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(54) **COMPOSITION FOR REGULATING PRODUCTION OF INTERFERING RIBONUCLEIC ACID**

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(57) **ABSTRACT**

Some embodiments of the present disclosure relate to one or more compositions that upregulate the production of one or more sequences of micro-interfering ribonucleic acid (miRNA). The sequences of miRNA may be complimentary to a sequence of target messenger RNA (mRNA) that encodes for translation of a target biomolecule and the miRNA can cause the target mRNA to be degraded or inactivated, thereby causing a decrease in bioavailability of the target biomolecule because it is degraded or inactivated by the miRNA, thereby decreasing the bioavailability of the target biomolecule within a subject that is administered the one or more compositions. In some embodiments of the present disclosure, the target biomolecule is a serotonin receptor, such as serotonin receptor 5HT1*a*, 5HT1*b*, 5HT1*d*, 5HT1*e*, 5HT1*f*, 5HT2*a*, 5HT2*b*, 5HT2*c*, 5HT3, 5HT4, 5HT6, or 5HT7.

Specification includes a Sequence Listing.

COMPOSITION FOR REGULATING PRODUCTION OF INTERFERING RIBONUCLEIC ACID

[0001] This application contains a Sequence Listing electronically submitted via Patent Center to the United States Patent and Trademark Office as an XML Document file entitled “A8149441US—Sequence Listing.xml” created on 2024 Feb. 12 and having a size of 110,545 bytes. The information contained in the Sequence Listing is incorporated by reference herein.

TECHNICAL FIELD

[0002] The present disclosure generally relates to compositions for regulating production of interfering ribonucleic acid (RNA). In particular, the present disclosure relates to compositions for regulating gene expression and therefore, the production of interfering RNA, that will suppress serotonin receptor expression.

BACKGROUND

[0003] Bioactive molecules, including complements and factors, are necessary for the homeostatic control of biological systems.

[0004] When bioactive molecules are over-expressed, under-expressed or mis-expressed, homeostasis is lost, and disease is often the result.

[0005] As such, it may be desirable to establish therapies, treatments and/or interventions that address when homeostasis and regulation of bioactive molecules is lost to prevent or treat the resulting disease.

SUMMARY

[0006] Some embodiments of the present disclosure relate to one or more compositions that upregulate the production of one or more sequences of micro-interfering ribonucleic acid (miRNA). The sequences of miRNA may be complimentary to a sequence of target messenger RNA (mRNA) that encodes for translation of a target biomolecule and the miRNA can cause the target mRNA to be degraded or inactivated, thereby causing a decrease in bioavailability of the target biomolecule because it is degraded or inactivated by the miRNA, thereby decreasing the bioavailability of the target biomolecule within a subject that is administered the one or more compositions. In some embodiments of the present disclosure, the target biomolecule is a serotonin receptor. In some embodiments of the present disclosure, the target biomolecule is a serotonin receptor such as serotonin receptor 5HT1a. In some embodiments of the present disclosure, the target biomolecule is a serotonin receptor such as serotonin receptor 5HT1b. In some embodiments of the present disclosure, the target biomolecule is a serotonin receptor such as serotonin receptor 5HT1c. In some embodiments of the present disclosure, the target biomolecule is a serotonin receptor such as serotonin receptor 5HT1d. In some embodiments of the present disclosure, the target biomolecule is a serotonin receptor such as serotonin receptor 5HT1e. In some embodiments of the present disclosure, the target biomolecule is a serotonin receptor such as serotonin receptor 5HT1f. In some embodiments of the present disclosure, the target biomolecule is a serotonin receptor such as serotonin receptor 5HT2a. In some embodiments of the present disclosure, the target biomolecule is a serotonin receptor such as serotonin receptor 5HT2b. In some embodiments of the present disclosure, the target biomolecule is a serotonin receptor such as serotonin receptor 5HT2c. In some embodiments of the present disclosure, the target biomolecule is a serotonin receptor such as serotonin receptor 5HT3. In some embodiments of the present disclosure, the target biomolecule is a serotonin receptor such as serotonin receptor 5HT4. In some embodiments of the present disclosure, the target biomolecule is a serotonin receptor such as serotonin receptor 5HT6. In some embodiments of the present disclosure, the target biomolecule is a serotonin receptor such as serotonin receptor 5HT7.

[0007] In some embodiments of the present disclosure the compositions comprise a plasmid of deoxyribonucleic acid (DNA) that includes one or more insert sequences of nucleic acids that encode for the production of miRNA and a backbone sequence of nucleic acids that facilitates introduction of the one or more insert sequences into one or more of a subject's cells where it is expressed and/or replicated. Expression of the one or more insert sequences by one or more cells of the subject results in an increased production of the miRNA and, therefore, decreased translation or production of the target biomolecule by one or more of the subject's cells.

[0008] Some embodiments of the present disclosure relate to compositions that upregulate the production of miRNA that degrades, or causes degradation of, or inactivates or causes the inactivation of, the target mRNA of the target biomolecule.

[0009] Some embodiments of the present disclosure relate to a recombinant plasmid (RP). In some embodiments of the present disclosure, the RP comprises a nucleotide sequence of SEQ ID NO. 1 and SEQ ID NO. 2. The RP comprises a nucleotide sequence encoding one or more nucleotide sequences encoding a miRNA sequence that targets the mRNA of serotonin receptor 5HT1a.

[0010] Some embodiments of the present disclosure relate to a recombinant plasmid. In some embodiments of the present disclosure, the RP comprises a nucleotide sequence of SEQ ID NO. 1 and SEQ ID NO. 3. The RP comprises a nucleotide sequence encoding one or more nucleotide sequences encoding a miRNA sequence that targets the mRNA of serotonin receptor 5HT1b.

[0011] Some embodiments of the present disclosure relate to a recombinant plasmid. In some embodiments of the present disclosure, the RP comprises a nucleotide sequence of SEQ ID NO. 1 and SEQ ID NO. 4. The RP comprises a nucleotide sequence encoding one or more nucleotide sequences encoding a miRNA sequence that targets the mRNA of serotonin receptor 5HT1d.

[0012] Some embodiments of the present disclosure relate to a recombinant plasmid. In some embodiments of the present disclosure, the RP comprises a nucleotide sequence of SEQ ID NO. 1 and SEQ ID NO. 5. The RP comprises a nucleotide sequence encoding one or more nucleotide sequences encoding a miRNA sequence that targets the mRNA of serotonin receptor 5HT1e.

[0013] Some embodiments of the present disclosure relate to a recombinant plasmid. In some embodiments of the present disclosure, the RP comprises a nucleotide sequence of SEQ ID NO. 1 and SEQ ID NO. 6. The RP comprises a nucleotide sequence encoding one or more nucleotide sequences encoding a miRNA sequence that targets the mRNA of serotonin receptor 5HT1f.

