DESIGNER EXTRACELLULAR VESICLES FOR TREATING EXCITOTOXICITY

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit of U.S. Provisional Application No. 62/990,783, filed March 17, 2020, which is hereby incorporated herein by reference in its entirety.

SEQUENCE LISTING

This application contains a sequence listing filed in electronic form as an ASCII.txt file entitled "321501_2470_Sequence_Listing_ST25" created on March 16, 2021. The content of the sequence listing is incorporated herein in its entirety.

BACKGROUND

Excitotoxicity is the pathological process by which nerve cells are damaged or killed by excessive stimulation by neurotransmitters such as glutamate and similar substances. This occurs when receptors for the excitatory neurotransmitter glutamate (glutamate receptors) such as the NMDA receptor and AMPA receptor are overactivated by glutamatergic storm. Excitotoxicity may be involved in spinal cord injury, stroke, traumatic brain injury, hearing loss (through noise overexposure or ototoxicity), and in neurodegenerative diseases of the central nervous system (CNS) such as multiple sclerosis, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Parkinson's disease, alcoholism or alcohol withdrawal and especially over-rapid benzodiazepine withdrawal, and also Huntington's disease.

SUMMARY

Disclosed herein are designer extracellular vesicles (EVs) functionalized with glutamate receptors (GluRs) that can selectively target injured regions of the CNS experiencing excitotoxicity. In some embodiments, the GluR is a metabotropic glutamate receptor (mGluR). For example, in some embodiments, the mGluR is a metabotropic glutamate receptor-1 (mGluR1), metabotropic glutamate receptor-3 (mGluR3), metabotropic glutamate receptor-4 (mGluR4), metabotropic glutamate receptor-7 (mGluR7), metabotropic glutamate receptor-8 (mGluR8), or any combination thereof. In some embodiments, the GluR is an ionotropic glutamate receptor (iGluR). iGluRs are found on pre- and postsynaptic cell membranes, primarily within the CNS1 and are divided into AMPA receptors, NMDA receptors and kainate receptors. These subfamilies are named according to their affinities for the synthetic agonists, AMPA (α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate),

NMDA (N-methyl-d-aspartate), and kainic acid (kainate). The delta receptor family has been classified as an iGluR by sequence homology.

In some embodiments, the GluR is linked to an exosomal or lysosomal transmembrane protein, e.g. expressed as a fusion protein.

GluR-decorated EVs preferentially anchor into injured areas of the CNS with a marked increase in extracellular glutamate associated with profuse neuro- and excitotoxicity. Therefore, GluR decoration can lead to enhanced homing in glutamate-rich areas of the CNS.

The disclosed GluR-decorated EVs can be used to deliver a wide variety of molecular cargo to the injured regions of the CNS. They can, for example, be used to selectively deliver proangiogenic, proneurogenic, or anti-infalmmatory molecular cargo to the brain, to boost vasculogenic and neurogenic repair processes, as well as modulating the inflammatory response after injury.

In some embodiments, the disclosed GluR-decorated EVs can be used for diagnostic applications to achieve targeted delivery of molecular beacon probes or other type of imaging agents.

In some embodiments, the disclosed GluR-decorated EVs can also serve as glutamate scavenging agents, helping to decrease the noxious concentration of free glutamate in injured regions of the brain, and aiding brain tissue recovery. For example, in some embodiments, GluR-decorated EVs can act as sink of free glutamate because it naturally binds to the receptors. In these embodiments, the dosage of GluR-decorated EVs given to the subject can be optimized to scavenge glutamate at a desired rate.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

FIG. 1A and 1B illustrate nanoscale engineering of designer extracellular vesicles for therapeutic applications.

FIGs. 2A to 2E show proneural designer EVs characterization. FIG. 2A shows transmission electron micrograph of designer EVs derived from primary mouse embryonic fibroblasts (PMEFs) and loaded with the proneural factors the ASCL1, BRN2, and MYT1L (i.e., ABM cocktail). FIG. 2B shows dynamic of proneural designer EV release showing a peak 24 hours after nanotransfection of PMEF, with a particle concentration in the order of ten billion particles per mL (*p-value=0.018). FIG. 2C shows the number of gene copies

packed inside these proneural designer EVs is approx. 3 orders of magnitude higher than that delivered to the donor cell for all three factors (*p-value=0.021). FIG. 2D shows proneural designer EVs are successfully captured by PMEFs in culture, with a peak in uptake 48 hours after treatment (*p-value ≤ 0.036). FIG. 2E Fluorescent image of PMEF cells incorporating fluorescently labeled (red) proneurogeninc-EVs 24 hours after treatment.

FIG. 3A shows confocal images of donor cells transfected with the mGluR8 or a sham vector, showing positive colocalization of cell membrane (green and white) and the transfected glutamate receptor (red) only for mGluR8 transfected cells. FIGs. 3B and 3C show Confocal images of EVs derived from sham or mGluR8 transfected donor cells, where EVs derived from GlutR8 transfected cells show co-localization of the EV membrane (green) with the targeting receptor (red). FIG. 3D Western blot of mGluR8-functionalized EVs showing positive protein expression compared to sham (control)-EVs. FIGs. 3E and 3F show characterization of designer EVs functionalized with mGRM4 and mGRM8 derived from PMEFs 24 hours after nanotransfection with plasmids encoding for each receptor or sham vector, with a particle concentration in the range of billions of EVs per mL and an average particle approximately 230 nm.

FIGs. 4A to 4C show functionalized designer EV to target the brain. FIG. 4A shows in vivo imaging of brains after intranasal delivery of fluorescently labeled designer EVs functionalized with mGluR8 or sham (control) EVs, showing significantly higher accumulation of functionalized EVs in the brain 24 hours after treatment. FIGs. 4B and 4C show immunofluorescence images of cerebellum and corpus callosum (sagittal cut) of brains of animals treated with fluorescently labeled (red) sham- or mGluR8-EVs, 24 hours post-intranasal instillation, and respective fluorescence intensity quantification (n=3, *p-value=0.0083).

FIG. 5 show characterization of relative expression of metabotropic glutamate receptors (mGluR4 and mGluR8) in designer EVs, these receptors are used to functionalize neurogenic designer EVs.

FIGs. 6A and 6B show designer EV biodistribution 24 hours after intranasal delivery showing higher accumulation in the brain of mice treated with designer EVs functionalized with mGluR8 vs. non-functionalized designer EVs, which accumulate in the liver tissue as they are cleared from the body.

FIGs. 7A and 7B show comparison of yield for in vitro-derived (using PMEF as donor cells) vs. in vivo-derived (using skin cells as donor cells) ABM- and control-designer EVs, showing that a significantly higher number of EVs are produced in vivo.

FIGs. 8A and 8B show efficiency of molecular loading of neurogenic factors ACL1, BRN2, and MYT1L (ABM) inside designer EVs vs. number of gene copies inside donor cells 24 hours after transfection.

FIG. 9A shows immunofluorescence images of primary neurons incorporating mGluR8 functionalized EVs (red) and control-EVs (green), showing preferential accumulation of GluR-8 EVs in postsynaptic regions (postsynaptic protein staining, PSD-95) (violet), with zoom-in regions for each type of sample (bottom) 7hours after treatment. FIG. 9B shows quantification of green (sham-EVs) or red (mGluR8-EVs) fluorescence intensity at the soma and neuronal projections (n=3, *p-value=0.024).

FIG. 10 shows mGluR4- and mGLuR8-functionalized designer EVs uptake by primary mouse embryonic neurons 8 hours after treatment.

FIG. 11A shows how prolonged culture studies suggest that PMEFs exposed to ABM-loaded EVs exhibit pro-neuronal conversions, as evidence by the increased in immunoreactivity for Tuj1 (green), a neuronal marker, relative to PMEFs exposed to control EVs as early as 7 days after treatment. These data suggest that the extent of plasmid DNA transfer from EVs to recipient cells falls within the same order of magnitude compared to direct electroporation. Additionally, the induction of Tuj1 immunoreactivity in fibroblast cultures suggest that ABM-loaded EVs could potentially be used to drive pro-neuronal responses/conversions in non-neuronal cells. FIG. 11B shows the quantification of Tuj1 fluorescence intensity 7- and 14-days post-treatment (n=3, *p-value=0.043, **p-value=0.004).

DETAILED DESCRIPTION

Before the present disclosure is described in greater detail, it is to be understood that this disclosure is not limited to particular embodiments described, and as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present disclosure will be limited only by the appended claims.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated

range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure, the preferred methods and materials are now described.

All publications and patents cited in this specification are herein incorporated by reference as if each individual publication or patent were specifically and individually indicated to be incorporated by reference and are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present disclosure is not entitled to antedate such publication by virtue of prior disclosure. Further, the dates of publication provided could be different from the actual publication dates that may need to be independently confirmed.

As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present disclosure. Any recited method can be carried out in the order of events recited or in any other order that is logically possible.

Embodiments of the present disclosure will employ, unless otherwise indicated, techniques of chemistry, biology, and the like, which are within the skill of the art.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to perform the methods and use the probes disclosed and claimed herein. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.), but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C, and pressure is at or near atmospheric. Standard temperature and pressure are defined as 20 °C and 1 atmosphere.

Before the embodiments of the present disclosure are described in detail, it is to be understood that, unless otherwise indicated, the present disclosure is not limited to particular materials, reagents, reaction materials, manufacturing processes, or the like, as such can vary. It is also to be understood that the terminology used herein is for purposes of

describing particular embodiments only, and is not intended to be limiting. It is also possible in the present disclosure that steps can be executed in different sequence where this is logically possible.

It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

The terms "extracellular vesicle" and "EV" are used herein to refer to a vesicle of about 10nm to 10μm in size consisting of fluid, macro-molecules, solutes, and metabolites from a cell contained by a lipid bilayer or micelle. In some cases, the EV is a cell-derived EV. The term "EV" also includes lipid vesicle engineered to contain bioactive molecules found in a cell-derived EVs. These terms encompass both exosomes and ectosomes. Exosomes are released on the exocytosis of multivesicular bodies (MVBs). Ectosomes are vesicles assembled at and released from the plasma membrane. In some cases, the EV is about 20nm to 10μm, 20nm to 1μm, 20 nm-500 nm, 30 nm-100nm, 30 nm-160nm, or 80-160 nm in size. In some embodiments, the EVs are exosomes that are about 20 to 150 nm in size.

Furthermore, the following terms shall have the definitions set out below. It is understood that in the event a specific term is not defined herein below, that term shall have a meaning within its typical use within context by those of ordinary skill in the art.

The term "subject" refers to any individual who is the target of administration or treatment. The subject can be a vertebrate, for example, a mammal. Thus, the subject can be a human or veterinary patient. The term "patient" refers to a subject under the treatment of a clinician, e.g., physician.

The term "therapeutically effective" refers to the amount of the composition used is of sufficient quantity to ameliorate one or more causes or symptoms of a disease or disorder. Such amelioration only requires a reduction or alteration, not necessarily elimination.

The term "pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit/risk ratio.

The terms "treat", "treating", and "treatment", etc., as used herein, refer to any action providing a benefit to a patient at risk for or afflicted by a disease state, condition or deficiency which may be improved using cellular compositions according to the present invention. Treating a condition includes improving the condition through lessening or

suppression of at least one symptom, delay in progression of the effects of the disease state or condition, including the prevention or delay in the onset of effects of the disease state or condition, etc. In the present application, treatment can involve reducing the impact of a spinal cord injury or stroke, including reversing and/or inhibiting the effects of such injury, reversing, improving, inhibiting and/or stabilizing a neurodegenerative disease such that the disease improves and/or does not progress or worsen. The term "prophylactic" is used to describe a method which "reduces the likelihood" that a particular result will occur, often the progression and/or worsening of a disease state and/or condition.

The term "autologous EV" is used to describe a population of EVs which are obtained from cells from a subject or patient to whom the EVs are to be administered.

Extracellular Vesicles

Disclosed herein are designer extracellular vesicles (EVs) functionalized with GluRs, which can selectively target injured regions of the CNS experiencing excitotoxicity.

Exosomes and microvesicles are EVs that differ based on their process of biogenesis and biophysical properties, including size and surface protein markers. Exosomes are homogenous small particles ranging from 40 to 150 nm in size and they are normally derived from the endocytic recycling pathway. In endocytosis, endocytic vesicles form at the plasma membrane and fuse to form early endosomes. These mature and become late endosomes where intraluminal vesicles bud off into an intra-vesicular lumen. Instead of fusing with the lysosome, these multivesicular bodies directly fuse with the plasma membrane and release exosomes into the extracellular space. Exosome biogenesis, protein cargo sorting, and release involve the endosomal sorting complex required for transport (ESCRT complex) and other associated proteins such as Alix and Tsg101. In contrast, microvesicles, are produced directly through the outward budding and fission of membrane vesicles from the plasma membrane, and hence, their surface markers are largely dependent on the composition of the membrane of origin. Further, they tend to constitute a larger and more heterogeneous population of extracellular vesicles, ranging from 150 to 1000 nm in diameter. However, both types of vesicles have been shown to deliver functional mRNA, miRNA and proteins to recipient cells.

