

1

**LIPOSOMES CONTAINING NOVEL
TARGETING AND/OR FUSOGENIC
PEPTIDES, PREPARATIONS CONTAINING
THEM AND THERAPEUTIC USE THEREOF**

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to cell-specific targeting peptides, to fusogenic peptides, to liposomes containing the cell-specific targeting peptides and/or fusogenic peptides, to methods of making the liposomes, and to therapeutic methods of using the liposomes to treat disorders.

2. Description of Related Art

For the past three decades, targeting drugs, oligonucleotides, and genes to a specific tissue have been one of the fundamental goals of the pharmaceutical industry. Until recently this has been a very elusive problem. One of the fundamental properties of living cells is their ability to sense and respond to their environment. This is accomplished by a specific set of receptors on the cell surface. Identifying the specific binding proteins and defining their specificity is a big stride towards resolving the problem of specific tissue targeting. The binding of targeting peptides to drugs raises many technical problems and in most cases affects the activity of the drug. The encapsulation of drugs in liposomes and other microcapsules give an important boost and opens new lines of research. Encapsulation can deliver a large number of drug molecules that are targeted towards a specific tissue, by protecting them from degradation by enzymes. Accomplishing this goal may reduce or even eliminate side effects by reducing the amount of drug needed and increasing its effectiveness due to its accumulation in the target tissue. Since most of the targeting peptides are water-soluble and the phospholipids are oil soluble, specific linkers and several steps are required for binding these two molecules. The situation gets more complicated when the conjugation of several peptides at different concentrations is required for the delivery of a gene.

Accordingly, it was an object of the present invention to provide a simplified means of incorporating targeting peptides into liposomes.

Another object was to identify new targeting peptides and their target cells.

SUMMARY OF THE INVENTION

These and other objects were met with the present invention, which relates in a first embodiment to an isolated and purified peptide comprising an amino acid sequence selected from the group consisting of:

- (a) SEQ ID NO: 1;
- (b) SEQ ID NO: 2;
- (c) SEQ ID NO: 3; and
- (d) SEQ ID NO: 4.

The present invention relates in a second embodiment to a liposome comprising at least one peptide, which comprises SEQ ID NO: 3.

The present invention relates in a third embodiment to a liposome comprising at least one peptide, which comprises SEQ ID NO: 4.

The present invention relates in a fourth embodiment to a method of preparing a liposome, wherein the method comprises the following steps:

- (a) preparing a solution comprising water, at least one of the abovementioned peptides, and other components of the liposome; and
- (b) manipulating the solution to form the liposome.

2

The present invention relates in a fifth embodiment to a pharmaceutical composition comprising:

- (a) a plurality of the abovementioned liposomes; and
- (b) a pharmaceutically acceptable carrier.

5 The present invention relates in a sixth embodiment to a method of treating a disorder in a patient in need thereof, wherein the method comprises administering to the patient at least one of the abovementioned liposomes in an amount effective to treat the disorder, wherein the liposome comprises a payload effective for the treating.

The present invention relates in a seventh embodiment to a method of delivering a therapeutic payload to lung cells, wherein the method comprises the following steps:

- (a) formulating at least one lung cell targeted liposome comprising SEQ ID NO: 1 and at least one therapeutic payload comprising at least one gene encoding a therapeutic protein, at least one antisense oligonucleotide, and/or at least one therapeutic drug; and
- (b) administering the at least one lung cell targeted liposome to a patient in need thereof.

The present invention relates in an eighth embodiment to a method of protecting a patient against the deleterious effects of nerve agent poisoning, wherein the method comprises the following steps:

- (a) formulating at least one lung cell targeted liposome comprising SEQ ID NO: 1 and comprising at least one cholinesterase gene; and
- (b) administering the at least one lung cell targeted liposome to the patient.

BRIEF DESCRIPTION OF THE DRAWINGS

One preferred embodiment or a small number of preferred embodiments of the invention will now be described with reference to the drawings, wherein:

FIG. 1 consists of a series of charts of flow-cytometry measurements of plasmid encapsulated in liposomes before and after purification on G-50 Sephadex column;

FIG. 2 consists of a series of images demonstrating the efficiency of the targeting peptides in binding to two of the most abundant cell types in the lungs—lung epithelial cells and mast cells;

FIG. 3 consists of two graphs demonstrating the fusion of liposomes conjugated with fusion peptide and targeting peptide;

FIG. 4 is a confocal microscope representation of the distribution of DNA (green) and liposome membrane (red) post transfection of RBL cell line;

FIG. 5 is a graph depicting secretion of AChE enzyme in A549 human lung epithelial cells; and

FIG. 6 is confocal microscope representation of the expression of GFP gene in lung epithelial cells post transfection.

It is understood that the references to the drawings herein are meant to be exemplary of the preferred embodiment(s) described, and that neither the drawings themselves, nor the reference numerals on the drawings are meant to be limiting of the invention in any respect.

DETAILED DESCRIPTION OF THE INVENTION

The peptides of SEQ ID NOS: 1-4 can be prepared by well known synthesis schemes, or can be obtained from well known commercial sources. One example of a well known commercial source is SynPep of Dublin, Calif.

By conjugating the N-terminal of either SEQ ID NO: 1 or SEQ ID NO: 2 to the carboxy group of 1,2-dioleoyl-sn-