[0014] Some embodiments of the present disclosure relate to a recombinant plasmid. In some embodiments of the present disclosure, the RP comprises a nucleotide sequence of SEQ ID NO. 1 and SEQ ID NO. 7. The RP comprises a nucleotide sequence encoding one or more nucleotide sequences encoding a miRNA sequence that targets the mRNA of serotonin receptor 5HT2a.

[0015] Some embodiments of the present disclosure relate to a recombinant plasmid. In some embodiments of the present disclosure, the RP comprises a nucleotide sequence of SEQ ID NO. 1 and SEQ ID NO. 8. The RP comprises a nucleotide sequence encoding one or more nucleotide sequences encoding a miRNA sequence that targets the mRNA of serotonin receptor 5HT2b.

[0016] Some embodiments of the present disclosure relate to a recombinant plasmid. In some embodiments of the present disclosure, the RP comprises a nucleotide sequence of SEQ ID NO. 1 and SEQ ID NO. 9. The RP comprises a nucleotide sequence encoding one or more nucleotide sequences encoding a miRNA sequence that targets the mRNA of serotonin receptor 5HT2c.

[0017] Some embodiments of the present disclosure relate to a recombinant plasmid. In some embodiments of the present disclosure, the RP comprises a nucleotide sequence of SEQ ID NO. 1 and SEQ ID NO. 10. The RP comprises a nucleotide sequence encoding one or more nucleotide sequences encoding a miRNA sequence that targets the mRNA of serotonin receptor 5HT3.

[0018] Some embodiments of the present disclosure relate to a recombinant plasmid. In some embodiments of the present disclosure, the RP comprises a nucleotide sequence of SEQ ID NO. 1 and SEQ ID NO. 11. The RP comprises a nucleotide sequence encoding one or more nucleotide sequences encoding a miRNA sequence that targets the mRNA of serotonin receptor 5HT4.

[0019] Some embodiments of the present disclosure relate to a recombinant plasmid. In some embodiments of the present disclosure, the RP comprises a nucleotide sequence of SEQ ID NO. 1 and SEQ ID NO. 12. The RP comprises a nucleotide sequence encoding one or more nucleotide sequences encoding a miRNA sequence that targets the mRNA of serotonin receptor 5HT6.

[0020] Some embodiments of the present disclosure relate to a recombinant plasmid. In some embodiments of the present disclosure, the RP comprises a nucleotide sequence of SEQ ID NO. 1 and SEQ ID NO. 13 The RP comprises a nucleotide sequence encoding one or more nucleotide sequences encoding a miRNA sequence that targets the mRNA of serotonin receptor 5HT7.

[0021] Some embodiments of the present disclosure relate to a method of making a composition/target cell complex. The method comprising a step of administering a RP comprising SEQ ID NO. 1 and one of SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO. 12, or SEQ ID NO. 13 to a target cell for forming the composition/target cell complex, wherein the composition/target cell complex causes the target cell to increase production of one or more sequences of miRNA that decreases production of a target biomolecule.

[0022] Embodiments of the present disclosure relate to at least one approach for inducing endogenous production of one or more sequences of miRNA that target and silence the mRNA of a target biomolecule, for example serotonin receptor 5HT1a. A first approach utilizes gene vectors containing nucleotide sequences for increasing the endogenous production of one or more sequences of miRNA, which are complete or partial sequences and/or combinations thereof, that target and silence the mRNA of serotonin receptor 5HT1a, which can be administered to a subject to increase the subject's production of one or more sequences of the miRNA.

[0023] Embodiments of the present disclosure relate to at least one approach for inducing endogenous production of one or more sequences of miRNA that target and silence the mRNA of a target biomolecule, for example serotonin receptor 5HT1b. A first approach utilizes gene vectors containing nucleotide sequences for increasing the endogenous production of one or more sequences of miRNA, which are complete or partial sequences and/or combinations thereof, that target and silence the mRNA of serotonin receptor 5HT1b, which can be administered to a subject to increase the subject's production of one or more sequences of the miRNA.

[0024] Embodiments of the present disclosure relate to at least one approach for inducing endogenous production of one or more sequences of miRNA that target and silence the mRNA of a target biomolecule, for example serotonin receptor 5HT1d. A first approach utilizes gene vectors containing nucleotide sequences for increasing the endogenous production of one or more sequences of miRNA, which are complete or partial sequences and/or combinations thereof, that target and silence the mRNA of serotonin receptor 5HT1d, which can be administered to a subject to increase the subject's production of one or more sequences of the miRNA.

[0025] Embodiments of the present disclosure relate to at least one approach for inducing endogenous production of one or more sequences of miRNA that target and silence the mRNA of a target biomolecule, for example serotonin receptor 5HT1e. A first approach utilizes gene vectors containing nucleotide sequences for increasing the endogenous production of one or more sequences of miRNA, which are complete or partial sequences and/or combinations thereof, that target and silence the mRNA of serotonin receptor 5HT1e, which can be administered to a subject to increase the subject's production of one or more sequences of the miRNA.

[0026] Embodiments of the present disclosure relate to at least one approach for inducing endogenous production of one or more sequences of miRNA that target and silence the mRNA of a target biomolecule, for example serotonin receptor 5HT1f. A first approach utilizes gene vectors containing nucleotide sequences for increasing the endogenous production of one or more sequences of miRNA, which are complete or partial sequences and/or combinations thereof, that target and silence the mRNA of serotonin receptor 5HT1f, which can be administered to a subject to increase the subject's production of one or more sequences of the miRNA.

[0027] Embodiments of the present disclosure relate to at least one approach for inducing endogenous production of one or more sequences of miRNA that target and silence the mRNA of a target biomolecule, for example serotonin receptor 5HT2a. A first approach utilizes gene vectors containing nucleotide sequences for increasing the endogenous production of one or more sequences of miRNA, which are complete or partial sequences and/or combinations thereof, that target and silence the mRNA of serotonin receptor 5HT2a, which can be administered to a subject to increase the subject's production of one or more sequences of the miRNA.

[0028] Embodiments of the present disclosure relate to at least one approach for inducing endogenous production of one or more sequences of miRNA that target and silence the mRNA of a target biomolecule, for example serotonin receptor 5HT2b. A first approach utilizes gene vectors containing nucleotide sequences for increasing the endogenous production of one or more sequences of miRNA, which are complete or partial sequences and/or combinations thereof, that target and silence the mRNA of serotonin receptor 5HT2b, which can be administered to a subject to increase the subject's production of one or more sequences of the miRNA.