The disclosed EVs can be obtained in some embodiments by culturing donor cells in cell culture medium under conditions and for a time sufficient to produce EVs, and isolating said EVs from the culture medium.

In some embodiments, the donor cell is autologous. In other embodiments, the donor cell is allogeneic. For example, these could be cells isolated from tissue biopsies (e.g., skin) or other cells derived from specific organs from matching donors.

In some embodiments, the donor cells can be any cell able to produce EVs, including (but not limited to) skin cells (e.g., fibroblasts, keratinocytes, skin stem cells), adipocytes, dendritic cells, peripheral blood mononuclear cells (PBMC), pancreatic cells (e.g., ductal epithelial cells), liver cells (e.g., hepatocytes), immune cells (e.g., T cells, macrophages, myeloid derived suppressor cells), Endothelial cells, or intervertebral disc cells.

As described in U.S. Patent Application Document No. 20140356382, "[e]xosomes produced from cells can be collected from the culture medium and/or cell tissue by any suitable method. Typically a preparation of EVs can be prepared from cell culture or tissue supernatant by centrifugation, filtration or combinations of these methods. For example, EVs can be prepared by differential centrifugation, that is low speed (<2,0000 g) centrifugation to pellet larger particles followed by high speed (>100,000 g) centrifugation to pellet EVs, size filtration with appropriate filters (for example, 0.22 μm filter), gradient ultracentrifugation (for example, with sucrose gradient) or a combination of these methods." It is noted that the contents of EVs, i.e., EVs in which the lipid bilayer has been removed or eliminated and the contents obtained may also be used to engineer artificial EVs.

Further, as described in U.S. Patent Application Document No. 20140356382, exogenous protein and/or peptide and other cargo can be introduced into the EVs by a number of different techniques including electroporation or the use of a transfection reagent. Electroporation conditions may vary depending on the charge and size of the biotherapeutic cargo. Typical voltages are in the range of 20V/cm to 1,000V/cm, such as 20V/cm to 100V/cm with capacitance typically between 25 μF and 250 μF, such as between 25 μF and 125 μF. A voltage in the range of 150 mV to 250 mV, particularly a voltage of 200 mV is preferred for loading EVs with an antibody. Alternatively, the EVs may be loaded with exogenous protein and/or peptide using a transfection reagent. Despite the small size of the EVs, conventional transfection agents may be used for transfection of EVs with protein and/or peptide. EVs may also be loaded by transforming or transfecting a host cell with a nucleic acid construct which expresses therapeutic protein or peptide of interest, such that the therapeutic protein or peptide is taken up into the EVs as the EVs are produced from the cell.

In illustrative embodiments, the EV-producing cells disclosed herein are cultured for about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 days or for as long as about 1, 2, 3, 4, 5, 6, 7, 8 weeks or about 1, 2, 3, 4, 5, or 6 months, depending on the cell and its ability to produce

EVs. The EV-producing cells may be cultured in suitable media and grown under conditions that are readily determined by one of ordinary skill in the art. Cell culture conditions may vary with cell type and the examples presented hereinafter illustrate suitable media and conditions.

EVs can be harvested at various time intervals (e.g. at about 2, 4, 6, 8 or 3, 6, 9, 12 day or longer intervals, depending upon the rate of production of EVs). Exemplary yields of EVs can range from at least about 1 ng EVs/1 million cells, at least about 10 ng EVs/1 million cells, at least about 50 ng EVs/1 million cells, at least about 100 ng EVs/1 million cells, at least about 500 ng EVs/1 million cells, at least about 750 ng EVs/1 million cells, at least about 800 ng EVs/1 million cells, at least about 900 ng EVs/1 million cells, at least about 1.0 µg EVs/1 million cells, at least about 1.5 µg EVs/1 million cells, at least about 2.0 µg EVs/1 million cells, at least about 2.5 µg EVs/1 million cells, at least e.g. about 3.0 µg EVs/1 million cells, at least about 5.0 µg EVs/1 million cells, and at least about 10.0 µg EVs/1 million cells, during a time period of about 24 hours to seven days of culture of proliferative and non-proliferative neural cells as otherwise described herein.

In certain embodiments, EVs are harvested and collected by ultracentrifugation or differential centrifugation or any combination thereof, pelleted EVs are collected, and, optionally, collected pelleted EVs are washed with a suitable medium. For example, a preparation of EVs can be prepared from cell culture or tissue supernatant by centrifugation, filtration or combinations of these methods. In some embodiments, the EVs can be prepared by differential centrifugation, that is low speed (<2,0000 g) centrifugation to pellet larger particles followed by high speed (>100,000 g) centrifugation to pellet EVs, size filtration with appropriate filters (for example, 0.22 µm filter), gradient ultracentrifugation (for example, with sucrose gradient) or a combination of these methods. EVs may be purified by differential centrifugation, micro and ultra-filtration, polymeric precipitation, microfluidic separation, immunocapture and size-exclusion chromatography. These and/or related methods for isolating and purifying EVs are described by Théry, *et al.*, *Current Protocols in Cell Biology,* (2006) 3.221-3.22.29, copyright 2006 by John Wiley & Sons, Inc.; Sokolova, et al., *Colloids and Surfaces* B: Biointerfaces, 2011, 87, 146-150; Wiklander, et al., *Journal of Extracellular Vesicles,* 2015, 4, 26316, pp. 1-13; and Böing, et al., *Journal of Extracellular Vesicles,* 2014, 3, 23430, pp. 1-11. Other methods for isolation may be developed such as electrical field radiofrequency and acoustics.

Glutamate Receptors

The disclosed designer extracellular vesicles (EVs) are functionalized with glutamate receptors (GluRs) that can selectively target injured regions of the CNS experiencing excitotoxicity. In some embodiments, the GluR is a metabotropic glutamate receptor (mGluR). For example, in some embodiments, the mGluR is a metabotropic glutamate receptor-1 (mGluR1), metabotropic glutamate receptor-3 (mGluR3), metabotropic glutamate receptor-4 (mGluR4), metabotropic glutamate receptor-7 (mGluR7), metabotropic glutamate receptor-8 (mGluR8), or any combination thereof. In some embodiments, the GluR is an ionotropic glutamate receptor (iGluR). iGluRs are found on pre- and postsynaptic cell membranes, primarily within the CNS1 and are divided into AMPA receptors, NMDA receptors and kainate receptors. These subfamilies are named according to their affinities for the synthetic agonists, AMPA (α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate), NMDA (N-methyl-d-aspartate), and kainic acid (kainate). The delta receptor family has been classified as an iGluR by sequence homology.

Therefore, in some embodiments, donor cells are modified with a transgene capable of expressing GluRs in the donor cell. In some embodiments, a polynucleotides comprising a GluR gene operably linked to an expression control sequence is delivered to the donor cell. In some embodiments, the polynucleotide is in a DNA vector. DNA vectors, such as viral vectors and non-viral vectors are known in the art and can be used to produce donor cells capable of producing the disclosed designer EVs. In some embodiments, the non-viral vector is a recombinant bacterial plasmid. In some embodiments, the non-viral vector has a pCDNA3 backbone. In some embodiments, the vector comprises an internal ribosome entry site (IRES).

Examples of mouse and human nucleic acid and amino acid sequences for GluRs for use in the disclosed compositions and methods are provided below.

In some embodiments, the mouse metabotropic glutamate receptor 4 (mGluR4) has an ORF nucleic acid sequence:

ATGTCCGGGAAGGGAGGCTGGGCCTGGTGGTGGGCCCGGCTGCCCCTCTGCC
TACTCCTCAGCCTTTATGGCTCCTGGGTGCCTTCATCCCTAGGAAAGCCCAAGGGTCA
CCCCCACATGAACTCTATCCGTATCGATGGAGACATCACCCTGGGAGGCCTGTTTCCC
GTCCACGGTCGCGGCTCCGAGGGCAAGGCCTGCGGCGAGTTGAAGAAGGAGAAAGGT
ATCCACCGGCTGGAGGCCATGCTCTTTGCCCTGGACCGCATCAACAACGACCCGGACC
TACTGCCCAACATCACGTTGGGCGCCCGCATTCTGGACACCTGCTCAAGGGATACCCA
CGCCCTGGAGCAGTCCCTGACCTTTGTGCAGGCGCTCATCGAGAAGGACGGCACGGA
GGTCCGCTGCGGCAGCGGGGGCCCACCCATCATCACCAAGCCTGAACGAGTGGTGGG

TGTCATCGGAGCTTCGGGGAGCTCCGTCTCGATCATGGTGGCCAACATCCTCCGCCTC
TTCAAGATCCCCCAGATCAGCTACGCCTCCACGGCTCCCGACTTGAGTGATAACAGCC
GCTATGACTTCTTCTCCCGGGTCGTGCCCTCGGACACATACCAGGCCCAGGCCATGGT
GGACATCGTCCGGGCCCTCAAGTGGAACTATGTGTCCACGCTGGCCTCAGAGGGTAG
CTACGGCGAGAGCGGCGTGGAGGCCTTTATCCAGAAGTCCCGAGAGAACGGAGGCGT
GTGCATTGCCCAGTCGGTGAAGATTCCACGGGAACCCAAGACCGGGGAGTTTGACAAG
ATCATCAAACGCCTTCTGGAAACGTCCAATGCCAGAGCCATCATCATCTTTGCCAACGA
GGATGATATCAGGAGGGTGCTGGAGGCAGCGCGCAGGGCCAACCAGACCGGCCACTT
CTTTTGGATGGGTTCTGATAGCTGGGGCTCCAAGAGCGCCCCCGTGCTGCGCCTTGAG
GAAGTGGCTGAAGGTGCAGTCACCATTCTTCCCAAGAGGACGTCTGTGCGAGGGTTTG
ACCGATACTTCTCCAGCCGCACGCTTGACAACAACAGGCGCAACATCTGGTTTGCTGA
GTTCTGGGAGGACAACTTCCATTGCAAGTTGAGCCGCCACGCGCTCAAGAAGGGAAGC
CACATCAAGAAGTGCACCAACCGAGAGCGCATCGGGCAGGACTCGGCCTACGAACAG
GAGGGGAAGGTGCAGTTTGTGATCGACGCCGTGTACGCCATGGGCCATGCTCTGCAC
GCCATGCATCGTGACCTGTGTCCCGGCCGCGTAGGACTCTGCCCTCGAATGGACCCT
GTGGATGGCACCCAGCTGCTTAAGTACATCAGAAACGTCAACTTCTCAGGCATCGCCG
GGAACCCGGTGACCTTCAACGAGAACGGAGACGCGCCAGGGCGTTATGACATCTACC
AGTACCAACGTCGCAACGGCTCGGCTGAGTACAAGGTCATCGGCTCATGGACAGACCA
CTTGCACCTCAGAATAGAGCGGATGCAGTGGCCAGGGAGTGGCCAGCAGCTGCCACG
CTCCATCTGCAGCCTGCCCTGCCAGCCAGGCGAGCGGAAGAAGACGGTGAAGGGCAT
GGCTTGCTGCTGGCACTGCGAGCCCTGCACGGGGTACCAGTACCAGGTGGACCGCTA
CACCTGTAAGACCTGCCCCTATGACATGCGGCCCACGGAGAACCGCACGAGCTGCCA
GCCCATACCCATTGTCAAGTTGGAGTGGGACTCACCCTGGGCTGTGCTGCCCCTCTTC
CTGGCTGTGGTGGGCATTGCTGCCACGCTGTTCGTGGTGGTCACTTTTGTGCGCTACA
ACGACACTCCGATCGTCAAGGCCTCGGGCCGGGAGCTGAGCTACGTCCTGCTGGCGG
GCATCTTTCTCTGCTATGCCACCACCTTCCTCATGATCGCAGAGCCTGACCTGGGGAC
CTGTTCACTCCGCCGCATCTTCCTGGGGCTTGGCATGAGCATCAGCTACGCGGCCCTG
CTGACCAAGACCAACCGCATCTACCGCATCTTTGAGCAGGGCAAGCGGTCGGTCAGCG
CCCCACGGTTCATCAGCCCCGCCTCGCAGCTGGCCATCACCTTCGTCCTCATCTCGCT
GCAGTTGCTTGGCATCTGCGTGTGGTTCGTGGTGGACCCCTCCCACTCGGTGGTGGAC
TTCCAGGACCAGCGGACACTTGACCCCCGCTTCGCCCGGGGTGTGCTCAAGTGTGAC
ATCTCGGACCTGTCGCTCATCTGCCTCCTGGGCTACAGCATGCTGCTGATGGTCACGT
GTACTGTGTATGCCATCAAGACTCGAGGCGTGCCTGAGACCTTCAATGAGGCCAAGCC
CATCGGCTTCACCATGTACACCACCTGCATCGTCTGGCTGGCCTTCATCCCCATCTTTT

TTGGCACCTCGCAGTCGGCTGACAAGGTAACCTCTGAGGCCCTGCCCGTGGAATTCAG
CCCGCCATTGCTGGCACATAAT (SEQ ID NO:1).

