myelosuppressive or myeloablative therapy, and a lack of optimal methods for *in vivo* selection of transduced cells.

Accordingly, there is a need in the art for improved methods of gene therapy and, in particular, for the treatment or prevention of hematopoietic disorders. The present invention offers solutions to these and other problems that plague the art.

SUMMARY

The present disclosure generally describes methods and compositions comprising a compound that increases prostaglandin EP receptor signaling for improving viral transduction efficiency. The compositions and methods further provide safer and more reliable 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.

In one aspect, the present invention refers to a method for increasing the transduction efficiency of cells cultured with a lentivirus comprising: culturing the cells and the lentivirus in a culture medium that comprises a compound that increases prostaglandin EP receptor signaling, wherein the cells are cultured with the lentivirus during, or prior to, culture with the compound that increases prostaglandin EP receptor signaling wherein (a) the compound that increases prostaglandin EP receptor signaling is selected from the group consisting of: PGA2; PGB2; PGD2; PGE1; PGE2; PGF2; PGI2; PGH2; PGJ2; and analogues thereof, (b) the compound that increases prostaglandin EP receptor signaling is selected from the group consisting of: 15d-PGJ2; delta12-PGJ2; 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 PGE2 receptor agonist; 16,16-dimethyl PGE2; 19(R)-hydroxy PGE2; 16,16-dimethyl PGE2 p-(pacetamidobenzamido) phenyl ester; 11-deoxy-16,16-dimethyl PGE2; 9-deoxy-9-methylene-16,16-dimethyl PGE2; 9-deoxy-9-methylene PGE2; Sulprostone; PGE2 serinol amide; PGE2 methyl ester; 16-phenyl tetranor PGE2; 15(S)¬15-methyl PGE2; 15(R)-15-methyl PGE2; 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; or (c) the compound that increases prostaglandin EP receptor signaling is selected from the group consisting of: prostaglandin E2(PGE2), or 16,16-dimethyl PGE2.

In various examples, it is contemplated, in part, a method for increasing the transduction efficiency of cells cultured with a retrovirus that comprises culturing the cells and the retrovirus in a culture medium that comprises one or more compounds that increase prostaglandin EP receptor signaling. In one example, the compound is a small molecule.

In one example, the cells are stem or progenitor cells.

In a particular example, the stem or progenitor cells are selected from the group consisting of: embryonic stem cells and induced pluripotent stem cells.

In a further example, the stem or progenitor cell 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.

In a certain example, the stem or progenitor cells are hematopoietic stem or progenitor cells.

In an additional example, 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.

In a further particular example, the cells are hematopoietic stem or hematopoietic progenitor cells.

In one example, at least about 50% of the hematopoietic stem or progenitor cells are transduced.

In another example, at least about 75% of the hematopoietic stem or progenitor cells are transduced.

In yet another example, at least about 90% of the hematopoietic stem or progenitor cells are transduced.

In particular example, any of the compositions or methods disclosed herein, comprise one or more compounds that increases prostaglandin EP receptor signaling 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.

In certain example, any of the compositions or methods disclosed herein, comprise one or more compounds that increases prostaglandin EP receptor signaling 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; Corey alcohol-A; Corey alcohol-B; Corey diol; 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.

In some example, any of the compositions or methods disclosed herein comprise one or more compounds that increase prostaglandin EP receptor signaling selected from the group consisting of: prostaglandin E2(PGE_2), or 16,16-dimethyl PGE_2.

In additional example, any of the methods disclosed herein further comprise culturing the cells and retrovirus in the presence of a histone deacetylase (HDAC) inhibitor.

In one example, 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 (CHAP1), MS-275, LBH589, and PXD-101.

In various example, any of the compositions or methods disclosed herein comprise a retrovirus that is a lentivirus.

In particular example, any of the compositions or methods disclosed herein comprise a retrovirus that is a Human immunodeficiency virus (HIV) virus.

In certain example, any of the compositions or methods disclosed herein comprise a retrovirus pseudotyped with a vesicular stomatitis virus G-protein (VSV-G) envelope protein.

In additional example, any of the methods disclosed herein comprise culturing the cells in the presence of the compound that increases prostaglandin EP receptor signaling prior to transduction.

In particular example, the cells are cultured with the compound that increases prostaglandin EP receptor signaling for at least about 2 hours.

In further example, the cells are cultured with the compound that increases prostaglandin EP receptor signaling for at least about 4 hours.

In certain example, the cells are cultured in the presence of the compound that increases prostaglandin EP receptor signaling during transduction.

In further example, the cells are cultured in the presence of the compound that increases prostaglandin EP receptor signaling for at least about twenty-four hours.

In additional example, the cells are cultured in the presence of the compound that increases prostaglandin EP receptor signaling during the first twenty-four hours of transduction.

In some example, the cells are cultured in the presence of the compound that increases prostaglandin EP receptor signaling during the first forty-eight hours of transduction.

In particular example, any of the compositions or methods disclosed herein comprise a retrovirus that comprises a vector comprising: a left (5') retroviral LTR; an expression control sequence operably linked to a gene of interest; and a right (3') retroviral LTR.