[0029] Embodiments of the present disclosure relate to at least one approach for inducing endogenous production of one or more sequences of miRNA that target and silence the mRNA of a target biomolecule, for example serotonin receptor 5HT2c. A first approach utilizes gene vectors containing nucleotide sequences for increasing the endogenous production of one or more sequences of miRNA, which are complete or partial sequences and/or combinations thereof, that target and silence the mRNA of serotonin receptor 5HT2c, which can be administered to a subject to increase the subject's production of one or more sequences of the miRNA.

[0030] Embodiments of the present disclosure relate to at least one approach for inducing endogenous production of one or more sequences of miRNA that target and silence the mRNA of a target biomolecule, for example serotonin receptor 5HT3. A first approach utilizes gene vectors containing nucleotide sequences for increasing the endogenous production of one or more sequences of miRNA, which are complete or partial sequences and/or combinations thereof, that target and silence the mRNA of serotonin receptor 5HT3 which can be administered to a subject to increase the subject's production of one or more sequences of the miRNA.

[0031] Embodiments of the present disclosure relate to at least one approach for inducing endogenous production of one or more sequences of miRNA that target and silence the mRNA of a target biomolecule, for example serotonin receptor 5HT4 A first approach utilizes gene vectors containing nucleotide sequences for increasing the endogenous production of one or more sequences of miRNA, which are complete or partial sequences and/or combinations thereof, that target and silence the mRNA of serotonin receptor 5HT4, which can be administered to a subject to increase the subject's production of one or more sequences of the miRNA.

[0032] Embodiments of the present disclosure relate to at least one approach for inducing endogenous production of one or more sequences of miRNA that target and silence the mRNA of a target biomolecule, for example serotonin receptor 5HT6. A first approach utilizes gene vectors containing nucleotide sequences for increasing the endogenous production of one or more sequences of miRNA, which are complete or partial sequences and/or combinations thereof, that target and silence the mRNA of serotonin receptor 5HT6, which can be administered to a subject to increase the subject's production of one or more sequences of the miRNA.

[0033] Embodiments of the present disclosure relate to at least one approach for inducing endogenous production of one or more sequences of miRNA that target and silence the mRNA of a target biomolecule, for example serotonin receptor 5HT7. A first approach utilizes gene vectors containing nucleotide sequences for increasing the endogenous production of one or more sequences of miRNA, which are complete or partial sequences and/or combinations thereof, that target and silence the mRNA of serotonin receptor 5HT7, which can be administered to a subject to increase the subject's production of one or more sequences of the miRNA.

DETAILED DESCRIPTION

[0034] Unless defined otherwise, all technical and scientific terms used therein have the meanings that would be commonly understood by one of skill in the art in the context of the present description. Although any methods and materials similar or equivalent to those described therein can also be used in the practice or testing of the present disclosure, the preferred methods and materials are now described. All publications mentioned therein are incorporated therein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0035] As used therein, the singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise. For example, reference to "a composition" includes one or more compositions and reference to "a subject" or "the subject" includes one or more subjects.

[0036] As used therein, the terms "about" or "approximately" refer to within about 25%, preferably within about 20%, preferably within about 15%, preferably within about 10%, preferably within about 5% of a given value or range. It is understood that such a variation is always included in any given value provided therein, whether or not it is specifically referred to.

[0037] As used therein, the term "ameliorate" refers to improve and/or to make better and/or to make more satisfactory.

[0038] As used therein, the term "cell" refers to a single cell as well as a plurality of cells or a population of the same cell type or different cell types. Administering a composition to a cell includes in vivo, in vitro and ex vivo administrations and/or combinations thereof.

[0039] As used therein, the term "complex" refers to an association, either direct or indirect, between one or more particles of a composition and one or more target cells. This association results in a change in the metabolism of the target cell. As used therein, the phrase "change in metabolism" refers to an increase or a decrease in the one or more target cells' production of one or more proteins, and/or any post-translational modifications of one or more proteins.

[0040] As used therein, the term "composition" refers to a substance that, when administered to a subject, causes one or more chemical reactions and/or one or more physical reactions and/or one or more physiological reactions and/or one or more biological reactions in the subject. In some embodiments of the present disclosure, the composition is a plasmid vector.

[0041] As used therein, the term "endogenous" refers to the production and/or modification of a molecule that originates within a subject.

[0042] As used therein, the term "exogenous" refers to a molecule that is within a subject but that did not originate within the subject. As used therein, the terms "production", "producing" and "produce" refer to the synthesis and/or replication of DNA, the transcription of one or more sequences of RNA, the translation of one or more amino acid sequences, the post-translational modifications of an amino acid sequence, and/or the production of one or more regulatory molecules that can influence the production and/or functionality of an effector molecule or an effector cell. For clarity, "production" is also used therein to refer to the functionality of a regulatory molecule, unless the context reasonably indicates otherwise.

[0043] As used therein, the term "subject" refers to any therapeutic target that receives the composition. The subject can be a vertebrate, for example, a mammal including a human. The term "subject" does not denote a particular age or sex. The term "subject" also refers to one or more cells of an organism, an in vitro culture of one or more tissue types, an in vitro culture of one or more cell types, ex vivo preparations, and/or a sample of biological materials such as tissue, and/or biological fluids.

[0044] As used therein, the term "target biomolecule" refers to a serotonin receptor that is found within a subject. A biomolecule may be endogenous or exogenous to a subject and when bioavailable the biomolecule may inhibit or stimulate a biological process within the subject.

[0045] As used therein, the term "target cell" refers to one or more cells and/or cell types that are deleteriously affected, either directly or indirectly, by a dysregulated biomolecule. The term "target cell" also refers to cells that are not deleteriously affected but that are the cells in which it is desired that the composition interacts.

[0046] As used therein, the term "therapeutically effective amount" refers to the amount of the composition used that is of sufficient quantity to ameliorate, treat and/or inhibit one or more of a disease, disorder or a symptom thereof. The "therapeutically effective amount" will vary depending on the composition used, the route of administration of the composition and the severity of the disease, disorder or symptom thereof. The subject's age, weight and genetic make-up may also influence the amount of the composition that will be a therapeutically effective amount.

[0047] As used therein, the terms "treat", "treatment" and "treating" refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing an occurrence of a disease, disorder or symptom thereof and/or the effect may be therapeutic in providing a partial or complete amelioration or inhibition of a disease, disorder, or symptom thereof. Additionally, the term "treatment" refers to any treatment of a disease, disorder, or symptom thereof in a subject and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; and (c) ameliorating the disease.