In some embodiments, the mouse metabotropic glutamate receptor 4 (mGluR4) has an amino acid sequence:

MSGKGGWAWWWARLPLCLLLSLYGSWVPSSLGKPKGHPHMNSIRIDGDITLGGLFPVHG
RGSEGKACGELKKEKGIHRLEAMLFALDRINNDPDLLPNITLGARILDTCSRDTHALEQSLTF
VQALIEKDGTEVRCGSGGPPIITKPERVVGVIGASGSSVSIMVANILRLFKIPQISYASTAPDL
SDNSRYDFFSRVVPSDTYQAQAMVDIVRALKWNYVSTLASEGSYGESGVEAFIQKSRENG
GVCIAQSVKIPREPKTGEFDKIIKRLLETSNARAIIIFANEDDIRRVLEAARRANQTGHFFWMG
SDSWGSKSAPVLRLEEVAEGAVTILPKRTSVRGFDRYFSSRTLDNNRRNIWFAEFWEDNF
HCKLSRHALKKGSHIKKCTNRERIGQDSAYEQEGKVQFVIDAVYAMGHALHAMHRDLCPG
RVGLCPRMDPVDGTQLLKYIRNVNFSGIAGNPVTFNENGDAPGRYDIYQYQRRNGSAEYK
VIGSWTDHLHLRIERMQWPGSGQQLPRSICSLPCQPGERKKTVKGMACCWHCEPCTGYQ
YQVDRYTCKTCPYDMRPTENRTSCQPIPIVKLEWDSPWAVLPLFLAVVGIAATLFVVVTFVR
YNDTPIVKASGRELSYVLLAGIFLCYATTFLMIAEPDLGTCSLRRIFLGLGMSISYAALLTKTN
RIYRIFEQGKRSVSAPRFISPASQLAITFVLISLQLLGICVWFVVDPSHSVVDFQDQRTLDPRF
ARGVLKCDISDLSLICLLGYSMLLMVTCTVYAIKTRGVPETFNEAKPIGFTMYTTCIVWLAFIPI
FFGTSQSADKVTSEALPVEFSPPLLAHN (SEQ ID NO:2).

In some embodiments, the mouse metabotropic glutamate receptor 8 (mGluR8) has an ORF nucleic acid sequence:

ATGGTTTGTGAGGGAAAGCGCTCAACCTCTTGCCCTTGTTTCTTCCTTTTGACTGCCAA
GTTCTACTGGATCCTCACAATGATGCAAAGAACTCACAGCCAGGAGTATGCGCATTCCA
TCCGCCTGGATGGGGACATCATTTTGGGGGGTCTTTTTCCTGTTCATGCCAAGGGAGA
AAGAGGGGTGCCTTGTGGGGACCTGAAGAAGGAAAAGGGCATCCACAGACTTGAGGC
CATGCTTTATGCAATCGACCAGATTAATAAGGACCCCGATCTCCTCTCCAATATCACTCT
GGGTGTCCGGATCCTTGACACATGTTCCAGGGACACCTATGCTTTGGAGCAGTCACTA
ACCTTCGTGCAGGCACTGATAGAGAAAGACGCGTCTGACGTGAAGTGTGCTAATGGAG
ACCCACCCATATTCACCAAGCCCGACAAGATTTCTGGTGTCATAGGTGCTGCAGCAAG
CTCCGTGTCCATCATGGTGGCTAACATTTTAAGACTTTTTAAGATACCTCAGATTAGCTA
TGCATCTACAGCCCCAGAGCTAAGTGACAACACCAGGTATGATTTCTTTTCTCGGGTGG
TCCCGCCTGACTCCTACCAAGCCCAAGCCATGGTGGACATTGTGACAGCCCTGGGATG
GAATTATGTGTCAACACTGGCTTCCGAGGGGAACTATGGAGAGAGTGGTGTTGAGGCC
TTCACTCAGATCTCAAGGGAGATTGGTGGTGTTTGCATTGCTCAATCACAGAAAATCCC
ACGTGAACCAAGACCTGGAGAATTCGAAAAAATTATCAAACGCCTGCTGGAGACACCCA
ACGCTCGCGCAGTGATTATGTTTGCCAATGAGGATGACATCAGGAGGATATTGGAAGC

AGCAAAAAAATTAAACCAGAGTGGGCATTTTCTATGGATTGGCTCAGATAGTTGGGGAT
CCAAAATAGCACCTGTCTATCAGCAGGAGGAGATCGCCGAAGGAGCTGTGACAATTTT
GCCCAAAAGAGCATCAATTGATGGGTTTGACCGATACTTTAGAAGCCGAACTCTTGCCA
ATAATCGAAGAAATGTGTGGTTTGCAGAATTTTGGGAGGAGAATTTTGGATGCAAATTA
GGATCACATGGGAAGAGGAACAGTCATATAAAGAAATGCACAGGGCTGGAGCGAATTG
CACGGGATTCATCTTACGAACAAGAAGGAAAGGTTCAATTTGTAATTGATGCAGTGTATT
CCATGGCTTATGCACTGCACAACATGCACAAAGAACTCTGCCCTGGTTACATAGGCCTT
TGCCCAAGGATGGTTACCATCGATGGGAAAGAGCTACTGGGTTACATCAGGGCCGTGA
ATTTTAATGGCAGCGCTGGTACACCTGTCACTTTTAATGAGAATGGAGATGCTCCGGGA
CGCTACGATATCTTCCAATATCAGATAAACAACAAAAGTACAGAATACAAAATCATCGGC
CACTGGACCAATCAACTTCACCTAAAAGTGGAAGACATGCAGTGGGCTAATAGAGAGC
ACACGCACCCAGCATCTGTCTGCAGCCTGCCGTGCAAGCCTGGGGAGAGGAAGAAAA
CCGTGAAAGGGGTCCCTTGCTGCTGGCACTGTGAACGCTGCGAGGGTTATAACTACCA
GGTGGACGAACTCTCCTGTGAACTCTGCCCTTTGGATCAGAGACCAAACATCAACCGC
ACTGGCTGCCAGAGGATTCCCATCATCAAGTTGGAGTGGCATTCACCCTGGGCCGTGG
TACCTGTGTTCATAGCAATATTGGGAATCATTGCCACCACCTTTGTGATTGTGACCTTTG
TCCGCTATAATGACACACCAATCGTGAGAGCTTCTGGGCGGGAACTTAGTTATGTGCTC
CTAACGGGGATTTTTCTCTGTTACTCAATCACTTTTTTGATGATTGCGGCACCTGACACA
ATCATCTGCTCTTTCCGAAGGATCTTCCTGGGACTTGGTATGTGTTTCAGCTATGCAGC
ACTTTTGACCAAAACAAACCGTATCCACCGAATATTCGAGCAAGGGAAGAAATCTGTCA
CAGCACCTAAGTTCATCAGCCCAGCATCCCAGCTGGTGATCACCTTCAGCCTCATCTCC
GTACAGCTCCTTGGAGTGTTTGTGTGGTTTGTCGTGGATCCCCCCCACACCATCATTGA
CTATGGAGAACAGCGAACACTGGATCCCGAGAACGCCAGGGGAGTGCTCAAGTGTGA
CATTTCCGATCTGTCACTCATTTGTTCACTGGGATACAGTATCCTCCTGATGGTCACTTG
TACTGTTTATGCCATTAAAACCAGAGGGGTTCCAGAAACGTTCAATGAAGCCAAACCTA
TTGGATTTACCATGTACACCACGTGCATCATTTGGTTAGCTTTCATTCCCATCTTTTTTG
GTACAGCCCAGTCAGCAGAAAAGATGTACATCCAGACAACAACACTTACTGTCTCCATG
AGTTTAAGTGCTTCAGTGTCTCTGGGAATGCTCTATATGCCCAAAGTTTATATTATAATTT
TTCATCCAGAGCAGAACGTTCAAAAACGCAAGAGAAGCTTCAAGGCTGTGGTCACGGC
CGCTACCATGCAAAGCAAACTGATCCAAAAGGGAAATGACAGACCAAACGGCGAGGTG
AAAAGTGAACTCTGTGAGAGTCTTGAAACCAACAGTAAGTCATCTGTAGACTTTCAGAT
GGTCAAGAGCGGGAGCACTTCC (SEQ ID NO:3).

In some embodiments, the mouse metabotropic glutamate receptor 8 (mGluR8) has an amino acid sequence:

MVCEGKRSTSCPCFFLLTAKFYWILTMMQRTHSQEYAHSIRLDGDIILGGLFPVHAKGERGV
PCGDLKKEKGIHRLEAMLYAIDQINKDPDLLSNITLGVRILDTCSRDTYALEQSLTFVQALIEK
DASDVKCANGDPPIFTKPDKISGVIGAAASSVSIMVANILRLFKIPQISYASTAPELSDNTRYD
FFSRVVPPDSYQAQAMVDIVTALGWNYVSTLASEGNYGESGVEAFTQISREIGGVCIAQSQ
KIPREPRPGEFEKIIKRLLETPNARAVIMFANEDDIRRILEAAKKLNQSGHFLWIGSDSWGSKI
APVYQQEEIAEGAVTILPKRASIDGFDRYFRSRTLANNRRNVWFAEFWEENFGCKLGSHGK
RNSHIKKCTGLERIARDSSYEQEGKVQFVIDAVYSMAYALHNMHKELCPGYIGLCPRMVTID
GKELLGYIRAVNFNGSAGTPVTFNENGDAPGRYDIFQYQINNKSTEYKIIGHWTNQLHLKVE
DMQWANREHTHPASVCSLPCKPGERKKTVKGVPCCWHCERCEGYNYQVDELSCELCPL
DQRPNINRTGCQRIPIIKLEWHSPWAVVPVFIAILGIIATTFVIVTFVRYNDTPIVRASGRELSY
VLLTGIFLCYSITFLMIAAPDTIICSFRRIFLGLGMCFSYAALLTKTNRIHRIFEQGKKSVTAPKFI
SPASQLVITFSLISVQLLGVFVWFVVDPPHTIIDYGEQRTLDPENARGVLKCDISDLSLICSLG
YSILLMVTCTVYAIKTRGVPETFNEAKPIGFTMYTTCIIWLAFIPIFFGTAQSAEKMYIQTTTLT
VSMSLSASVSLGMLYMPKVYIIIFHPEQNVQKRKRSFKAVVTAATMQSKLIQKGNDRPNGEV
KSELCESLETNSKSSVDFQMVKSGSTS (SEQ ID NO:4).

In some embodiments, the human metabotropic glutamate receptor 4 (mGluR4) has an ORF nucleic acid sequence:

ATGCCTGGGAAGAGAGGCTTGGGCTGGTGGTGGGCCCGGCTGCCCCTTTGCCTGCTC
CTCAGCCTTTACGGCCCCTGGATGCCTTCCTCCCTGGGAAAGCCCAAAGGCCACCCTC
ACATGAATTCCATCCGCATAGATGGGGACATCACACTGGGAGGCCTGTTCCCGGTGCA
TGGCCGGGGCTCAGAGGGCAAGCCCTGTGGAGAACTTAAGAAGGAAAAGGGCATCCA
CCGGCTGGAGGCCATGCTGTTCGCCCTGGATCGCATCAACAACGACCCGGACCTGCT
GCCTAACATCACGCTGGGCGCCCGCATTCTGGACACCTGCTCCAGGGACACCCATGC
CCTCGAGCAGTCGCTGACCTTTGTGCAGGCGCTCATCGAGAAGGATGGCACAGAGGT
CCGCTGTGGCAGTGGCGGCCCACCCATCATCACCAAGCCTGAACGTGTGGTGGGTGT
CATCGGTGCTTCAGGGAGCTCGGTCTCCATCATGGTGGCCAACATCCTTCGCCTCTTC
AAGATACCCCAGATCAGCTACGCCTCCACAGCGCCAGACCTGAGTGACAACAGCCGCT
ACGATTTCTTCTCCCGCGTGGTGCCCTCGGACACGTACCAGGCCCAGGCCATGGTGGA
CATCGTCCGTGCCCTCAAGTGGAACTATGTGTCCACAGTGGCCTCGGAGGGCAGCTAT
GGTGAGAGCGGTGTGGAGGCCTTCATCCAGAAGTCCCGTGAGGACGGGGGCGTGTGC
ATCGCCCAGTCGGTGAAGATACCACGGGAGCCCAAGGCAGGCGAGTTCGACAAGATC
ATCCGCCGCCTCCTGGAGACTTCGAACGCCAGGGCAGTCATCATCTTTGCCAACGAGG
ATGACATCAGGCGTGTGCTGGAGGCAGCACGAAGGGCCAACCAGACAGGCCATTTCTT
CTGGATGGGCTCTGACAGCTGGGGCTCCAAGATTGCACCTGTGCTGCACCTGGAGGA
GGTGGCTGAGGGTGCTGTCACGATCCTCCCCAAGAGGATGTCCGTACGAGGCTTCGA

