NANOCARRIERS FOR LUNG INFLAMMATION THERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. Provisional Application No. 62/747,987, filed October 19, 2018, which is hereby incorporated herein by reference in its entirety.

SEQUENCE LISTING

[0002] This application contains a sequence listing filed in electronic form as an ASCII.txt file entitled “321501-2340 Sequence Listing_ST25” created on October 18, 2019. The content of the sequence listing is incorporated herein in its entirety.

BACKGROUND

[0003] Acute Respiratory Distress Syndrome (ARDS) is characterized by severe hypoxemia, diffuse bilateral pulmonary infiltrates, and decreased pulmonary compliance. Currently there are no approved treatments for ARDS.

SUMMARY

[0003a] In an aspect of the present disclosure, there is provided a fusion protein comprising surfactant protein-A (SPA) or CD200, and an exosomal or lysosomal transmembrane protein.

[0003b] In another aspect of the present disclosure, there is provided an extracellular vesicle comprising the fusion protein disclosed herein.

[0003c] In a further aspect of the present disclosure, there is provided a cell comprising a nucleic acid encoding the fusion protein disclosed herein.

[0003d] In another aspect of the present disclosure, there is provided a method of producing an extracellular vesicle, comprising culturing the cell disclosed herein under conditions suitable for vesicle secretion, and isolating extracellular vesicles secreted by the cell.

[0003e] In a further aspect of the present disclosure, there is provided a use of an extracellular vesicle disclosed herein in the manufacture of a medicament for treating a lung disorder in a subject.

[0003f] In another aspect of the present disclosure, there is provided an extracellular vesicle disclosed herein for use in treating a lung disorder in a subject.

[0004] Disclosed herein is a nanocarrier system to effectively target lung cells loaded with anti-inflammatory cargo to modulate lung inflammation during ARDS. These nanocarriers can be obtained after transfection of cells in vitro or tissues in vivo using diverse transfection techniques (e.g. bulk electroporation, nanoelectroporation, tissue nanotransfection, viral transfection). The disclosed nanocarriers are custom-made extracellular vesicles (EVs) loaded with anti-inflammatory cargo, such as micro-RNA 146a, and are functionalized for targeted delivery via decoration with ligands for specific receptors in the lung microenvironment.

[0005] As shown in Figure 1, loading EVs with anti-inflammatory cargo, and/or decoration with cell-targeting ligands, can be achieved by transfecting, for example, plasmid DNA encoding for the specific cargo or ligand. The cargo could be varied depending on the inflammatory pathway that needs to be regulated, and the surface decoration can be varied depending on the target cell type.

[0006] For example, type II pneumocytes and lung macrophages can be targeted using plasmid genes that can encode for surfactant protein-A (SPA) and membrane glycoprotein CD200, respectively. EVs functionalized with SPA interact

with receptor P63/CKAP4 on type II cells, while EVs functionalized with CD200 preferentially interact with the CD200R receptor in alveolar macrophages.

[0007] The EV can be functionalized with SDF1 which will interact with the receptor CXCR7 in the lung vasculature. This can be valuable, for example, when the EVs are delivered by intravascular methods.

[0008] As shown in Figure 2, surface-decorated EVs can also be loaded with anti-inflammatory cargo that are membrane-permeable pharmacological compounds (e.g., Y-27632 Rho-inhibitor, Calbiochem) that can diffuse into the EVs via a concentration gradient.

[0009] The details of one or more examples of the present disclosure are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the present disclosure will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

[0010] FIG. 1 is a schematic representation of production of custom-made nanocarriers loaded with anti-inflammatory cargo and/or decorated with cell-targeting ligands after transfection of cells or tissues.

[0011] FIG. 2 is a schematic representation of surface-decorated EVs loaded with other anti-inflammatory cargo, for example, a membrane-permeable pharmacological compound.

[0012] FIGs. 3A to 3E show in vitro-derived designer EV characterization. FIG. 3A and 3B shows designer EVs loaded with miR-146a isolated 24 hours after nanotransfection of primary mouse embryonic fibroblasts (PMEFs), with a particle size in the range of 200 nm and EV concertation in the range of billions of particles per mL. FIG. 3C shows qRT-PCR characterization of miR-146A loaded designer EVs obtained from mouse dendritic cells 48 hours after nanotransfection with plasmids encoding for miR-146A and a sham/control plasmid (*p-value=0.041). FIG. 3D shows relative miR-146A expression in A549 lung epithelial cells and differentiated THP-1 monocytes treated with these miR-146A or sham designer EVs (*p-value<0.001). FIG. 3E shows C57BL/6 mouse primary alveolar epithelial cells after 48 hours of treatment with fluorescently labeled miR-146a designer EVs derived from PMEFs, where white arrows highlights EV uptake.

[0013] FIGs. 4A to 4F show in vivo-derived designer EV characterization. FIG. 4A is a schematic representation of tissue nanotransfection (TNT) of skin in vivo and subsequent isolation of designer EVs. FIG. 4B shows particle concentration in the order of ten trillion EVs per cm^2 of skin. FIG. 4C shows average particle size in

WHAT IS CLAIMED IS:

1. A fusion protein comprising surfactant protein-A (SPA) or CD200, and an exosomal or lysosomal transmembrane protein.
2. An extracellular vesicle comprising the fusion protein of claim 1.
3. The extracellular vesicle of claim 2, further comprising a therapeutic cargo.
4. The extracellular vesicle of claim 3, wherein the therapeutic cargo comprises miR146a.
5. The extracellular vesicle of claim 3 or 4, wherein the therapeutic cargo comprises Y-27632.
6. A cell comprising a nucleic acid encoding the fusion protein of claim 1.
7. The cell of claim 6, further comprising a nucleic acid encoding a therapeutic RNA.
8. A method of producing an extracellular vesicle, comprising culturing the cell of claim 6 or 7 under conditions suitable for vesicle secretion, and isolating extracellular vesicles secreted by the cell.
9. The method of claim 8, further comprising loading the extracellular vesicle with a therapeutic drug.
10. Use of an extracellular vesicle of any one of claims 3 to 5 in the manufacture of a medicament for treating a lung disorder in a subject.
11. The use of claim 10, wherein the lung disorder comprises Acute Respiratory Distress Syndrome (ARDS).
12. An extracellular vesicle of any one of claims 3 to 5 for use in treating a lung disorder in a subject.
13. The extracellular vesicle for use of claim 12, wherein the lung disorder comprises Acute Respiratory Distress Syndrome (ARDS).