[0048] As used therein, the terms "unit dosage form" and "unit dose" refer to a physically discrete unit that is suitable as a unitary dose for patients. Each unit contains a predetermined quantity of the composition and optionally, one or more suitable pharmaceutically acceptable carriers, one or more excipients, one or more additional active ingredients, or combinations thereof. The amount of composition within each unit is a therapeutically effective amount.

[0049] Where a range of values is provided therein, 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.

[0050] In some embodiments of the present disclosure, a composition is a recombinant plasmid (RP) for introducing genetic material, such as one or more nucleotide sequences, into a target cell for reproduction or transcription of an insert that comprises one or more nucleotide sequences that are carried within the RP. In some embodiments of the present disclosure, the RP is delivered without a carrier, by a viral vector, by a protein coat, or by a lipid vesicle. In some embodiments of the present disclosure, the vector is an adeno-associated virus (AAV) vector.

[0051] In some embodiments of the present disclosure, the insert comprises one or more nucleotide sequences that encode for production of at least one sequence of miRNA that decreases the production of target biomolecules. The miRNA may, directly or indirectly, bind to and degrade the target mRNA or otherwise inactivate the target mRNA so that less or none of the target-biomolecule protein is produced.

[0052] In some embodiments of the present disclosure, the target biomolecule is serotonin receptor 5HT1a.

[0053] In some embodiments of the present disclosure, the target biomolecule is serotonin receptor 5HT1b.

[0054] In some embodiments of the present disclosure, the target biomolecule is serotonin receptor 5HT1d.

[0055] In some embodiments of the present disclosure, the target biomolecule is serotonin receptor 5HT1e.

[0056] In some embodiments of the present disclosure, the target biomolecule is serotonin receptor 5HT1f.

[0057] In some embodiments of the present disclosure, the target biomolecule is serotonin receptor 5HT2a.

[0058] In some embodiments of the present disclosure, the target biomolecule is serotonin receptor 5HT2b.

[0059] In some embodiments of the present disclosure, the target biomolecule is serotonin receptor 5HT2c.

[0060] In some embodiments of the present disclosure, the target biomolecule is serotonin receptor 5HT3.

[0061] In some embodiments of the present disclosure, the target biomolecule is serotonin receptor 5HT4.

[0062] In some embodiments of the present disclosure, the target biomolecule is serotonin receptor 5HT6.

[0063] In some embodiments of the present disclosure, the target biomolecule is serotonin receptor 5HT7.

[0064] In some embodiments of the present disclosure, the insert comprises one or more nucleotide sequences that each encode for one or more miRNA sequences that may be complimentary to and degrade, or cause degradation of, mRNA of the target biomolecule.

[0065] Some embodiments of the present disclosure relate to a composition that can be administered to a subject with a condition that results, directly or indirectly, from the production of a dysregulated biomolecule. When a therapeutically effective amount of the composition is administered to the subject, the subject may change production and/or functionality of one or more biomolecules.

[0066] In some embodiments of the present disclosure, the subject may respond to receiving the therapeutic amount of the composition by changing production and/or functionality of one or more intermediary molecules by changing production of one or more DNA sequences, one or more RNA sequences, and/or one or more proteins that regulate the levels and/or functionality of the one or more intermediary molecules. The one or more intermediary molecules regulate the subject's levels and/or functionality of the one or more biomolecules.

[0067] In some embodiments of the present disclosure, administering a therapeutic amount of the composition to a subject upregulates the production, functionality or both one or more sequences of miRNA that each target the mRNA of one or more target biomolecules. In some embodiments of the present disclosure, there are one, two, three, four, five, or six miRNA sequences that each are complimentary to and degrade, or cause degradation of, one biomolecule, such as the mRNA of serotonin receptor 5HT1a, serotonin receptor 5HT1b, serotonin receptor 5HT1d, serotonin receptor 5HT1e, serotonin receptor 5HT1f, serotonin receptor 5HT2a, serotonin receptor 5HT2b, serotonin receptor 5HT2c, serotonin receptor 5HT3, serotonin receptor 5HT4, serotonin receptor 5HT6, or serotonin receptor 5HT7. In some embodiments of the present disclosure, the composition may comprise multiple copies of the same nucleotide sequence of miRNA.

[0068] In some embodiments of the present disclosure, the composition is an RP that may be used for gene therapy. The gene therapy is useful for increasing the subject's endogenous production of one or more sequences of miRNA that target the mRNA of a target biomolecule. For example, the RP can contain one or more nucleotide sequences that cause increased production of one or more nucleotide sequences that cause an increased production of one or more miRNA sequences that are each complimentary to and degrade, or cause degradation of, or inactivate, or cause inactivation of, one biomolecule, such as serotonin receptor 5HT1a, serotonin receptor 5HT1b, serotonin receptor 5HT1d, serotonin receptor 5HT1e, serotonin receptor 5HT1f, serotonin receptor 5HT2a, serotonin receptor 5HT2b, serotonin receptor 5HT2c, serotonin receptor 5HT3, serotonin receptor 5HT4, serotonin receptor 5HT6, or serotonin receptor 5HT7.

[0069] In some embodiments of the present disclosure, the delivery vehicle of the RP used for gene therapy may be a vector that is comprised of a virus that can be enveloped, or not (unenveloped), replication effective or not (replication ineffective), or combinations thereof. In some embodiments of the present disclosure, the vector is a virus that is not enveloped and not replication effective. In some embodiments of the present disclosure, the vector is a virus of the *Parvoviridae* family. In some embodiments of the present disclosure, the vector is a virus of the genus *Dependoparvovirus*. In some embodiments of the present disclosure, the vector is an adeno-associated virus (AAV). In some embodiments of the present disclosure, the vector is a recombinant AAV. In some embodiments of the present disclosure, the vector is a recombinant AAV6.2FF.

[0070] In some embodiments of the present disclosure, the delivery vehicle of the RP used for gene therapy may be a protein coat.

[0071] In some embodiments of the present disclosure, the delivery vehicle of the RP used for gene therapy may be a lipid vesicle.

[0072] The embodiments of the present disclosure also relate to administering a therapeutically effective amount of the composition. In some embodiments of the present disclosure, the therapeutically effective amount of the composition that is administered to a patient is between about 10 and about 1×10^{16} $TCID_{50}$/kg (50% tissue culture infective dose per kilogram of the patient's body mass). In some embodiments of the present disclosure, the therapeutically effective amount of the composition that is administered to the patient is about 1×10^{13} $TCID_{50}$/kg. In some embodiments of the present disclosure, the therapeutically effective amount of the composition that is administered to a patient is measured in TPC/kg (total particle count of the composition per kilogram of the patient's body mass). In some embodiments the therapeutically effective amount of the composition is between about 10 and about 1×10^{16} TCP/kg.