CCGCTACTTCTCCAGCCGCACGCTGGACAACAACCGGCGCAACATCTGGTTTGCCGAG
TTCTGGGAGGACAACTTCCACTGCAAGCTGAGCCGCCACGCCCTCAAGAAGGGCAGC
CACGTCAAGAAGTGCACCAACCGTGAGCGAATTGGGCAGGATTCAGCTTATGAGCAGG
AGGGGAAGGTGCAGTTTGTGATCGATGCCGTGTACGCCATGGGCCACGCGCTGCACG
CCATGCACCGTGACCTGTGTCCCGGCCGCGTGGGGCTCTGCCCGCGCATGGACCCTG
TAGATGGCACCCAGCTGCTTAAGTACATCCGAAACGTCAACTTCTCAGGCATCGCAGG
GAACCCTGTGACCTTCAATGAGAATGGAGATGCGCCTGGGCGCTATGACATCTACCAA
TACCAGCTGCGCAACGATTCTGCCGAGTACAAGGTCATTGGCTCCTGGACTGACCACC
TGCACCTTAGAATAGAGCGGATGCACTGGCCGGGGAGCGGGCAGCAGCTGCCCCGCT
CCATCTGCAGCCTGCCCTGCCAACCGGGTGAGCGGAAGAAGACAGTGAAGGGCATGC
CTTGCTGCTGGCACTGCGAGCCTTGCACAGGGTACCAGTACCAGGTGGACCGCTACAC
CTGTAAGACGTGTCCCTATGACATGCGGCCCACAGAGAACCGCACGGGCTGCCGGCC
CATCCCCATCATCAAGCTTGAGTGGGGCTCGCCCTGGGCCGTGCTGCCCCTCTTCCTG
GCCGTGGTGGGCATCGCTGCCACGTTGTTCGTGGTGATCACCTTTGTGCGCTACAACG
ACACGCCCATCGTCAAGGCCTCGGGCCGTGAACTGAGCTACGTGCTGCTGGCAGGCA
TCTTCCTGTGCTATGCCACCACCTTCCTCATGATCGCTGAGCCCGACCTTGGCACCTGC
TCGCTGCGCCGAATCTTCCTGGGACTAGGGATGAGCATCAGCTATGCAGCCCTGCTCA
CCAAGACCAACCGCATCTACCGCATCTTCGAGCAGGGCAAGCGCTCGGTCAGTGCCC
CACGCTTCATCAGCCCCGCCTCACAGCTGGCCATCACCTTCAGCCTCATCTCGCTGCA
GCTGCTGGGCATCTGTGTGTGGTTTGTGGTGGACCCCTCCCACTCGGTGGTGGACTTC
CAGGACCAGCGGACACTCGACCCCCGCTTCGCCAGGGGTGTGCTCAAGTGTGACATC
TCGGACCTGTCGCTCATCTGCCTGCTGGGCTACAGCATGCTGCTCATGGTCACGTGCA
CCGTGTATGCCATCAAGACACGCGGCGTGCCCGAGACCTTCAATGAGGCCAAGCCCAT
TGGCTTCACCATGTACACCACTTGCATCGTCTGGCTGGCCTTCATCCCCATCTTCTTTG
GCACCTCGCAGTCGGCCGACAAGCTGTACATCCAGACGACGACGCTGACGGTCTCGG
TGAGTCTGAGCGCCTCGGTGTCCCTGGGAATGCTCTACATGCCCAAAGTCTACATCAT
CCTCTTCCACCCGGAGCAGAATGTGCCCAAGCGCAAGCGCAGCCTCAAAGCCGTCGTT
ACGGCGGCCACCATGTCCAACAAGTTCACGCAGAAGGGCAACTTCCGGCCCAACGGA
GAGGCCAAGTCTGAGCTCTGCGAGAACCTTGAGGCCCCAGCGCTGGCCACCAAACAG
ACTTACGTCACTTACACCAACCATGCAATC (SEQ ID NO:5).

In some embodiments, the human metabotropic glutamate receptor 4 (mGluR4) has an amino acid sequence:

MPGKRGLGWWWARLPLCLLLSLYGPWMPSSLGKPKGHPHMNSIRIDGDITLGGLFPVHGR
GSEGKPCGELKKEKGIHRLEAMLFALDRINNDPDLLPNITLGARILDTCSRDTHALEQSLTFV
QALIEKDGTEVRCGSGGPPIITKPERVVGVIGASGSSVSIMVANILRLFKIPQISYASTAPDLS

DNSRYDFFSRVVPSDTYQAQAMVDIVRALKWNYVSTVASEGSYGESGVEAFIQKSREDGG
VCIAQSVKIPREPKAGEFDKIIRRLLETSNARAVIIFANEDDIRRVLEAARRANQTGHFFWMG
SDSWGSKIAPVLHLEEVAEGAVTILPKRMSVRGFDRYFSSRTLDNNRRNIWFAEFWEDNFH
CKLSRHALKKGSHVKKCTNRERIGQDSAYEQEGKVQFVIDAVYAMGHALHAMHRDLCPGR
VGLCPRMDPVDGTQLLKYIRNVNFSGIAGNPVTFNENGDAPGRYDIYQYQLRNDSAEYKVI
GSWTDHLHLRIERMHWPGSGQQLPRSICSLPCQPGERKKTVKGMPCCWHCEPCTGYQY
QVDRYTCKTCPYDMRPTENRTGCRPIPIIKLEWGSPWAVLPLFLAVVGIAATLFVVITFVRYN
DTPIVKASGRELSYVLLAGIFLCYATTFLMIAEPDLGTCSLRRIFLGLGMSISYAALLTKTNRIY
RIFEQGKRSVSAPRFISPASQLAITFSLISLQLLGICVWFVVDPSHSVVDFQDQRTLDPRFAR
GVLKCDISDLSLICLLGYSMLLMVTCTVYAIKTRGVPETFNEAKPIGFTMYTTCIVWLAFIPIFF
GTSQSADKLYIQTTTLTVSVSLSASVSLGMLYMPKVYIILFHPEQNVPKRKRSLKAVVTAATM
SNKFTQKGNFRPNGEAKSELCENLEAPALATKQTYVTYTNHAI (SEQ ID NO:6).

In some embodiments, the human metabotropic glutamate receptor 8 (mGluR8) has an ORF nucleic acid sequence:

ATGGTATGCGAGGGAAAGCGATCAGCCTCTTGCCCTTGTTTCTTCCTCTTGACCGCCAA
GTTCTACTGGATCCTCACAATGATGCAAAGAACTCACAGCCAGGAGTATGCCCATTCCA
TACGGGTGGATGGGGACATTATTTTGGGGGGTCTCTTCCCTGTCCACGCAAAGGGAGA
GAGAGGGGTGCCTTGTGGGGAGCTGAAGAAGGAAAAGGGGATTCACAGACTGGAGGC
CATGCTTTATGCAATTGACCAGATTAACAAGGACCCTGATCTCCTTTCCAACATCACTCT
GGGTGTCCGCATCCTCGACACGTGCTCTAGGGACACCTATGCTTTGGAGCAGTCTCTA
ACATTCGTGCAGGCATTAATAGAGAAAGATGCTTCGGATGTGAAGTGTGCTAATGGAGA
TCCACCCATTTTCACCAAGCCCGACAAGATTTCTGGCGTCATAGGTGCTGCAGCAAGCT
CCGTGTCCATCATGGTTGCTAACATTTTAAGACTTTTTAAGATACCTCAAATCAGCTATG
CATCCACAGCCCCAGAGCTAAGTGATAACACCAGGTATGACTTTTTCTCTCGAGTGGTT
CCGCCTGACTCCTACCAAGCCCAAGCCATGGTGGACATCGTGACAGCACTGGGATGGA
ATTATGTTTCGACACTGGCTTCTGAGGGGAACTATGGTGAGAGCGGTGTGGAGGCCTT
CACCCAGATCTCGAGGGAGATTGGTGGTGTTTGCATTGCTCAGTCACAGAAAATCCCA
CGTGAACCAAGACCTGGAGAATTTGAAAAAATTATCAAACGCCTGCTAGAAACACCTAA
TGCTCGAGCAGTGATTATGTTTGCCAATGAGGATGACATCAGGAGGATATTGGAAGCA
GCAAAAAAACTAAACCAAAGTGGGCATTTTCTCTGGATTGGCTCAGATAGTTGGGGATC
CAAAATAGCACCTGTCTATCAGCAAGAGGAGATTGCAGAAGGGGCTGTGACAATTTTGC
CCAAACGAGCATCAATTGATGGATTTGATCGATACTTTAGAAGCCGAACTCTTGCCAAT
AATCGAAGAAATGTGTGGTTTGCAGAATTCTGGGAGGAGAATTTTGGCTGCAAGTTAGG
ATCACATGGGAAAAGGAACAGTCATATAAAGAAATGCACAGGGCTGGAGCGAATTGCT
CGGGATTCATCTTATGAACAGGAAGGAAAGGTCCAATTTGTAATTGATGCTGTATATTCC

ATGGCTTACGCCCTGCACAATATGCACAAAGATCTCTGCCCTGGATACATTGGCCTTTG
TCCACGAATGAGTACCATTGATGGGAAAGAGCTACTTGGTTATATTCGGGCTGTAAATT
TTAATGGCAGTGCTGGCACTCCTGTCACTTTTAATGAAAACGGAGATGCTCCTGGACGT
TATGATATCTTCCAGTATCAAATAACCAACAAAAGCACAGAGTACAAAGTCATCGGCCA
CTGGACCAATCAGCTTCATCTAAAAGTGGAAGACATGCAGTGGGCTCATAGAGAACATA
CTCACCCGGCGTCTGTCTGCAGCCTGCCGTGTAAGCCAGGGGAGAGGAAGAAAACGG
TGAAAGGGGTCCCTTGCTGCTGGCACTGTGAACGCTGTGAAGGTTACAACTACCAGGT
GGATGAGCTGTCCTGTGAACTTTGCCCTCTGGATCAGAGACCCAACATGAACCGCACA
GGCTGCCAGCTTATCCCCATCATCAAATTGGAGTGGCATTCTCCCTGGGCTGTGGTGC
CTGTGTTTGTTGCAATATTGGGAATCATCGCCACCACCTTTGTGATCGTGACCTTTGTC
CGCTATAATGACACACCTATCGTGAGGGCTTCAGGACGCGAACTTAGTTACGTGCTCCT
AACGGGGATTTTTCTCTGTTATTCAATCACGTTTTTAATGATTGCAGCACCAGATACAAT
CATATGCTCCTTCCGACGGGTCTTCCTAGGACTTGGCATGTGTTTCAGCTATGCAGCCC
TTCTGACCAAAACAAACCGTATCCACCGAATATTTGAGCAGGGGAAGAAATCTGTCACA
GCGCCCAAGTTCATTAGTCCAGCATCTCAGCTGGTGATCACCTTCAGCCTCATCTCCGT
CCAGCTCCTTGGAGTGTTTGTCTGGTTTGTTGTGGATCCCCCCCCACATCATCATTGACT
ATGGAGAGCAGCGGACACTAGATCCAGAGAAGGCCAGGGGAGTGCTCAAGTGTGACA
TTTCTGATCTCTCACTCATTTGTTCACTTGGATACAGTATCCTCTTGATGGTCACTTGTA
CTGTTTATGCCATTAAAACGAGAGGTGTCCCAGAGACTTTCAATGAAGCCAAACCTATT
GGATTTACCATGTATACCACCTGCATCATTTGGTTAGCTTTCATCCCCATCTTTTTTGGT
ACAGCCCAGTCAGCAGAAAAGATGTACATCCAGACAACAACACTTACTGTCTCCATGAG
TTTAAGTGCTTCAGTATCTCTGGGCATGCTCTATATGCCCAAGGTTTATATTATAATTTTT
CATCCAGAACAGAATGTTCAAAAACGCAAGAGGAGCTTCAAGGCTGTGGTGACAGCTG
CCACCATGCAAAGCAAACTGATCCAAAAAGGAAATGACAGACCAAATGGCGAGGTGAA
AAGTGAACTCTGTGAGAGTCTTGAAACCAACACTTCCTCTACCAAGACAACATATATCA
GTTACAGCAATCATTCAATC (SEQ ID NO:7).