In certain example, any of the compositions or methods disclosed herein comprise a retrovirus that comprises a vector comprising: a left (5') HIV-1 LTR; a Psi packaging sequence (Ψ+); an HIV-1 central polypurine tract/DNA flap (cPPT/FLAP); a rev response element (RRE); a β-globin promoter and a β-globin locus control region (LCR) operably linked to a gene of interest; and a right (3') retroviral LTR that comprises: one or more insulator elements, or a rabbit β-globin polyA sequence (rβgpA). In various embodiments, the hematopoietic stem or progenitor cells are administered to a patient suffering from a hemoglobinopathy.

In various particular example, the hemoglobinopathy is β-thalassemia or sickle cell disease.

In certain example, any of the compositions or methods disclosed herein comprise a vector comprising: a left (5') HIV-1 LTR; a Psi (Ψ) packaging signal; a cPPT/FLAP; an RRE; a MND promoter, operably linked to a polynucleotide encoding a human ABCD1 polypeptide; a right (3') HIV-1 LTR; and a rabbit β-globin polyadenylation sequence. In various certain embodiments, the hematopoietic stem or progenitor cells are administered to a patient suffering from an adrenoleukodystrophy or an adrenomyeloneuropathy.

In various example, the retrovirus is replication defective.

CLAIMS

1. A method for increasing the transduction efficiency of cells cultured with a lentivirus comprising: culturing the cells and the lentivirus in a culture medium that comprises a compound that increases prostaglandin EP receptor signaling,
wherein the cells are cultured with the lentivirus during, or prior to, culture with the compound that increases prostaglandin EP receptor signaling
wherein
(a) the compound that increases prostaglandin EP receptor signaling is selected from the group consisting of: PGA2; PGB2; PGD2; PGE1; PGE2; PGF2; PGI2; PGH2; PGJ2; and analogues thereof,
(b) the compound that increases prostaglandin EP receptor signaling is selected from the group consisting of: 15d-PGJ2; delta12-PGJ2; 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 PGE2 receptor agonist; 16,16-dimethyl PGE2; 19(R)-hydroxy PGE2; 16,16-dimethyl PGE2 p-(pacetamidobenzamido) phenyl ester; 11-deoxy-16,16-dimethyl PGE2; 9-deoxy-9-methylene-16,16-dimethyl PGE2; 9-deoxy-9-methylene PGE2; Sulprostone; PGE2 serinol amide; PGE2 methyl ester; 16-phenyl tetranor PGE2; 15(S)15-methyl PGE2; 15(R)-15-methyl PGE2; 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;
or
(c) the compound that increases prostaglandin EP receptor signaling is selected from the group consisting of: prostaglandin E2(PGE2), or 16,16-dimethyl PGE2.

2. The method of claim 1, wherein
(a) the cells are stem or progenitor cells;
(b) 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; or

(c) the cells are hematopoietic stem or hematopoietic progenitor cells.

3. The method of claim 2(a), wherein

(a) the stem or progenitor cells are selected from the group consisting of: embryonic stem cells and induced pluripotent stem cells;

(b) 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; or

(c) the stem or progenitor cells are hematopoietic stem or progenitor cells.

4. The method of claim 2(c), wherein

(a) at least about 50% of the hematopoietic stem or progenitor cells are transduced;

(b) at least about 75% of the hematopoietic stem or progenitor cells are transduced; or

(c) at least about 90% of the hematopoietic stem or progenitor cells are transduced.

5. The method of any one of claims 1 to 4, further comprising culturing the cells and lentivirus in the presence of a histone deacetylase (HDAC) inhibitor,
wherein the HDAC inhibitor is optionally 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 (CHAPI), MS-275, LBH589, and PXD-101.

6. The method of any one of claims 1 to 5, wherein the lentivirus is optionally a Human immunodeficiency virus (HIV) virus and/or wherein the lentivirus is optionally pseudotyped with a vesicular stomatitis virus G-protein (VSV-G) envelope protein.

7. The method of any one of claims 1 to 6, wherein the cells are cultured in the presence of the compound that increases prostaglandin EP receptor signaling during transduction, optionally for at least about twenty-four hours.

8. The method of claim 7, 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 or during the first forty-eight hours of transduction.

9. The method of any one of claims 1 to 8, wherein the lentivirus comprises a vector comprising:

a) a left (5') lentviral LTR;

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

and

c) a right (3') lentiviral LTR.

10. The method of any one of claims 4 to 9, wherein

(I) the lentivirus comprises a vector comprising:

a) a left (5') HIV- 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') lentiviral LTR that comprises:

i) one or more insulator elements, or

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

(II) the hematopoietic stem or progenitor cells are to be administered to a patient suffering from a hemoglobinopathy, wherein the hemoglobinopathy is optionally β-thalassemia or sickle cell disease; or

(III) the lentivirus comprises 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.

11. The method of any one of claims 1 to 10, wherein the lentivirus is replication.