[0073] Some embodiments of the present disclosure relate to an adeno-associated virus (AAV) genome consisting of a RP that when operable inside a target cell will cause the target cell to produce a miRNA sequence that downregulates production of a biomolecule, with examples being serotonin receptor 5HT1a, serotonin receptor 5HT1b, serotonin receptor 5HT1d, serotonin receptor 5HT1e, serotonin receptor 5HT1f, serotonin receptor 5HT2a, serotonin receptor 5HT2b, serotonin receptor 5HT2c, serotonin receptor 5HT3, serotonin receptor 5HT4, serotonin receptor 5HT6, or serotonin receptor 5HT7. The RP is comprised of AAV2 inverted terminal repeats (ITRs), a composite CASI promoter, a human growth hormone (HGH) signal peptide followed by a miRNA expression cassette containing up to six different miRNAs targeting the mRNA of serotonin receptor 5HT1a, serotonin receptor 5HT1b, serotonin receptor 5HT1d, serotonin receptor 5HT1e, serotonin receptor 5HT1f, serotonin receptor 5HT2a, serotonin receptor 5HT2b, serotonin receptor 5HT2c, serotonin receptor 5HT3, serotonin receptor 5HT4, serotonin receptor 5HT6, or serotonin receptor 5HT7, followed by a Woodchuck Hepatitis Virus post-transcriptional regulatory element (WPRE) and a Simian virus 40 (SV40) polyadenylation (polyA) signal.

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SEQ ID NO. 1 (backbone sequence No. 1):
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CGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAG

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ACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTCGCTTTCCCCCTC
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-continued

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CTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCCGCCTCCCCGCCTAAGCTT

ATCGATACCGTCGAGATCTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAA

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GAATTTTAACAAAATATTAACGTTTACAATTTAAATATTTGCTTATACAATCTTCCTGTT

TTTGGGGCTTTTCTGATTATCAACCGGGGTACATATGATTGACATGCTAGTTTTACGATT

ACCGTTCATCGATTCTCTTGTTTGCTCCAGACTCTCAGGCAATGACCTGATAGCCTTTGT

AGAGACCTCTCAAAAATAGCTACCCTCTCCGGCATGAATTTATCAGCTAGAACGGTTGAA

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ACACATTACTCAGGCATTGCATTTAAAATATATGAGGGTTCTAAAAATTTTTATCCTTGC

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ATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACC

GTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAA

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GGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGAT

GAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGA

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GAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAAC

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GGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAAC

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TAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGA

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GCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTC

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CGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCG

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GCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCAGCTGCGCGCTCGCTCGCTCACT

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GAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCTTGTAGTTAATG

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TGACTGACCGCGTTACTAAAACAGGTAAGTCCGGCCTCCGCGCCGGGTTTTGGCGCCTCC

CGCGGGCGCCCCCCTCCTCACGGCGAGCGCTGCCACGTCAGACGAAGGGCGCAGCGAGCG

TCCTGATCCTTCCGCCCGGACGCTCAGGACAGCGGCCCGCTGCTCATAAGACTCGGCCTT

AGAACCCCAGTATCAGCAGAAGGACATTTTAGGACGGGACTTGGGTGACTCTAGGGCACT

GGTTTTCTTTCCAGAGAGCGGAACAGGCGAGGAAAAGTAGTCCCTTCTCGGCGATTCTGC

GGAGGGATCTCCGTGGGGCGGTGAACGCCGATGATGCCTCTACTAACCATGTTCATGTTT

TCTTTTTTTTTCTACAGGTCCTGGGTGACGAACAGGGTACC 3'

SEQ ID NO. 2 (miRNA expression cassette No. 2-serotonin receptor 5HT1a):
5' GCCACCATGGCCACCGGCTCTCGCACAAGCCTGCTGCTGGCTTTCGGACTGCTGTGC

CTGCCTTGGCTCCAGGAGGGCTCCGCCGCTAGCATCGATACCGTCGCTATGTGCTGGAGG

CTTGCTGAAGGCTGTATGCTGATCAATCGGATTGCGGTAATCGCGTTTTGGCCTCTGACT

GACGCGATTACCGATCCGATTGATCAGGACACAAGGCCTGTTACTAGCACTCACATGGAA

CAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTATGCTGATCTTTGCTAAATTGGT

GCACGCGTTTTGGCCTCTGACTGACGCGTGCACCATTAGCAAAGATCAGGACACAAGGCC

TGTTACTAGCACTCACATGGAACAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTA

TGCTGACTTCAATCACAATTCCAGCGCCGTTTTGGCCTCTGACTGACGGCGCTGGAAGTG

ATTGAAGTCAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCCTCTCTAG

AAT 3'

SEQ ID NO. 3 (miRNA expression cassette No. 3-serotonin receptor 5HT1b):
5' GCCACCATGGCCACCGGCTCTCGCACAAGCCTGCTGCTGGCTTTCGGACTGCTGTGC

CTGCCTTGGCTCCAGGAGGGCTCCGCCGCTAGCATCGATACCGTCGCTATGTGCTGGAGG

CTTGCTGAAGGCTGTATGCTGTAATCTTTCGCTGGCTGCAGTTCGTTTTGGCCTCTGACT

GACGAACTGCAGCGCGAAAGATTACAGGACACAAGGCCTGTTACTAGCACTCACATGGAA

CAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTATGCTGTGTTAATGCTGATGTCA

CGCTGCGTTTTGGCCTCTGACTGACGCAGCGTGACCAGCATTAACACAGGACACAAGGCC

TGTTACTAGCACTCACATGGAACAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTA

TGCTGTTCACCTGGTTAACACATACACCGTTTTGGCCTCTGACTGACGGTGTATGTGAAC

CAGGTGAACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCCTCTCTAG

AAT 3'

SEQ ID NO. 4 (miRNA expression cassette No. 4-serotonin receptor 5HT1d):
5' GCCACCATGGCCACCGGCTCTCGCACAAGCCTGCTGCTGGCTTTCGGACTGCTGTGC

CTGCCTTGGCTCCAGGAGGGCTCCGCCGCTAGCATCGATACCGTCGCTATGTGCTGGAGG

CTTGCTGAAGGCTGTATGCTGATTTCTTCCTGTGCGCTTTCGCCGTTTTGGCCTCTGACT

GACGGCGAAAGCGCAGGAAGAAATCAGGACACAAGGCCTGTTACTAGCACTCACATGGAA

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CAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTATGCTGAGAATAATCAGATCAGC

ACGCTCGTTTTGGCCTCTGACTGACGAGCGTGCTGCTGATTATTCTCAGGACACAAGGCC

TGTTACTAGCACTCACATGGAACAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTA

TGCTGTAATCAGGCTGAATTCAGATAGCGTTTTGGCCTCTGACTGACGCTATCTGAACAG

CCTGATTACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCCTCTCTAG

AAT 3'