In some embodiments, the human metabotropic glutamate receptor 8 (mGluR8) has an amino acid sequence:

MVCEGKRSASCPCFFLLTAKFYWILTMMQRTHSQEYAHSIRVDGDIILGGLFPVHAKGERG
VPCGELKKEKGIHRLEAMLYAIDQINKDPDLLSNITLGVRILDTCSRDTYALEQSLTFVQALIE
KDASDVKCANGDPPIFTKPDKISGVIGAAASSVSIMVANILRLFKIPQISYASTAPELSDNTRY
DFFSRVVPPDSYQAQAMVDIVTALGWNYVSTLASEGNYGESGVEAFTQISREIGGVCIAQS
QKIPREPRPGEFEKIIKRLLETPNARAVIMFANEDDIRRILEAAKKLNQSGHFLWIGSDSWGS
KIAPVYQQEEIAEGAVTILPKRASIDGFDRYFRSRTLANNRRNVWFAEFWEENFGCKLGSH
GKRNSHIKKCTGLERIARDSSYEQEGKVQFVIDAVYSMAYALHNMHKDLCPGYIGLCPRMS

TIDGKELLGYIRAVNFNGSAGTPVTFNENGDAPGRYDIFQYQITNKSTEYKVIGHWTNQLHL
KVEDMQWAHREHTHPASVCSLPCKPGERKKTVKGVPCCWHCERCEGYNYQVDELSCEL
CPLDQRPNMNRTGCQLIPIIKLEWHSPWAVVPVFVAILGIIATTFVIVTFVRYNDTPIVRASGR
ELSYVLLTGIFLCYSITFLMIAAPDTIICSFRRVFLGLGMCFSYAALLTKTNRIHRIFEQGKKSV
TAPKFISPASQLVITFSLISVQLLGVFVWFVVDPPHIIIDYGEQRTLDPEKARGVLKCDISDLSL
ICSLGYSILLMVTCTVYAIKTRGVPETFNEAKPIGFTMYTTCIIWLAFIPIFFGTAQSAEKMYIQT
TTLTVSMSLSASVSLGMLYMPKVYIIIFHPEQNVQKRKRSFKAVVTAATMQSKLIQKGNDRP
NGEVKSELCESLETNTSSTKTTYISYSNHSI (SEQ ID NO:8).

In some embodiments, the disclosed designer EVs are functionalized with GluRs having at least 65%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 2, 4, 6, or 8.

Examples of commercially available constructs for expression of GluRs in donor cells include mGluR4 (NM_001013385; Mouse Tagged ORF Clone; OriGene Technologies, Inc., CAT#: MR210868), mGluR8 (NM_008174; Mouse Tagged ORF Clone; OriGene Technologies, Inc., CAT#: MR211148), Metabotropic Glutamate Receptor 4 (mGluR4) (NM_000841; Human Tagged ORF Clone; OriGene Technologies, Inc., CAT#: RC223673) and Metabotropic Glutamate Receptor 8 (mGluR8) (NM_000845; Human Tagged ORF Clone; OriGene Technologies, Inc., CAT#: RC210813).

In some embodiments, the polynucleotides are delivered to the donor cells for EVs, intracellularly via a gene gun, a microparticle or nanoparticle suitable for such delivery, transfection by electroporation, three-dimensional nanochannel electroporation, a tissue nanotransfection device, a liposome suitable for such delivery, or a deep-topical tissue nanoelectroinjection device. In some embodiments, a viral vector can be used. However, in other embodiments, the polynucleotides are not delivered virally.

Electroporation is a technique in which an electrical field is applied to cells in order to increase permeability of the cell membrane, allowing cargo (e.g., reprogramming factors) to be introduced into cells. Electroporation is a common technique for introducing foreign DNA into cells.

Transmembrane Domain

In some embodiments, the GluR is linked to an exosomal or lysosomal transmembrane protein, e.g. expressed as a fusion protein. Design strategies for producing exosomes is described in Liu C, et al. Theranostics. 2019 9(4):1015–1028, which is incorporated by reference for the teaching of transmembrane proteins that can be used to

guide fusion proteins into exosomes. Therefore, in some embodiments, the transmembrane protein is selected from the group consisting of CD63, CD9, CD81, CD53, CD82, CD37 (Tetraspanins), Alix (endosome-associated proteins), flotillin-1 (lipid raft-associated protein), TSG101 (Component of the ESCRT-I complex), ARRDC (Arrestin family of protein), Palmitoylated tdTomato (Tandem dimer Tomato fused at NH2-termini with a palmitoylation signal for EV membrane labelling), Lactadherin C1C2 domain (Membrane glycoprotein), EGF VIII (Transmembrane glycoprotein), PDGFR TM domain (Cell surface tyrosine kinase receptor), HIV-1 Nef (mut) (Released in extracellular vesicles), VSVG (Vesicular stomatitis virus glycoprotein), LAMP2B (Lysosome-Associated Membrane Glycoprotein 2), LAMP1 (Lysosome-Associated Membrane Glycoprotein 1), ALIX-1 (Cytosolic protein that associates with MVB by interacting with ESCRT-III subunit SNF7), HSP70 (Heat Shock Protein), HSP90 (Heat Shock Protein), MHC (Anchored in the membrane), SCAMPs (Secretory Carrier-Associated Membrane Protein 18), ApoE (Apolipoprotein E), and WW tag (Recognized by the L-domain-containing protein Ndfip1, resulting in ubiquitination and loading into exosomes).

Also disclosed are polynucleotides comprising nucleic acid sequences encoding a transmembrane protein suitable for guiding the APC-targeting ligand into an exosome. Examples of this type of proteins include tetraspanins CD9, CD63, and CD81.

Therefore, in some embodiments, the transmembrane protein is CD9 and comprises the amino acid sequence:
MPVKGGTKCIKYLLFGFNFIFWLAGIAVLAIGLWLRFDSQTKSIFEQETNNNNSSFYTGVYILI
GAGALMMLVGFLGCCGAVQESQCMLGLFFGFLLVIFAIEIAAAIWGYSHKDEVIKEVQEFYK
DTYNKLKTKDEPQRETLKAIHYALNCCGLAGGVEQFISDICPKKDVLETFTVKSCPDAIKEVF
DNKFHIIGAVGIGIAVVMIFGMIFSMILCCAIRRNREMV (SEQ ID NO:9), or an amino acid sequence that has at least 65%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:9. Therefore, in some embodiments, the nucleic acid sequence encoding the APC-targeting ligand is encoded by the nucleic acid sequence:
GACCAGCCTACAGCCGCCTGCATCTGTATCCAGCGCCAGGTCCCGCCAGTCCCAGCT
GCGCGCGCCCCCCAGTCCCGCACCCGTTCGGCCCAGGCTAAGTTAGCCCTCACCATG
CCGGTCAAAGGAGGCACCAAGTGCATCAAATACCTGCTGTTCGGATTTAACTTCATCTT
CTGGCTTGCCGGGATTGCTGTCCTTGCCATTGGACTATGGCTCCGATTCGACTCTCAG
ACCAAGAGCATCTTCGAGCAAGAAACTAATAATAATAATTCCAGCTTCTACACAGGAGT
CTATATTCTGATCGGAGCCGGCGCCCTCATGATGCTGGTGGGCTTCCTGGGCTGCTGC

GGGGCTGTGCAGGAGTCCCAGTGCATGCTGGGACTGTTCTTCGGCTTCCTCTTGGTGA
TATTCGCCATTGAAATAGCTGCGGCCATCTGGGGATATTCCCACAAGGATGAGGTGATT
AAGGAAGTCCAGGAGTTTTACAAGGACACCTACAACAAGCTGAAAACCAAGGATGAGC
CCCAGCGGGAAACGCTGAAAGCCATCCACTATGCGTTGAACTGCTGTGGTTTGGCTGG
GGGCGTGGAACAGTTTATCTCAGACATCTGCCCCAAGAAGGACGTACTCGAAACCTTC
ACCGTGAAGTCCTGTCCTGATGCCATCAAAGAGGTCTTCGACAATAAATTCCACATCAT
CGGCGCAGTGGGCATCGGCATTGCCGTGGTCATGATATTTGGCATGATCTTCAGTATG
ATCTTGTGCTGTGCTATCCGCAGGAACCGCGAGATGGTCTAGAGTCAGCTTACATCCCT
GAGCAGGAAAGTTTACCCATGAAGATTGGTGGGATTTTTTGTTTGTTTGTTTTGTTTTGT
TTGTTGTTTGTTGTTTGTTTTTTTGCCACTAATTTTAGTATTCATTCTGCATTGCTAGATAA
AAGCTGAAGTTACTTTATGTTTGTCTTTTAATGCTTCATTCAATATTGACATTTGTAGTTG
AGCGGGGGGTTTGGTTTGCTTTGGTTTATATTTTTTCAGTTGTTTGTTTTTGCTTGTTATA
TTAAGCAGAAATCCTGCAATGAAAGGTACTATATTTGCTAGACTCTAGACAAGATATTGT
ACATAAAAGAATTTTTTTGTCTTTAAATAGATACAAATGTCTATCAACTTTAATCAAGTTGT
AACTTATATTGAAGACAATTTGATACATAATAAAAAATTATGACAATGTCAAAAAAAAAAA
AAAA (SEQ ID NO:10), or a nucleic acid sequence that hybridizes to a nucleic acid sequence consisting of SEQ ID NO:10 under stringent hybridization conditions.

In some embodiments, the transmembrane protein is CD63 and comprises the amino acid sequence:

MAVEGGMKCVKFLLYVLLLAFCACAVGLIAVGVGAQLVLSQTIIQGATPGSLLPVVIIAVGVFL
FLVAFVGCCGACKENYCLMITFAIFLSLIMLVEVAAAIAGYVFRDKVMSEFNNNFRQQMENY
PKNNHTASILDRMQADFKCCGAANYTDWEKIPSMSKNRVPDSCCINVTVGCGINFNEKAIH
KEGCVEKIGGWLRKNVLVVAAAALGIAFVEVLGIVFACCLVKSIRSGYEVM (SEQ ID NO:11),
or an amino acid sequence that has at least 65%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:11. Therefore, in some embodiments, the nucleic acid sequence encoding the APC-targeting ligand is encoded by the nucleic acid sequence:

ATGGCGGTGGAAGGAGGAATGAAATGTGTGAAGTTCTTGCTCTACGTCCTCCTGCTGG
CCTTTTGCGCCTGTGCAGTGGGACTGATTGCCGTGGGTGTCGGGGCACAGCTTGTCCT
GAGTCAGACCATAATCCAGGGGGCTACCCCTGGCTCTCTGTTGCCAGTGGTCATCATC
GCAGTGGGTGTCTTCCTCTTCCTGGTGGCTTTTGTGGGCTGCTGCGGGGCCTGCAAGG
AGAACTATTGTCTTATGATCACGTTTGCCATCTTTCTGTCTCTTATCATGTTGGTGGAGG
TGGCCGCAGCCATTGCTGGCTATGTGTTTAGAGATAAGGTGATGTCAGAGTTTAATAAC
AACTTCCGGCAGCAGATGGAGAATTACCCGAAAAACAACCACACTGCTTCGATCCTGG

ACAGGATGCAGGCAGATTTTAAGTGCTGTGGGGCTGCTAACTACACAGATTGGGAGAA
AATCCCTTCCATGTCGAAGAACCGAGTCCCCGACTCCTGCTGCATTAATGTTACTGTGG
GCTGTGGGATTAATTTCAACGAGAAGGCGATCCATAAGGAGGGCTGTGTGGAGAAGAT
TGGGGGCTGGCTGAGGAAAAATGTGCTGGTGGTAGCTGCAGCAGCCCTTGGAATTGCT
TTTGTCGAGGTTTTGGGAATTGTCTTTGCCTGCTGCCTCGTGAAGAGTATCAGAAGTGG
CTACGAGGTGATG (SEQ ID NO:12), or a nucleic acid sequence that hybridizes to a nucleic acid sequence consisting of SEQ ID NO:12 under stringent hybridization conditions.

Therefore, in some embodiments, the transmembrane protein is CD81 and comprises the amino acid sequence:

MGVEGCTKCIKYLLFVFNFVFWLAGGVILGVALWLRHDPQTTNLLYLELGDKPAPNTFYVGI
YILIAVGAVMMFVGFLGCYGAIQESQCLLGTFFTCLVILFACEVAAGIWGFVNKDQIAKDVKQ
FYDQALQQAVVDDDANNAKAVVKTFHETLDCCGSSTLTALTTSVLKNNLCPSGSNIISNLFK
EDCHQKIDDLFSGKLYLIGIAAIVVAVIMIFEMILSMVLCCGIRNSSVY (SEQ ID NO:13), or an amino acid sequence that has at least 65%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:13. Therefore, in some embodiments, the nucleic acid sequence encoding the APC-targeting ligand is encoded by the nucleic acid sequence:

GGCCAGAGAGCGAGCGCGCAACGGCGGCGACGGCGGCGACCCCACCGCGCATCCTG
CCAGGCCTCCGGCGCCCAGCGCCCCACGCGCCCCCGCGCCCCCGCGCCCCCGCGCC
CCTTTCTTCGCGCCCCCGCCCCTCGGCCCGCCAGGCCCCCTTGCCGGCCACCCGCCA
GGCCCCGCGCCGGCCCGCCCGCCGCCCAGGACCGGCCCGCGCCCCGCAGGCCGCC
CGCCGCCCGCGCCGCCATGGGAGTGGAGGGCTGCACCAAGTGCATCAAGTACCTGCT
CTTCGTCTTCAATTTCGTCTTCTGGCTGGCTGGAGGCGTGATCCTGGGTGTGGCCCTGT
GGCTCCGCCATGACCCGCAGACCACCAACCTCCTGTATCTGGAGCTGGGAGACAAGCC
CGCGCCCAACACCTTCTATGTAGGCATCTACATCCTCATCGCTGTGGGCGCTGTCATGAT
GTTCGTTGGCTTCCTGGGCTGCTACGGGGCCATCCAGGAATCCCAGTGCCTGCTGGGG
ACGTTCTTCACCTGCCTGGTCATCCTGTTTGCCTGTGAGGTGGCCGCCGGCATCTGGG
GCTTTGTCAACAAGGACCAGATCGCCAAGGATGTGAAGCAGTTCTATGACCAGGCCCTA
CAGCAGGCCGTGGTGGATGATGACGCCAACAACGCCAAGGCTGTGGTGAAGACCTTCC
ACGAGACGCTTGACTGCTGTGGCTCCAGCACACTGACTGCTTTGACCACCTCAGTGCT
CAAGAACAATTTGTGTCCCTCGGGCAGCAACATCATCAGCAACCTCTTCAAGGAGGACT
GCCACCAGAAGATCGATGACCTCTTCTCCGGGAAGCTGTACCTCATCGGCATTGCTGCC
ATCGTGGTCGCTGTGATCATGATCTTCGAGATGATCCTGAGCATGGTGCTGTGCTGTGG
CATCCGGAACAGCTCCGTGTACTGAGGCCCCGCAGCTCTGGCCACAGGGACCTCTGCA

