therapy for advanced Parkinson's disease (PD), NLX-P101. Study participants who received NLX-P101 experienced statistically significant and clinically meaningful improvements in off-medication motor scores compared to control subjects who received sham surgery. In the trial, this benefit was seen at one month and continued virtually unchanged throughout the six month blinded study period. The results also demonstrated a positive safety profile for NLX-P101, with no serious adverse events related to the gene therapy or surgical procedure reported. Patients enrolled in the trial had moderate to advanced PD and were not adequately responsive to current therapies.

In 2009, a French group of scientists reported using hematopoietic stem cell mediated gene therapy to successfully treat X-linked adrenoleukodystrophy

(ALD). Autologous stem cells were removed from the patients, genetically corrected *ex vivo* and then re-infused into the patients after they had received myeloablative treatment. Over a span of 24 to 30 months of follow-up, polyclonal reconstitution, with 9 to 14% of granulocytes, monocytes, and T and B lymphocytes expressing the ALD protein was detected. These results strongly suggest that hematopoietic stem cells were transduced in the patients. Beginning 14 to 16 months after infusion of the genetically corrected cells, progressive cerebral demyelination in the two patients stopped.

Recent progress in the field of gene therapy has raised the hope that patients afflicted with hemoglobinopathies such as β-thalassemia and sickle cell anemia will benefit from novel therapeutic approaches. Transplantation of hematopoietic cells (HCs) modified with lentiviral vectors carrying the β-globin gene has resulted in long-term correction of several mouse models of hemoglobin disorders Imren *et al.*, *Proc Natl Acad Sci U S A*. 2002;99(22):14380-14385; Malik *et al.*, *Ann NY Acad Sci*. 2005;1054:238-249; May *et al.*, *Nature*. 2000;406(6791):82-86; Pawliuk *et al.*, *Science*. 2001;294(5550): 2368-2371), but in contrast, has led to transfusion independence in only one β-thalassemic patient (Cavazzana-Calvo *et al.*, *Nature*. 2010;467(7313):318-322).

Although the main advantages of infusing genetically modified autologous cells are to avoid the risks of GVHD and immunosuppressive pretransplant conditioning as well as to address the lack of compatible donors, current therapy faces at least three substantive caveats: the requirement for toxic myeloablation (Dunbar *et al,*. *Hum Gene Ther*. 1998;9(17):2629-2640); current gene transfer methods are unable to transduce more than a fraction of hematopoietic stem cells (HSCs) (Santoni de Sio and Naldini, *Methods Mol Biol*. 2009;506:59-70); and various *in vivo* selection strategies available suffer from suboptimal efficacy and safety (Beard *et al.*, *J Clin Invest*. 2010;120(7):2345-2354; Cornetta *et al.*, *Cancer Gene Ther*. 2006;13(9):886-895; Milsom *et al.*, *Cancer Res*. 2008;68(15): 6171-6180). For example, in disorders amenable to hematopoietic stem cell therapy, *e.g.,* sickle cell disease, β-thalassemia, adrenoleukodystrophy, and adrenomyeloneuropathy, limitations include, but are not limited to, inefficient transduction of hematopoietic stem or progenitor cells, the requirement for toxic