SEQ ID NO. 5 (miRNA expression cassette No. 5-serotonin receptor 5HTle):
5' GCCACCATGGCCACCGGCTCTCGCACAAGCCTGCTGCTGGCTTTCGGACTGCTGTGC

CTGCCTTGGCTCCAGGAGGGCTCCGCCGCTAGCATCGATACCGTCGCTATGTGCTGGAGG

CTTGCTGAAGGCTGTATGCTGATAATCACCGCTGCAGGTTCAGCGTTTTGGCCTCTGACT

GACGCTGAACCTGGCGGTGATTATCAGGACACAAGGCCTGTTACTAGCACTCACATGGAA

CAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTATGCTGTTCAATCGCGTATTGGT

AATCGCGTTTTGGCCTCTGACTGACGCGATTACCAACGCGATTGAACAGGACACAAGGCC

TGTTACTAGCACTCACATGGAACAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTA

TGCTGTGATCATGCTGAAAATGGTGCACGTTTTGGCCTCTGACTGACGTGCACCATTCAG

CATGATCACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCCTCTCTAG

AAT 3'

SEQ ID NO. 6 (miRNA expression cassette No. 6-serotonin receptor 5HT1f):
5' GCCACCATGGCCACCGGCTCTCGCACAAGCCTGCTGCTGGCTTTCGGACTGCTGTGC

CTGCCTTGGCTCCAGGAGGGCTCCGCCGCTAGCATCGATACCGTCGCTATGTGCTGGAGG

CTTGCTGAAGGCTGTATGCTGAGGTAATATCCTGACGCTCAGCCGTTTTGGCCTCTGACT

GACGGCTGAGCGTGGATATTACCTCAGGACACAAGGCCTGTTACTAGCACTCACATGGAA

CAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTATGCTGTACAGAATCAGATAATC

AGCGCCGTTTTGGCCTCTGACTGACGGCGCTGATTCTGATTCTGTACAGGACACAAGGCC

TGTTACTAGCACTCACATGGAACAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTA

TGCTGTCATGTTTAAAAATTCGCTGCGCGTTTTGGCCTCTGACTGACGCGCAGCGAATTT

AAACATGACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCCTCTCTAG

AAT 3'

SEQ ID NO. 7 (miRNA expression cassette No. 7-serotonin receptor 5HT2a):
5' GCCACCATGGCCACCGGCTCTCGCACAAGCCTGCTGCTGGCTTTCGGACTGCTGTGC

CTGCCTTGGCTCCAGGAGGGCTCCGCCGCTAGCATCGATACCGTCGCTATGTGCTGGAGG

CTTGCTGAAGGCTGTATGCTGATGAATCGGGTTGTCTGAATCGCGTTTTGGCCTCTGACT

GACGCGATTCAGAACCCGATTCATCAGGACACAAGGCCTGTTACTAGCACTCACATGGAA

CAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTATGCTGAACACTTTGCTATATCA

TCCTGCGTTTTGGCCTCTGACTGACGCAGGATGATAGCAAAGTGTTCAGGACACAAGGCC

TGTTACTAGCACTCACATGGAACAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTA

TGCTGTTCTGTTCGTTAAGCTAATGCTCGTTTTGGCCTCTGACTGACGAGCATTAGCAAC

GAACAGAACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCCTCTCTAG

AAT 3'

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SEQ ID NO. 8 (miRNA expression cassette No. 8-serotonin receptor 5HT2b):
5' GCCACCATGGCCACCGGCTCTCGCACAAGCCTGCTGCTGGCTTTCGGACTGCTGTGC

CTGCCTTGGCTCCAGGAGGGCTCCGCCGCTAGCATCGATACCGTCGCTATGTGCTGGAGG

CTTGCTGAAGGCTGTATGCTGGAGCATTAGCAATGCGAACAGAAGTTTTGGCCTCTGACT

GACTTCTGTTCGTTGCTAATGCTCCAGGACACAAGGCCTGTTACTAGCACTCACATGGAA

CAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTATGCTGAAACATAATGGATTCAG

CAGCGCGTTTTGGCCTCTGACTGACGCGCTGCTGACCATTATGTTTCAGGACACAAGGCC

TGTTACTAGCACTCACATGGAACAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTA

TGCTGTTATCTTTGCGAAGCTGCCATCCGTTTTGGCCTCTGACTGACGGATGGCAGCCGC

AAAGATAACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCCTCTCTAG

AAT 3'

SEQ ID NO. 9 (miRNA expression cassette No. 9-serotonin receptor 5HT2c):
5' GCCACCATGGCCACCGGCTCTCGCACAAGCCTGCTGCTGGCTTTCGGACTGCTGTGC

CTGCCTTGGCTCCAGGAGGGCTCCGCCGCTAGCATCGATACCGTCGCTATGTGCTGGAGG

CTTGCTGAAGGCTGTATGCTGGCTCCTCCACTTGGTGGTTTGGTTTTGGCCTCTGACTGA

CGCGGCAACATTCTGGTGATTACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACA

AATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTATGCTGTCATAATCGCTATTTGGTG

CGGCGTTTTGGCCTCTGACTGACGCCGCACCAAAGCGATTATGACAGGACACAAGGCCTG

TTACTAGCACTCACATGGAACAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTATG

CTGTTCTGATCCTGAAGTTCGGGTTCGTTTTGGCCTCTGACTGACGAACCCGAACCAGGA

TCAGAACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCCTCTCTAGAA

T 3'

SEQ ID NO. 10 (miRNA expression cassette No. 10-serotonin receptor 5HT3):
5' GCCACCATGGCCACCGGCTCTCGCACAAGCCTGCTGCTGGCTTTCGGACTGCTGTGC

CTGCCTTGGCTCCAGGAGGGCTCCGCCGCTAGCATCGATACCGTCGCTATGTGCTGGAGG

CTTGCTGAAGGCTGTATGCTGAAATCTTCCGGTGGTTCCACTGCGTTTTGGCCTCTGACT

GACGCAGTGGAACCCGGAAGATTTCAGGACACAAGGCCTGTTACTAGCACTCACATGGAA

CAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTATGCTGATATCCTGAATATGGTA

TGCAGCGTTTTGGCCTCTGACTGACGCTGCATACCATTCAGGATATCAGGACACAAGGCC

TGTTACTAGCACTCACATGGAACAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTA

TGCTGTTTAAAGCTCAAACGCGTTCGCCGTTTTGGCCTCTGACTGACGGCGAACGCGTGA

GCTTTAAACAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCCTCTCTAG

AAT 3'