GTGCCCCCTAAGTGACCCGGACACTTCCGAGGGGGCCATCACCGCCTGTGTATATAAC
GTTTCCGGTATTACTCTGCTACACGTAGCCTTTTTACTTTTGGGGTTTTGTTTTTGTTCTG
AACTTTCCTGTTACCTTTTCAGGGCTGACGTCACATGTAGGTGGCGTGTATGAGTGGAG
ACGGGCCTGGGTCTTGGGGACTGGAGGGCAGGGGTCCTTCTGCCCTGGGGTCCCAG
GGTGCTCTGCCTGCTCAGCCAGGCCTCTCCTGGGAGCCACTCGCCCAGAGACTCAGC
TTGGCCAACTTGGGGGGCTGTGTCCACCCAGCCCGCCCGTCCTGTGGGCTGCACAGC
TCACCTTGTTCCCTCCTGCCCCGGTTCGAGAGCCGAGTCTGTGGGCACTCTCTGCCTT
CATGCACCTGTCCTTTCTAACACGTCGCCTTCAACTGTAATCACAACATCCTGACTCCGT
CATTTAATAAAGAAGGAACATCAGGCATGCTA SEQ ID NO:14), or a nucleic acid sequence that hybridizes to a nucleic acid sequence consisting of SEQ ID NO:14 under stringent hybridization conditions.

Cargo

The disclosed GluR-decorated EVs can be used to deliver a wide variety of molecular cargo to the injured regions of the CNS. They can, for example, be used to selectively deliver proangiogenic, proneurogenic, anti-infalmmatory, or neuroprotective molecular cargo to the brain, to boost vasculogenic and neurogenic repair processes, as well as modulating the inflammatory response after injury.

In some embodiments, the GluR-decorated EVs are coupled with other nanostructures (e.g., gold nanoparticles, magnetic nanoparticles, quantum dots, carbon nanotubes) either via encapsulation of chemical coupling (e.g., surface decoration) to enable additional therapeutic (e.g., hyperthermia, phototherapy, etc.) or theranostics applications.

In some embodiments, the disclosed GluR-decorated EVs can be used for diagnostic applications to achieve targeted delivery of a wide array of diagnostic agents, including molecular probes for nucleic acids or proteins, contrast/imaging agents (e.g., magnetic nanoparticles, plasmonic gold nanoparticles, quantum dots), etc.

Pharmaceutical Compositions

Disclosed is a pharmaceutical compositions containing therapeutically effective amounts of one or more of the disclosed EVs and a pharmaceutically acceptable carrier. Formulations containing the disclosed EVs may take the form of liquid, solid, semi-solid or lyophilized powder forms, such as, for example, solutions, suspensions, emulsions, sustained-release formulations, tablets, capsules, powders, suppositories, creams, ointments, lotions, aerosols, patches or the like, preferably in unit dosage forms suitable for simple administration of precise dosages.

Pharmaceutical compositions typically include a conventional pharmaceutical carrier and/or excipient and may additionally include other medicinal agents, carriers, adjuvants, additives and the like. The weight percentage ratio of the EVs to the one or more excipients can be between about 20:1 to about 1:60, or between about 15:1 to about 1:45, or between about 10:1 to about 1:40, or between about 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1 or 1:1 to about 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:15, 1:20, 1:25, 1:30, or 1:35, and preferably is about 20:1, 19:1, 18:1, 17:1, 16:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1 or 5:1. In some embodiments, the disclosed composition comprises between about 1 µg to about 1 g or more of total EVs, about 500 µg about 500 mg, about 1 mg to about 500 mg of total EVs, about 5 to about 500 mg, about 10 to about 500 mg, about 25 to about 500 mg, about 50 mg to about 350 mg, about 75 mg to about 450 mg, about 50 mg to about 450 mg, or about 75 mg to about 325 mg or about 100 mg to about 650 mg of total EVs and may optionally contain one or more suitable pharmaceutical carriers, additives and/or excipients.

An injectable composition for parenteral administration (e.g. intravenous, intramuscular, intrathecal intracerebrospinal fluid, or intranasal), will typically contain the EVs and optionally additional components in a suitable i.v. solution, such as sterile physiological salt solution. The composition may also be formulated as a suspension in an aqueous emulsion.

Liquid compositions can be prepared by dissolving or dispersing the pharmaceutical composition comprising the EVs, and optional pharmaceutical adjuvants, in a carrier, such as, for example, aqueous saline, aqueous dextrose, glycerol, or ethanol, to form a solution or suspension. For use in an oral liquid preparation, the composition may be prepared as a solution, suspension, emulsion, or syrup, being supplied either in liquid form or a dried form suitable for hydration in water or normal saline. In the case of intranasal, intratracheal or intrapulmonary administration, the compositions may be provided as liquid composition which can be sprayed into the nose, trachea and/or lungs.

For oral administration, such excipients include pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, gelatin, sucrose, magnesium carbonate, and the like. If desired, the composition may also contain minor amounts of non-toxic auxiliary substances such as wetting agents, emulsifying agents, or buffers.

When the composition is employed in the form of solid preparations for oral administration, the preparations may be tablets, granules, powders, capsules or the like. In a tablet formulation, the composition is typically formulated with additives, e.g. an excipient such as a saccharide or cellulose preparation, a binder such as starch paste or methyl

cellulose, a filler, a disintegrator, and other additives typically used in the manufacture of medical preparations.

Methods for preparing such dosage forms are known or are apparent to those skilled in the art; for example, see Remington's Pharmaceutical Sciences (17th Ed., Mack Pub. Co. 1985). The composition to be administered will contain a quantity of the selected compound in a pharmaceutically effective amount for therapeutic use in a biological system, including a patient or subject according to the present invention.

Intravenous formulations can comprise the EVs described herein, an isotonic medium and one or more substances preventing aggregation of the EVs. Example intravenous/ intrathecal/ intracerebrospinal fluid formulations may contain saline solutions (e.g. normal saline (NS); about 0.91% w/v of NaCl, about 300 mOsm/L) and/or dextrose 4% in 0.18% saline, and optionally 1%, 2% or 3% human serum albumin. In addition, the EVs may be disrupted to obtain the contents and the contents used in compositions according to the present invention.

In exemplary embodiments, formulations of the invention may comprise about 50 ng EVs/ml intravenous/intrathecal/intracerebrospinal fluid medium, including about 100 ng, 200 ng, 300 ng, 400 ng, 500 ng, 600 ng, 700 ng, 800 ng, 900 ng, 1.0 µg, 1.5 µg, 2.0 µg, 2.5 µg, 3.0 µg, 5.0 µg, 10.0, 15.0 µg, 20.0 µg, 100 µg, or more EVs/ml intravenous/intrathecal/intracerebrospinal fluid medium for use in treating spinal cord injury, stroke, traumatic brain injury and/or neurodegenerative diseases.

In some embodiments, intravenous formulations may comprise about 0.1 µg EVs/ml medium, about 0.2 µg EVs/ml intravenous medium, about 0.3 µg EVs/ml intravenous medium, about 0.4 µg EVs/ml intravenous medium, about 0.5 µg EVs/ml intravenous medium, about 0.6 µg EVs/ml intravenous medium, about 0.7 µg EVs/ml intravenous medium, about 0.8 µg EVs/ml intravenous medium, about 0.9 µg EVs/ml intravenous medium, about 1.0 µg EVs/ml intravenous medium, about 1.5 µg EVs/ml intravenous medium, about 2.0 µg EVs/ml intravenous medium, about 2.5 µg EVs/ml intravenous medium, such as at least e.g. about 3.0 µg EVs/ml intravenous medium, such as e.g. at least about 5.0 µg EVs/ml intravenous medium, about 10.0 µg EVs/ml intravenous medium, 15.0 µg EVs/ml intravenous medium or about 20.0 µg or more EVs/ml intravenous medium.

In some embodiments, the pharmaceutical composition is in a dosage form comprising at least 25 mg of EVs, at least 50 mg of EVs, at least 60 mg of EVs, at least 75 mg of EVs, at least 100 mg of EVs, at least 150 mg of EVs, at least 200 mg of EVs, at least 250 mg of EVs, at least 300 mg of EVs, about 350 mg of EVs, about 400 mg of EVs, about 500 mg of EVs, about 750 mg of EVs, about 1g (1,000mg) or more of EVs, alone or in

combination with a therapeutically effective amount of at least one additional bioactive agent, which agent may be useful in the treatment of spinal cord injury, stroke, traumatic brain injury and/or neurodegenerative disease. In some embodiments, the pharmaceutical composition comprises between about 10 mg to about 750 mg, about 25 mg to about 650 mg, or between about 30 mg to about 500 mg, or about 35 mg to about 450 mg, most often about 50 to about 500 mg of EVs.

In some embodiments, an intravenous formulation comprises the EVs described herein, an isotonic medium, and one or more substances preventing aggregation of the EVs. Intravenous formulations may therefore contain saline solutions (e.g. normal saline (NS); about 0.91% w/v of NaCl, about 300 mOsm/L) and/or dextrose 4% in 0.18% saline, and optionally 1%, 2% or 3% human serum albumin.

In some embodiments, the composition comprising the disclosed EVs further comprises one more neurotrophic agents. The composition can further comprises one or more agents selected from the group consisting of leukemia inhibitory factor (LIF), brain-derived neurotrophic factor (BDNF), epidermal growth factor receptor (EGF), basic fibroblast growth factor (bFGF), FGF-6, glial-derived neurotrophic factor (GDNF), granulocyte colony-stimulating factor (GCSF), hepatocyte growth factor (HGF), IFN-γ, insulin-like growth factor binding protein (IGFBP-2), IGFBP-6, IL-1ra, IL-6, IL-8, monocyte chemotactic protein (MCP-1), mononuclear phagocyte colony-stimulating factor (M-CSF), neurotrophic factors (NT3), tissue inhibitor of metalloproteinases (TIMP-1), TIMP-2, tumor necrosis factor (TNF-β), vascular endothelial growth factor (VEGF), VEGF-D, urokinase plasminogen activator receptor (uPAR), bone morphogenetic protein 4 (BMP4), IL1-a, IL-3, leptin, stem cell factor (SCF), stromal cell-derived factor-1 (SDF-1), platelet derived growth factor-BB (PDGFBB), transforming growth factors beta (TGFβ-1) and TGFβ-3.

In some embodiments, the disclosed EVs are contained in or on a biocompatible scaffold, such as a hydrogel. Suitable hydrogels include temperature dependent hydrogels that solidify or set at body temperature, e.g., PLURONICS™; hydrogels crosslinked by ions, e.g., sodium alginate; hydrogels set by exposure to either visible or ultraviolet light, e.g., polyethylene glycol polylactic acid copolymers with acrylate end groups; and hydrogels that are set or solidified upon a change in pH, e.g., TETRONICS™. Examples of materials that can be used to form these different hydrogels include polysaccharides such as alginate, polyphosphazenes, and polyacrylates, which are cross-linked ionically, or block copolymers such as PLURONICS™ (also known as POLOXAMERS™), which are poly(oxyethylene)-poly(oxypropylene) block polymers solidified by changes in temperature, or TETRONICS™

(also known as POLOXAMINES™), which are poly(oxyethylene)-poly(oxypropylene) block polymers of ethylene diamine solidified by changes in pH.

Suitable hydrogels also include undefined extracellular matrix derived hydrogels that originated from tissues including but not limited to bladder intestine, blood and brain.

In some embodiments, the disclosed EVs are contained in or on a biocompatible scaffold comprising collagen, fibrin, silk, agarose, alginate, hyaluronan, chitosan, a biodegradable polyester such as polylactic-co-glycolic acid, polylacic acid, or polyglycolic acid, polyethylene glycol, polyvinylpyrrolidone, polyethersulfone, a peptide-based biomaterial, glycose amino glycan, fibronectin, laminin, or any combination thereof.

