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(54) Title: LYMPHOCYTE TARGETED LENTIVIRAL VECTORS

(57) Abstract: Provided herein are lentiviral vectors comprising a mutated, heterologous envelope protein, a targeting protein, and at least one transgene for delivery to and expression by a cell characterized by the targeting protein. Also provided are methods and materials for producing the lentiviral vectors described herein, methods for transducing target cells, and cells transduced by lentiviral vectors according to the present disclosure.

LYMPHOCYTE TARGETED LENTIVIRAL VECTORS

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 930207_401WO_SEQUENCE_LISTING.txt. The text file is 158 KB, was created on February 25, 2022, and is being submitted electronically via EFS-Web.

BACKGROUND

Lentiviral vectors play a critical role in gene-modified cell therapies, particularly T cell therapies. Recently approved T cell therapies rely on retroviral vectors to transduce the therapeutic molecule (e.g., chimeric antigen receptor (CAR)) into T lymphocytes. An associated risk to CAR T cell production is the transduction of other cell types with the transgene. The use of integrating vectors with broad cell tropism, e.g., lentiviral vectors pseudotyped with a VSV-G envelope protein, can represent a serious, though rare, safety concern.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

FIG. 1 is a schematic representation of helper plasmids suitable for use in a third generation LVV production system.

FIGS. 2A-2B depict graphs (FIG. 2A) and FACS plots (FIG. 2B) showing on-target and off-target entry of Jurkat T cells and Raji B cells by lentiviral vectors bearing mutated VSV-G envelope to abolish LDL receptor binding (Trop-002, Trop-051, Trop-052, Trop-055, and Trop-061) and T cell targeting protein CD80. In FIG. 2A, on-target entry is the left bar and off-target is the right bar of each sample. Binding of the T cell targeting protein to its cognate ligand on T cells leads to entry of the lentiviral vector, and subsequent expression of reporter green fluorescent protein (GFP) is measured.

FIGS. 3A-3B depicts graphs showing: (FIG. 3A) T cell targeting protein CD80 expressed from the VSV-G packaging plasmid is expressed at relatively equivalent levels as the mutated VSV-G on the surface of HEK293 producer cells; and (FIG. 3B) LVV generated with this approach can transduce targeted Jurkat T cells but do not transduce Raji B cells.

FIG. 4 depicts graphs showing: (top row) expression levels of CD80 and mutated VSV-G on the surface of HEK293T producer cells using LVV generated by cloning CD80 targeting protein into the Rev packaging plasmid or into the mutated VSV-G packaging plasmid; and (bottom) LVV generated by cloning CD80 targeting protein into the Rev packaging plasmid or into the mutated VSV-G packaging plasmid transduce targeted Jurkat T cells.

FIG. 5 depicts (left) CD80 targeting protein expression on surface of HEK293T producer cells using a five plasmid packaging system as a function of CD80 plasmid concentration; and (right) LVV generated with the five plasmid packaging system transduce targeted Jurkat T cells and transduction efficiency was associated with CD80 packaging plasmid concentration.

FIGS. 6A-6D depict titers of lymphocyte targeting LVV produced in adherent HEK293 or suspension HEK293 producer cells. LVV harvested from HEK293 adherent cell culture medium by centrifugation (FIG. 6A) or by anion exchange chromatography followed by tangential flow filtration (FIG. 6B). LVV harvested from HEK293 suspension cell culture medium by anion exchange chromatography (FIG. 6C). Concentration by AEX/TFF resulted in LVV preparations with a high level of purity and recovery (FIG. 6D).

FIG. 7 depicts a schematic for testing T cell transduction in PBMCs from healthy human donors with LVV comprising a BCMA CAR transgene or T cell targeting LVV (anti-CD3 and CD80) comprising a BCMA CAR trasngene. Graphs shown on lower right indicate that even at low MOI, the T cell targeting LVV transduced T cells at a higher level than standard LVV and that T cell targeting LVV is capable of transducing T cells without the presence of IL-2 and exogenous activating antibodies (anti-CD3 and anti-CD28) in contrast to standard LVV.

FIG. 8 depicts graphs showing T cell expansion from PBMCs obtained from three different donors and transduced using standard LVV or T cell redirected LVV (anti-CD3 and CD80) in the presence or absence of exogenous activating anti-CD3 and anti-CD28 antibodies

FIG. 9 depcits a schematic for testing T cell transduction in PBMCs from healthy human donors with LVV comprising a CD19 CAR transgene or T cell targeting LVV (anti-CD3 and CD80) comprising a CD19 CAR trasngene. Graphs shown on lower right show that the T cell targeting LVV transduced T cells at a higher level than standard LVV and that T cell targeting LVV is capable of transducing T cells without the presence of exogenous activating antibodies (anti-CD3 and anti-CD28) in contrast to standard LVV.

FIG. 10 depicts graphs showing levels of T cell transduction efficiency and T cell activation with anti-CD3 targeting proteins (12F6 in VH-VL orientation and VL-VH orientation) used to generate T cell targeting LVV.

FIG. 11 depicts graphs showing that BCMA CAR T cells exhibited increased expression of T cell effector cytokines (TNFα – left; TNFα and IFNγ – right) after culture with BCMA-positive cell lines that is not observed with BCMA-negative cell lines whether generated by standard LVV or T cell-redirected LVV (anti-CD3 and CD80).

FIGS. 12A-12B depict graphs showing *in vivo* delivery of transgene using T cell targeting LVV: (FIG. 12A) T cell targeting LVV (anti-CD3 and CD80) specificly transduce human T cells (CD3+) and not human B cells (CD20+) in humanized mouse model (n=5); and (FIG. 12B) T cell targeting LVV transduce both CD8+ and CD8- (CD4) T cells compared to standard LVV which did not.

FIG. 13 depicts graphs showing that CD80 targeting LVVs enhance transduction of CD4 T cells compared to standard LVVs.

FIG. 14 depicts graphs showing that T cell targeting (anti-CD3 and CD80) LVVs transduce target Jurkat T cells but do not transduce off-target tumor cells (Raji, Ramos, Jeko-1, and NALM-6) compared to standard LVV.

DETAILED DESCRIPTION

Engineered lentiviral vectors are described herein. The lentiviral vectors include a mutated, heterologous envelope protein, a targeting protein, and at least one transgene for delivery to and expression by a cell characterized by the targeting protein. In some embodiments, the targeting protein is selected to target an immune cell, including, for example a lymphocyte or a T cell. In certain such embodiments, the lentiviral vectors described herein are capable of selectively targeting and efficiently transducing resting lymphocytes, e.g., T cells. In some embodiments, lentiviral vectors described herein are capable of transducing and/or activating T cells in the absence of an exogenous T cell stimulating agent. In some embodiments, lentiviral vectors described herein enhance transduction of CD4 T cells compared to standard lentiviral vectors.

In some embodiments, the lentiviral vectors incorporating a mutated env and a targeting protein as described herein are capable of producing a high titer LVV product, as compared to standard LVV incorporating another fusogenic env protein (e.g., cocal env, paramyxovirus env, truncated VSV-G env).

Also provided are methods and materials for producing the lentiviral vectors described herein, methods for transducing target cells, and cells transduced by lentiviral vectors according to the present disclosure. In some embodiments, a lentiviral vector as described herein and/or cells transduced by such a vector may be used in treating a disease or disorder responsive to the presence of cells expressing the transgene delivered by the vector.

Definitions

Prior to setting forth this disclosure in more detail, it may be helpful to an understanding thereof to provide definitions of certain terms to be used herein.

In the present description, any concentration range, percentage range, ratio range, or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated. Also, any number range recited

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