SEQ ID NO. 11 (miRNA expression cassette No. 11-serotonin receptor 5HT4):
5' GCCACCATGGCCACCGGCTCTCGCACAAGCCTGCTGCTGGCTTTCGGACTGCTGTGC

CTGCCTTGGCTCCAGGAGGGCTCCGCCGCTAGCATCGATACCGTCGCTATGTGCTGGAGG

CTTGCTGAAGGCTGTATGCTGTAATAAAGGTCTGGGAATCACCCGTTTTGGCCTCTGACT

GACGGGTGATTCCGACCTTTATTACAGGACACAAGGCCTGTTACTAGCACTCACATGGAA

CAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTATGCTGTAATACGCCAGATCACC

ATCAGCGTTTTGGCCTCTGACTGACGCTGATGGTGCTGGCGTATTACAGGACACAAGGCC

TGTTACTAGCACTCACATGGAACAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTA

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TGCTGATACAGAAACGAAGGTTCAGGCCGTTTTGGCCTCTGACTGACGGCCTGAACCCGT

TTCTGTATCAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCCTCTCTAG

AAT 3'

SEQ ID NO. 12 (miRNA expression cassette No. 12-serotonin receptor 5HT6):
5' GCCACCATGGCCACCGGCTCTCGCACAAGCCTGCTGCTGGCTTTCGGACTGCTGTGC

CTGCCTTGGCTCCAGGAGGGCTCCGCCGCTAGCATCGATACCGTCGCTATGTGCTGGAGG

CTTGCTGAAGGCTGTATGCTGTCAGATCGCTGTGGTAAACAGGCGTTTTGGCCTCTGACT

GACGCCTGTTTACCAGCGATCTGACAGGACACAAGGCCTGTTACTAGCACTCACATGGAA

CAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTATGCTGAGAATCAGATCAGATAG

CGATCCGTTTTGGCCTCTGACTGACGGATCGCTATCTGCTGATTCTCAGGACACAAGGCC

TGTTACTAGCACTCACATGGAACAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTA

TGCTGAAACATGCCAACAGCAGAATGCCGTTTTGGCCTCTGACTGACGGCATTCTGCTGG

GCATGTTTCAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCCTCTCTAG

AAT 3'

SEQ ID NO. 13 (miRNA expression cassette No. 13-serotonin receptor 5HT7):
5' GCCACCATGGCCACCGGCTCTCGCACAAGCCTGCTGCTGGCTTTCGGACTGCTGTGC

CTGCCTTGGCTCCAGGAGGGCTCCGCCGCTAGCATCGATACCGTCGCTATGTGCTGGAGG

CTTGCTGAAGGCTGTATGCTGACAATCAGATATGGTTGCTCGGCGTTTTGGCCTCTGACT

GACGCCGAGCAACTATCTGATTGTCAGGACACAAGGCCTGTTACTAGCACTCACATGGAA

CAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTATGCTGTTTCACAATGCATCGTT

CAGCGCGTTTTGGCCTCTGACTGACGCGCTGAACGGCATTGTGAAACAGGACACAAGGCC

TGTTACTAGCACTCACATGGAACAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTA

TGCTGACAATAATGCCAACAGGGTGGTCGTTTTGGCCTCTGACTGACGACCACCCTGGGC

ATTATTGTCAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCCTCTCTAG

AAT 3'

SEQ ID NO. 14 = SEQ ID NO. 1 + SEQ ID NO. 2
5' AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTT

GCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCC

CGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAG

TTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTGACGCAACCCCC

ACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTCGCTTTCCCCCTC

CCTATTGCCACGGCGGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGG

CTGTTGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAATCATCGTCCTTTCCTTGGCTG

CTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCCTTCTGCTACGTCCCTTCGGCC

CTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCGT

CTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCCGCCTCCCCGCCTAAGCTT

ATCGATACCGTCGAGATCTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAA

TAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTC

CAAACTCATCAATGTATCTTATCATGTCTGGATCTCGACCTCGACTAGAGCATGGCTACG

TAGATAAGTAGCATGGCGGGTTAATCATTAACTACAAGGAACCCCTAGTGATGGAGTTGG

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GCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTAC

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CGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGC

TTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATC

GCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACT

CTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGG

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GAATTTTAACAAAATATTAACGTTTACAATTTAAATATTTGCTTATACAATCTTCCTGTT

TTTGGGGCTTTTCTGATTATCAACCGGGGTACATATGATTGACATGCTAGTTTTACGATT

ACCGTTCATCGATTCTCTTGTTTGCTCCAGACTCTCAGGCAATGACCTGATAGCCTTTGT

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CCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGC

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GTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAA

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ACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCG

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GAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGA

GCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCAC

AGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCAT

GAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAAC

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GAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAAC

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CTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTG

GTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACT

GGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAAC

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ACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATT

TAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGA

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GCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTC

TGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGG

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GGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGG

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TTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCC

TGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCG

AACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACC

GCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCAGCTGCGCGCTCGCTCGCTCACT

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ATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGT

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ATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCA

TCGCTATTACCATGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCC

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CGGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGGGGGGCGAGGGGGGGGCGGGGCG

AGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATG

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CGCGGGCGCCCCCCTCCTCACGGCGAGCGCTGCCACGTCAGACGAAGGGCGCAGCGAGCG

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CTCGCACAAGCCTGCTGCTGGCTTTCGGACTGCTGTGCCTGCCTTGGCTCCAGGAGGGCT

CCGCCGCTAGCATCGATACCGTCGCTATGTGCTGGAGGCTTGCTGAAGGCTGTATGCTGA

TCAATCGGATTGCGGTAATCGCGTTTTGGCCTCTGACTGACGCGATTACCGATCCGATTG

ATCAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAATGGCCTCTAGCCTGGAGG

CTTGCTGAAGGCTGTATGCTGATCTTTGCTAAATTGGTGCACGCGTTTTGGCCTCTGACT

GACGCGTGCACCATTAGCAAAGATCAGGACACAAGGCCTGTTACTAGCACTCACATGGAA

CAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTATGCTGACTTCAATCACAATTCC

AGCGCCGTTTTGGCCTCTGACTGACGGCGCTGGAAGTGATTGAAGTCAGGACACAAGGCC

TGTTACTAGCACTCACATGGAACAAATGGCCTCTCTAGAAT 3'

SEQ ID NO. 15 = SEQ ID NO. 1 + SEQ ID NO. 3

5' AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTT

GCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCC

CGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAG

TTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTGACGCAACCCCC

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ACAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTATGCTGTTCACCTGGTTAACAC

ATACACCGTTTTGGCCTCTGACTGACGGTGTATGTGAACCAGGTGAACAGGACACAAGGC

CTGTTACTAGCACTCACATGGAACAAATGGCCTCTCTAGAAT 3'

SEQ ID NO. 16 = SEQ ID NO. 1 + SEQ ID NO. 4
5' AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTT

GCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCC

CGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAG

TTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTGACGCAACCCCC

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AGATAGCGTTTTGGCCTCTGACTGACGCTATCTGAACAGCCTGATTACAGGACACAAGGC

CTGTTACTAGCACTCACATGGAACAAATGGCCTCTCTAGAAT 3'

SEQ ID NO. 17 = SEQ ID NO. 1 + SEQ ID NO. 5
5' AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTT

GCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCC

CGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAG

TTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTGACGCAACCCCC

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SEQ ID NO. 18 = SEQ ID NO. 1 + SEQ ID NO. 6
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TGTTACTAGCACTCACATGGAACAAATGGCCTCTCTAGAAT 3'

SEQ ID NO. 19 = SEQ ID NO. 1 + SEQ ID NO. 7

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SEQ ID NO. 20 = SEQ ID NO. 1 + SEQ ID NO. 8
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GCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCC

CGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAG

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CAAATGGCCTCTAGCCTGGAGGCTTGCTGAAGGCTGTATGCTGTTATCTTTGCGAAGCTG

CCATCCGTTTTGGCCTCTGACTGACGGATGGCAGCCGCAAAGATAACAGGACACAAGGCC

TGTTACTAGCACTCACATGGAACAAATGGCCTCTCTAGAAT 3'

SEQ ID NO. 21 = SEQ ID NO. 1 + SEQ ID NO. 9
5' AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTT

GCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCC

CGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAG

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GGTTCGTTTTGGCCTCTGACTGACGAACCCGAACCAGGATCAGAACAGGACACAAGGCCT

GTTACTAGCACTCACATGGAACAAATGGCCTCTCTAGAAT 3'

SEQ ID NO. 22 = SEQ ID NO. 1 + SEQ ID NO. 10
5' AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTT

GCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCC

CGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAG

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TGTTACTAGCACTCACATGGAACAAATGGCCTCTCTAGAAT 3'

SEQ ID NO. 23 = SEQ ID NO :. 1 + SEQ ID NO. 11
5' AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTT

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CTGTTACTAGCACTCACATGGAACAAATGGCCTCTCTAGAAT 3'

SEQ ID NO. 24 = SEQ ID NO. 1 + SEQ ID NO. 12
5' AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTT

GCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCC

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AATGCCGTTTTGGCCTCTGACTGACGGCATTCTGCTGGGCATGTTTCAGGACACAAGGCC

TGTTACTAGCACTCACATGGAACAAATGGCCTCTCTAGAAT 3'

SEQ ID NO. 25 = SEQ ID NO. 1 + SEQ ID NO. 13

5' AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTT

GCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCC

CGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAG

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[0074] As will be appreciated by those skilled in the art, because the recombinant plasmid is a circular vector, the one or more sequences of the miRNA expression cassettes may be connected at the 3' end of SEQ ID NO. 1, as shown in SEQ ID NO. 14, SEQ ID NO. 15, SEQ ID NO. 16, SEQ ID NO. 17, SEQ ID NO. 18, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24 and SEQ ID NO. 25, or at the 5' end of SEQ ID NO. 1.

[0075] As will be appreciated by those skilled in the art, a perfect match of nucleotides with each of the miRNA expression cassette sequences is not necessary in order to have the desired result of decreased bioavailability of the target biomolecule as a result of the target cell producing the miRNA sequence that will bind to and degrade the mRNA of the target biomolecule. In some embodiments of the present disclosure, about 80% to about 100% nucleotide sequence matching with each of the miRNA expression cassettes causes the desired result. In some embodiments of the present disclosure, about 85% to about 100% nucleotide sequence matching with each of the miRNA expression cassettes causes the desired result. In some embodiments of the present disclosure, about 90% to about 100% nucleotide sequence matching with each of the miRNA expression cassettes causes the desired result. In some embodiments of the present disclosure, about 95% to about 100% nucleotide sequence matching with each of the miRNA expression cassettes causes the desired result.

Example 1—Expression Cassette

[0076] Expression cassettes for expressing miRNA were synthesized. The synthesized miRNA expression cassettes were cloned into the pAVA-00200 plasmid backbone containing the CASI promoter, multiple cloning site (MCS), Woodchuck Hepatitis Virus post-transcriptional regulatory element (WPRE), and Simian virus 40 (SV40) polyadenylation (polyA) sequence, all flanked by the AAV2 inverted terminal repeats (ITR). pAVA-00200 was cut with the restriction enzymes KpnI and XbaI in the MCS and separated on a 1% agarose gel. The band of interest was excised and purified using a gel extraction kit. Each miRNA expression cassette was amplified by polymerase chain reaction (PCR) using Taq polymerase and the PCR products were gel purified and the bands on interest were also excised and purified using a gel extraction kit. These PCR products contained the miRNA expression cassettes in addition to 15 base pair 5' and 3' overhangs that aligned with the ends of the linearized pAVA-00200 backbone. Using in-fusion cloning, the amplified miRNA expression cassettes were integrated with the pAVA-00200 backbone via homologous recombination. The resulting RP contained the following: 5' ITR, CASI promoter, miRNA expression cassette, WPRE, SV40 polyA and ITR 3'.

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FEATURE                Location/Qualifiers
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                        organism = synthetic construct
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SEQ ID NO: 7            moltype = DNA  length = 540
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                        organism = synthetic construct
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SEQ ID NO: 8            moltype = DNA  length = 540
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SEQ ID NO: 9            moltype = DNA  length = 538
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                        organism = synthetic construct
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SEQ ID NO: 11           moltype = DNA  length = 540
FEATURE                 Location/Qualifiers
source                  1..540
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SEQ ID NO: 12           moltype = DNA  length = 540
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SEQ ID NO: 13           moltype = DNA  length = 540
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SEQ ID NO: 14           moltype = DNA  length = 6339
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                        organism = synthetic construct
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1. A composition that comprises a recombinant plasmid (RP) that comprises a sequence of nucleotides that is SEQ ID NO. 2.

2. (canceled)

3. The composition of claim 1, wherein the RP is encapsulated in a protein coat, a lipid vesicle, or any combination thereof.

4. The composition of claim 1, wherein the RP is encapsulated in a viral vector.

5. The composition of claim 4, wherein the viral vector is one of: a double stranded DNA virus, a single stranded DNA virus, a single stranded RNA virus, or a double stranded RNA virus.

6. The composition of claim 4, wherein the viral vector is an adeno-associated virus.

7. (canceled)

8. (canceled)

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