In some cases, the hydrogel is produced by cross-linking the anionic salt of alginic acid, a carbohydrate polymer isolated from seaweed, with ions, such as calcium cations. The strength of the hydrogel increases with either increasing concentrations of calcium ions or alginate. For example, U.S. Pat. No. 4,352,883 describes the ionic cross-linking of alginate with divalent cations, in water, at room temperature, to form a hydrogel matrix.

EVs are mixed with an alginate solution, the solution is delivered to an already implanted support structure and then solidifies in a short time due to the presence in vivo of physiological concentrations of calcium ions. Alternatively, the solution is delivered to the support structure prior to implantation and solidified in an external solution containing calcium ions.

In general, these polymers are at least partially soluble in aqueous solutions, e.g., water, or aqueous alcohol solutions that have charged side groups, or a monovalent ionic salt thereof. There are many examples of polymers with acidic side groups that can be reacted with cations, e.g., poly(phosphazenes), poly(acrylic acids), and poly(methacrylic acids). Examples of acidic groups include carboxylic acid groups, sulfonic acid groups, and halogenated (preferably fluorinated) alcohol groups. Examples of polymers with basic side groups that can react with anions are poly(vinyl amines), poly(vinyl pyridine), and poly(vinyl imidazole).

Polyphosphazenes are polymers with backbones consisting of nitrogen and phosphorous atoms separated by alternating single and double bonds. Each phosphorous atom is covalently bonded to two side chains. Polyphosphazenes that can be used have a majority of side chains that are acidic and capable of forming salt bridges with di- or trivalent cations. Examples of acidic side chains are carboxylic acid groups and sulfonic acid groups.

Bioerodible polyphosphazenes have at least two differing types of side chains, acidic side groups capable of forming salt bridges with multivalent cations, and side groups that hydrolyze under in vivo conditions, e.g., imidazole groups, amino acid esters, glycerol, and

glucosyl. Bioerodible or biodegradable polymers, i.e., polymers that dissolve or degrade within a period that is acceptable in the desired application (usually in vivo therapy), will degrade in less than about five years and most preferably in less than about one year, once exposed to a physiological solution of pH 6-8 having a temperature of between about 25° C. and 38° C. Hydrolysis of the side chain results in erosion of the polymer. Examples of hydrolyzing side chains are unsubstituted and substituted imidizoles and amino acid esters in which the side chain is bonded to the phosphorous atom through an amino linkage.

Methods for synthesis and the analysis of various types of polyphosphazenes are described in U.S. Pat. Nos. 4,440,921, 4,495,174, and 4,880,622. Methods for the synthesis of the other polymers described above are known to those skilled in the art. See, for example Concise Encyclopedia of Polymer Science and Engineering, J. I. Kroschwitz, editor (John Wiley and Sons, New York, N.Y., 1990). Many polymers, such as poly(acrylic acid), alginates, and PLURONICS™, are commercially available.

Water soluble polymers with charged side groups are cross-linked by reacting the polymer with an aqueous solution containing multivalent ions of the opposite charge, either multivalent cations if the polymer has acidic side groups, or multivalent anions if the polymer has basic side groups. Cations for cross-linking the polymers with acidic side groups to form a hydrogel include divalent and trivalent cations such as copper, calcium, aluminum, magnesium, and strontium. Aqueous solutions of the salts of these cations are added to the polymers to form soft, highly swollen hydrogels.

Anions for cross-linking the polymers to form a hydrogel include divalent and trivalent anions such as low molecular weight dicarboxylate ions, terepthalate ions, sulfate ions, and carbonate ions. Aqueous solutions of the salts of these anions are added to the polymers to form soft, highly swollen hydrogels, as described with respect to cations.

For purposes of preventing the passage of antibodies into the hydrogel, but allowing the entry of nutrients, a useful polymer size in the hydrogel is in the range of between 10,000 D and 18,500 D.

Temperature-dependent, or thermosensitive, hydrogels have so-called “reverse gelation” properties, i.e., they are liquids at or below room temperature, and gel when warmed to higher temperatures, e.g., body temperature. Thus, these hydrogels can be easily applied at or below room temperature as a liquid and automatically form a semi-solid gel when warmed to body temperature. As a result, these gels are especially useful when the support structure is first implanted into a patient, and then filled with the hydrogel-EV composition. Examples of such temperature-dependent hydrogels are PLURONICS™

(BASF-Wyandotte), such as polyoxyethylene-polyoxypropylene F-108, F-68, and F-127, poly(N-isopropylacrylamide), and N-isopropylacrylamide copolymers.

These copolymers can be manipulated by standard techniques to affect their physical properties such as porosity, rate of degradation, transition temperature, and degree of rigidity. For example, the addition of low molecular weight saccharides in the presence and absence of salts affects the lower critical solution temperature (LCST) of typical thermosensitive polymers. In addition, when these gels are prepared at concentrations ranging between 5 and 25% (W/V) by dispersion at 4°C., the viscosity and the gel-sol transition temperature are affected, the gel-sol transition temperature being inversely related to the concentration.

U.S. Pat. No. 4,188,373 describes using PLURONIC™ polyols in aqueous compositions to provide thermal gelling aqueous systems. U.S. Pat. Nos. 4,474,751, '752, '753, and 4,478,822 describe drug delivery systems which utilize thermosetting polyoxyalkylene gels; with these systems, both the gel transition temperature and/or the rigidity of the gel can be modified by adjustment of the pH and/or the ionic strength, as well as by the concentration of the polymer.

pH-dependent hydrogels are liquids at, below, or above specific pH values, and gel when exposed to specific pHs, e.g., 7.35 to 7.45, the normal pH range of extracellular fluids within the human body. Thus, these hydrogels can be easily delivered to an implanted support structure as a liquid and automatically form a semi-solid gel when exposed to body pH. Examples of such pH-dependent hydrogels are TETRONICS™ (BASF-Wyandotte) polyoxyethylene-polyoxypropylene polymers of ethylene diamine, poly(diethyl aminoethyl methacrylate-g-ethylene glycol), and poly(2-hydroxymethyl methacrylate). These copolymers can be manipulated by standard techniques to affect their physical properties.

Hydrogels that are solidified by either visible or ultraviolet light can be made of macromers including a water soluble region, a biodegradable region, and at least two polymerizable regions as described in U.S. Pat. No. 5,410,016. For example, the hydrogel can begin with a biodegradable, polymerizable macromer including a core, an extension on each end of the core, and an end cap on each extension. The core is a hydrophilic polymer, the extensions are biodegradable polymers, and the end caps are oligomers capable of cross-linking the macromers upon exposure to visible or ultraviolet light, e.g., long wavelength ultraviolet light.

Examples of such light solidified hydrogels include polyethylene oxide block copolymers, polyethylene glycol polylactic acid copolymers with acrylate end groups, and 10K polyethylene glycol-glycolide copolymer capped by an acrylate at both ends. As with the

PLURONIC™ hydrogels, the copolymers comprising these hydrogels can be manipulated by standard techniques to modify their physical properties such as rate of degradation, differences in crystallinity, and degree of rigidity.

Methods

Application of the disclosed GluR-functionalized designer EVs can be translated to a wide variety of conditions involving CNS injuries. According to the CDC, stroke is the leading cause of long-term disability in the US, with more than 795,000 cases reported yearly and an estimated annual cost of $34 billion. Ischemic strokes, which have the highest incidence rate (~87%), result in significant cellular death (e.g. vascular, neuronal) and inflammation. Currently, there is only one FDA-approved treatment for ischemic stroke, and it is an acute measure limited only to quickly restoring blood flow. However, its implementation is restricted to the first 4.5 hours, and therefore more than 95% of patients are not eligible for it. As such, there is still a need for effective therapies to attenuate tissue damage and aid repair post-stroke.

Therefore, also disclosed is a method of treating a subject with a with CNS comprising administering to the subject an effective amount of a composition containing a population of the designer EVs disclosed herein.

In some embodiments, the CNS injury is a spinal cord injury, stroke, traumatic brain injury or a neurodegenerative disease, such as Alzheimer's disease, Parkinson's disease, a Parkinson's-related disorder, Huntington's disease, prion disease, motor neuron disease (MND), spinocerebellar ataxia (SCA) or spinal muscular atrophy (SMA), or multiple sclerosis (MS).

In some embodiments, the designer EV serves as glutamate scavenging agents, helping to decrease the noxious concentration of free glutamate in injured regions of the brain, and aiding brain tissue recovery. In some embodiments, the designer EVs contain therapeutic cargo, such as a proangiogenic, proneurogenic, or anti-infalmmatory molecular cargo to boost vasculogenic and neurogenic repair processes, as well as modulating the inflammatory response after injury.

The term "spinal cord injury" is used to describe a spinal cord injury which results in a temporary or permanent change in the normal motor, autonomic or sensory function of the cord. The damage often results from physical trauma, such as sports injuries, slip and fall accidents or motor vehicular accidents but can also result from diseases such as spina bifida, Friedrich's ataxis and/or transverse myelitis. Injury to the spinal cord resulting in a loss of function does not have to be the result of complete severing of the spinal cord.

Depending on where the spinal cord and its nerve roots are damaged, the symptoms and degree of injury can vary widely, from pain to incontinence to paralysis. Spinal cord injuries are described at various levels of incomplete to complete injury, resulting in a total loss of function. The spinal cord injury can result in paraplegia or tetraplegia.

Traditional treatment of spinal cord injuries starts with stabilizing the spine and controlling inflammation associated with the spin cord damage to prevent further damage. Other interventions can vary widely depending on the location and extent of the injury. In many cases, using conventional therapy, spinal cord injuries require substantial, long-term physical therapy and rehabilitation, especially if the injury interferes with activities of daily life.

Spinal cord injury can be classified into three types based on its cause: mechanical forces, toxic, and ischemic, from lack of blood flow. Spinal cord damage can also be divided into primary and second injury. Primary injury is caused by the cell death that occurs immediately in the original injury (physical trauma, exposure to toxins, or ischemia), and secondary injury is caused by the resultant cascades that are caused by the original insult and cause further tissue damage. These secondary injury pathways include inflammation, swelling, neurotransmitter deficiencies/imbalances, the results of ischemia and cell suicide. The present invention may be used to treat all forms of spinal cord injury, including complete and incomplete injuries, ischemia, spinal cord injury without radiographic abnormality, central cord syndrome, anterior cord syndrome, Brown-Séquard syndrome, posterior cord syndrome, tabes dorsalis and conus medullaris, among others.

The term “stroke” is used to describe a cerebrovascular accident (CVA), cerebrovascular insult (CVI), or brain attack, occurs when poor blood flow to the brain results in cell death. There are two main types of stroke: ischemic, due to lack of blood flow, and hemorrhagic, due to bleeding. Both of these types of stroke result in part of the brain not functioning properly. Signs and symptoms of a stroke may include an inability to move or feel on one side of the body, problems understanding or speaking, a sense of spinning, or loss of vision to one side, among others. Signs and symptoms often appear soon after the stroke has occurred. If symptoms last less than one or two hours it is known as a transient ischemic attack. Hemorrhagic strokes may also be associated with a severe headache. The symptoms of a stroke can be permanent. Long term complications of stroke may include pneumonia or loss of bladder control The main risk factor for stroke is high blood pressure. Other risk factors include tobacco smoking, obesity, high blood cholesterol, diabetes mellitus, previous transient ischemic attack (TIA), and atrial fibrillation, among others. An ischemic stroke is typically caused by blockage of a blood vessel. A hemorrhagic stroke is

caused by bleeding either directly into the brain or into the space surrounding the brain. Bleeding may occur due to a brain aneurysm. Both ischemic and hemorrhagic stroke are treated pursuant to the present invention.

The term “traumatic brain injury” (TBI) is used to describe an injury to the brain caused by movement of the brain within the skill or an injury to the brain caused by a foreign object. Causes of TBI may include falls, a motor vehicle crash or being struck by or with an object. TBI may also be caused by a penetrating object- an injury to the brain caused by a foreign object entering the skull. Causes may include firearm injuries or being struck with a sharp object. TBI may cause a concussion, a period of unconsciousness (coma) or amnesia. TBI may impair one or more of cognitive function (e.g., attention and memory), motor function (e.g., extremity weakness, impaired coordination and balance), sensation (e.g., hearing, vision, impaired perceptin and touch and emotion (e.g., depression, anxiety, aggression, impulse control, personality changes).

The term “neurodegenerative disease” is used throughout the specification to describe a disease which is caused by damage to the central nervous system and which damage can be reduced and/or alleviated through transplantation of neural cells according to the present invention to damaged areas of the brain and/or spinal cord of the patient. Exemplary neurodegenerative diseases which may be treated using the neural cells and methods according to the present invention include for example, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (Lou Gehrig's disease), Alzheimer's disease, lysosomal storage disease (“white matter disease” or glial/demyelination disease, as described, for example by Folkerth, *J. Neuropath. Exp. Neuro.,* 58, 9, Sep., 1999), Tay Sachs disease (beta hexosamimidase deficiency), other genetic diseases, multiple sclerosis, brain injury or trauma caused by ischemia, accidents, environmental insult, etc., spinal cord damage, ataxia and alcoholism. In addition, the present invention may be used to reduce and/or eliminate the effects on the central nervous system of a stroke or a heart attack in a patient, which is otherwise caused by lack of blood flow or ischemia to a site in the brain of said patient or which has occurred from physical injury to the brain and/or spinal cord. The term neurodegenerative diseases also includes neurodevelopmental disorders including for example, autism and related neurological diseases such as schizophrenia, among numerous others.

The herein disclosed compositions, including pharmaceutical composition, may be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated.

Methods of treating subjects involve administration of a pharmaceutical composition comprising an effective amount of EVs described herein and optionally at least one additional bioactive (e.g. an agent which is useful in the treatment of a neurodegenerative disease, stroke and/or spinal cord injury) agent. For example, the compositions could be formulated so that a therapeutically effective dosage of between about 0.01, 0.1, 1, 5, 10, 15, 20, 25, 30 , 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90 or 100 mg/kg of patient/day or in some embodiments, greater than 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 or 200 mg/kg of the disclosed EVs can be administered to a patient receiving these compositions.

The dose of EVs administered to a subject can be less than 10 µg, less than 25 µg, less than 50 µg, less than 75 µg, less than 0.10 mg, less than 0.25 mg, less than 0.5 mg, less than 1 mg, less than 2.5 mg, less than 5 mg, less than 10 mg, less than 15 mg, less than 20 mg, less than 50 mg, less than 75 mg, less than 100 mg, less than 500 mg, less than 750 mg, less than 1 g or more than 1 g. Administration may be by numerous routes of administration, but intravenous, intrathecal, intranasal and/or intracerebrospinal fluid are often used as routes of administration.

In some embodiments, the disclosed EVs are administered within 24 after a stroke or trauma. However, in some embodiments, the EVs are administered at least 1, 2, 3, or 4 weeks after a stroke or trauma. In some embodiments, the disclosed EVs are administered in multiple doses 1, 2, 3, or more days apart. In some cases, such as cases of neurodegenerative disease, the EVs are administered continuously (e.g., once every 1, 2, 3, or 4 weeks) over the course of the disease.

EVs may be loaded with small molecules, antisense oligonucleotides, siRNAs, peptides, proteins or antibodies that target, peptides or peptide *translation products which are involved in neurodegenerative processes.*

In certain embodiments, the disclosed EVs are loaded with additional bioactive agents or are co-administered with additional bioactive agents, especially agents which are useful in the treatment of neurodegenerative diseases.

The term “coadministered”, “coadministration” or “combination therapy” is used to describe a therapy in which at least two active compounds/compositions in effective amounts are used to treat neural injury and/or a neurodegenerative disease. Although the term co-administration preferably includes the administration of EVs and at least one additional active compound to the subject at the same time, it is not necessary that the compounds/compositions be administered to the patient simultaneously, only that effective amounts of the individual compounds/compositions be present in the patient at the same time. Thus, the term co-administration includes an administration in which the EVs and the

bioactive agent(s) are administered at approximately the same time (contemporaneously), or from about one to several minutes to about eight hours, about 30 minutes to about 6 hours, about an hour to about 4 hours, or even much earlier than the other compound/composition as otherwise described herein including up to a day or substantially more.

Agents which may be loaded or coadministered along with EVs may include, for example aricept, namenda, donepezil, excelon, razadyne, glantamine, rivastigmine, memantine, ergoloid, namzaric and mixtures thereof for Alzheimer's disease, biperiden, apomorphine, trihexyphenidyl, carbidopa/levodopa, rasagline, belladona, levodopa, benztropine, entacapone, selegiline, rivastigmine, pramipexole, rotigotine, bromocriptine, pergolide, ropinirole, carbidopa/entacapone/levodopa, amantadine, tolcopone, trihexiphenidyl and mixtures thereof, for Parkinson's disease, tetrabenazine, haloperidol, chlorpromazine, olanzapine, fluoxetine, sertraline, nortriptyline, benzodiazpines, paroxetine, venlafaxin, beta-blockers, lithium, valproate, carbamazepine, botulinum toxin and mixtures thereof for the treatment of Huntington's disease, anticholinergic drugs, anticonvulsants, antidepressants, benzodiazepines, decongestants, muscle relaxants, pain medications, stimulants and mixtures thereof for the treatment of motor neuron disease, selective serotonin reuptake inhibitors (SSRI's), selective norepinephrine-serotoning reuptake inhibitors (SNRI's), acetazolamide, baclofen, clonazepam, flunarizine, gabapentin, meclizine, memantine, ondansetron, scopolamine, modafinil, armodafinil, amantadine, atomoxetine, buproprion, carnitine, creatine, modafinil, armodafinil, pyrudistigmine, selegiline, venlafaxine, desvenlafaxine, buspirone, riluzole, verenicline, memantine, baclofen, tizanidine, cymbalta, lyrica, acetazolamide, carbamazepine, clonazepam, isoniazid, droxidopa, ephedrine, fludrocortisones, midodrine, levodopa, pramipexole, fluoxetine, n-acetylcysteine, baclofen, dantrolene sodium, diazepam, ropinirole, tizanidine, trihexylphenidyl, clonazepine, flunarazine, levetiracetam, primidone, topiramate, valproic acid, phenytoin, 4-aminopyridine and mixtures thereof for the treatment of spinocerebellar ataxia and riluzole for the treatment of spinal muscular atrophy. Agents for the treatment of stroke include salicylates, such as aspirin, a thrombolytic agent (alteplase) and a platelet aggregation inhibitor (clopidogrel), among others.

More generally, non-steroidal anti-inflammatory drugs (NSAIDS) and other anti-inflammatory agents may be used in the treatment of neurodegenerative diseases as described herein.

The activities of EVs described herein can be evaluated by methods known in the art. The amount of EVs required for use in treatment can vary not only with the particular cell from which the EVs are prepared, but also with the route of administration, the nature of the

condition being treated and the age and condition of the patient and can be ultimately at the discretion of the attendant physician or clinician. In general, however, a dose can be in the range of from about 0.01 mg/kg to about 10 mg/kg of body weight per day.

Identifying EVs useful in the present methods for treating a spinal cord injury, stroke, traumatic brain injury and/or a neurodegenerative disease which occurs by modulating the activity and expression of a disease-related protein and biologically active fragments thereof can be made by screening EV activity in any of a variety of screening techniques. The screening can be made for whole EVs or their contents. Fragments employed in such screening tests may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The blocking or reduction of biological activity or the formation of binding complexes between the disease-related protein, the EVs and/or one or more components of the EVs may be measured by methods available in the art.

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

EXAMPLES

Example 1:

Experiments in which mGluR4/mGluR8-decorated EVs were delivered intranasally into C57BL6 mice suggest that such decoration strategy favored homing to brain tissue. In vitro studies with primary co-cultures of glia and neurons further indicate selective uptake of mGluR4/mGluR8-decorated EVs by neurons only shortly after exposure (i.e. approximately 8 hours), followed by more widespread uptake by neurons and glia alike after approximately 24 hours. While mGluR4/mGluR8 decoration seems to help to narrow down the range of action of these EVs to brain tissue (minimizing off-target side effects), we believe that neuronal tissue injury, such as stroke events, will help to further guide mGluR4/mGluR8-decorated EVs primarily to stroke-affected areas due to the fact that stroke insults lead to a marked increase in extracellular glutamate associated with profuse neuro- and excitotoxicity.

FIGs. 1A and 1B illustrate nanoscale engineering of designer extracellular vesicles for therapeutic applications.

Table 1. Examples of molecular cargo and mechanism of action of designer EV therapies for ischemic stroke

Proposed Therapy	Plasmids	Rationale

Designer EVs loaded with pro-neurogenic factors	ASCL1, BRN2, and MYT1L (ABM)	• Shown to promote neuronal phenotype[1] • May induce direct cell reprogramming of resident/ scaring glia into neurons at the infarcted area, helping to repair damaged nerve tissue
Designer EVs loaded with pro-angiogenic factors	FOXC2, ETV2, or FLI1	• Shown to promote vasculogenesis in vivo[2,4] • May induce direct cell reprogramming of resident/ scaring glia into functionalized endothelium at the infarcted area, [2] helping to re-establish proper tissue perfusion
Designer EVs loaded with anti-inflammatory cargo	IL-4 and IL-10	• IL-4 is secreted by ischemic neurons to favor an anti-inflammatory phenotype and increased phagocytic activity in microglia.[5] • IL-10 has the potential to reduce infarct size when administered to the lateral ventricle after stroke.[6]

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FIGs. 2A to 2E show proneural designer EVs characterization. FIG. 2A shows transmission electron micrograph of designer EVs derived from primary mouse embryonic fibroblasts (PMEFs) and loaded with the proneural factors the ASCL1, BRN2, and MYT1L (i.e., ABM cocktail). FIG. 2B shows dynamic of proneural designer EV release showing a peak 24 hours after nanotransfection of PMEF, with a particle concentration in the order of ten billion particles per mL (*p-value=0.018). FIG. 2C shows the number of gene copies packed inside these proneural designer EVs is approx. 3 orders of magnitude higher than that delivered to the donor cell for all three factors (*p-value=0.021). FIG. 2D shows proneural designer EVs are successfully captured by PMEFs in culture, with a peak in uptake 48 hours after treatment (*p-value ≤ 0.036). FIG. 2E Fluorescent image of PMEF cells incorporating fluorescently labeled (red) proneurogeninc-EVs 24 hours after treatment.

FIG. 3A shows confocal images of donor cells transfected with the mGluR8 or a sham vector, showing positive colocalization of cell membrane (green and white) and the transfected glutamate receptor (red) only for mGluR8 transfected cells. FIGs. 3B and 3C show Confocal images of EVs derived from sham or mGluR8 transfected donor cells, where EVs derived from GlutR8 transfected cells show co-localization of the EV membrane (green) with the targeting receptor (red). FIG. 3D Western blot of mGluR8-functionalized EVs showing positive protein expression compared to sham (control)-EVs.

[0001] FIGs. 4A to 4C show functionalized designer EV to target the brain. FIG. 4A shows in vivo imaging of brains after intranasal delivery of fluorescently labeled designer EVs functionalized with mGluR8 or sham (control) EVs, showing significantly higher accumulation of functionalized EVs in the brain 24 hours after treatment. FIGs. 4B and 4C show immunofluorescence images of cerebellum and corpus callosum (sagittal cut) of brains of animals treated with fluorescently labeled (red) sham- or mGluR8-EVs, 24 hours post-intranasal instillation, and respective fluorescence intensity quantification (n=3, *p-value=0.0083).

Example 2:

[0002] FIGs. 3E and 3F show characterization of designer EVs functionalized with mGRM4 and mGRM8 derived from PMEFs 24 hours after nanotransfection with plasmids encoding for each receptor or sham vector, with a particle concentration in the range of billions of EVs per mL and an average particle approximately 230 nm.

[0003] FIG. 5 show characterization of relative expression of metabotropic glutamate receptors (mGluR4 and mGluR8) in designer EVs, these receptors are used to functionalize neurogenic designer EVs.

[0004] FIGs. 6A and 6B show designer EV biodistribution 24 hours after intranasal delivery showing higher accumulation in the brain of mice treated with designer EVs functionalized with mGluR8 vs. non-functionalized designer EVs, which accumulate in the liver tissue as they are cleared from the body.

[0005] FIGs 7A and 7B show comparison of yield for in vitro-derived (using PMEF as donor cells) vs. in vivo-derived (using skin cells as donor cells) ABM- and control-designer EVs, showing that a significantly higher number of EVs are produced in vivo.

[0006] FIGs. 8A and 8B show efficiency of molecular loading of neurogenic factors ACL1, BRN2, and MYT1L (ABM) inside designer EVs vs. number of gene copies inside donor cells 24 hours after transfection.

[0007] FIG. 9A shows immunofluorescence images of primary neurons incorporating mGluR8 functionalized EVs (red) and control-EVs (green), showing preferential accumulation of GluR-8 EVs in postsynaptic regions (postsynaptic protein staining, PSD-95) (violet), with zoom-in regions for each type of sample (bottom) 7hours after treatment. FIG. 9B shows quantification of green (sham-EVs) or red (mGluR8-EVs.

[0008] FIG. 10 shows mGluR4- and mGLuR8-functionalized designer EVs uptake by primary mouse embryonic neurons 8 hours after treatment.

[0009] FIG. 11A shows how prolonged culture studies suggest that PMEFs exposed to ABM-loaded EVs exhibit pro-neuronal conversions, as evidence by the increased in immunoreactivity for Tuj1 (green), a neuronal marker, relative to PMEFs exposed to control EVs as early as 7 days after treatment. These data suggest that the extent of plasmid DNA transfer from EVs to recipient cells falls within the same order of magnitude compared to direct electroporation. Additionally, the induction of Tuj1 immunoreactivity in fibroblast cultures suggest that ABM-loaded EVs could potentially be used to drive pro-neuronal responses/conversions in non-neuronal cells. FIG. 11B shows the quantification of Tuj1 fluorescence intensity 7- and 14-days post-treatment (n=3, *p-value=0.043, **p-value=0.004).

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed invention belongs. Publications cited herein and the materials for which they are cited are specifically incorporated by reference.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.