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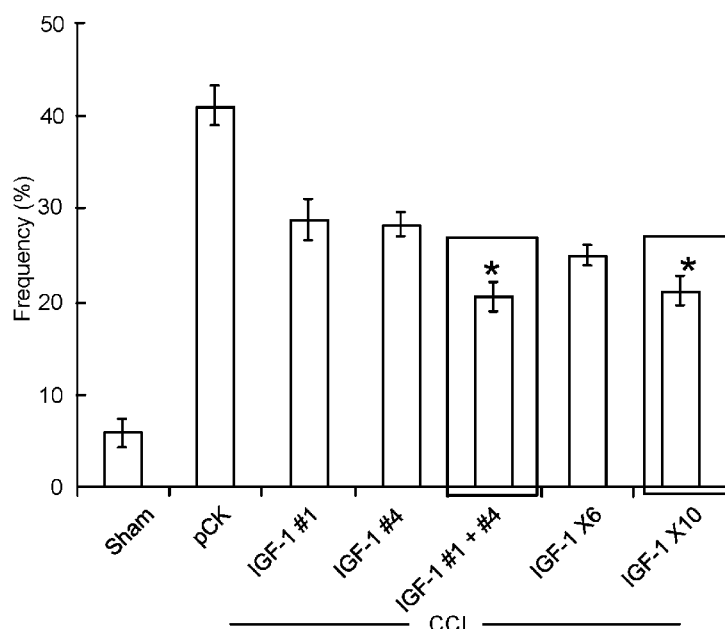


FIG 5B

(57) Abstract: The present invention relates to a DNA construct encoding one or more human IGF-1 isoforms that can be used for treatment of neuropathy. Further provided herein are a pharmaceutical composition including the DNA construct as an active ingredient and a method of administering the DNA construct for treatment of neuropathy. The present invention provides a safe and effective way of treating neuropathic patients.



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TREATMENT OF NEUROPATHY WITH DNA CONSTRUCTS EXPRESSING IGF-1 ISOFORMS

1. CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/699,662, filed July 17, 2018, which is hereby incorporated by reference in its entirety.

2. SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 2, 2019, is named 38917US_CRF_sequencelisting.txt, and is 47,265 bytes in size.

3. BACKGROUND

[0003] Neuropathy is a chronic pathologic condition resulting from nerve damage. Neuropathy is a common consequence of diabetes, with neuropathy in a diabetic patient specifically referred to as diabetic neuropathy. Neuropathy can also be caused by nerve damage caused by infections (*e.g.*, herpes, with the associated neuropathy arising after infection known as post-herpetic neuralgia; HIV/AIDS; Lyme disease; leprosy; syphilis; and shingles); autoimmune diseases (*e.g.*, rheumatoid arthritis, systemic lupus, and Guillain-Barre syndrome); genetic or inherited disorders (*e.g.*, Friedreich's ataxia and Charcot-Marie-Tooth disease); amyloidosis; uremia; exposure to toxins, poisons or drugs; trauma; or injury. In some cases, the cause is not known, in which case the neuropathy is referred to as idiopathic neuropathy.

[0004] Regardless of the cause, neuropathy is associated with characteristic symptoms that depend, in part, on the anatomic site of nerve damage (*e.g.*, peripheral neuropathy, cranial neuropathy, autonomic neuropathy, focal neuropathy), such as pain (neuropathic pain), other sensory defects (*e.g.*, anesthetics, including partial or complete loss of feeling; and paresthesias, including numbness, tingling, *etc.*), motor defects (*e.g.*, weakness, loss of reflexes, loss of muscle mass, cramping, loss of dexterity, *etc.*), and autonomic dysfunction (*e.g.*, nausea, vomiting, impotence, dizziness, constipation, diarrhea, *etc.*).

[0005] Neuropathy is routinely treated with measures that manage associated symptoms, and when the etiology is known, by treating the underlying cause of neuropathy. For example, pain

medications, or medical treatments for diabetes, autoimmune diseases, infections, or vitamin deficiencies have been used. However, these methods do not treat the nerve damage itself.

[0006] There is, therefore, a need for an effective treatment method that can prevent and repair nerve damages associated with neuropathy.

[0007] Various growth factors have been suggested as possible agents for treating neuropathy, and Kessler and colleagues recently reported a successful double-blind, placebo-controlled, phase 2 human clinical trial of nonviral hepatocyte growth factor (HGF) gene therapy in diabetic peripheral neuropathy. Kessler *et al.*, *Annals Clin. Transl. Neurology* 2(5):465-478 (2015). See also U.S. Pat. No. 9,963,493, incorporated herein by reference in its entirety.

[0008] Given the wide range of etiologies that cause neuropathy and the wide range of neuropathy clinical presentations, despite the clinical success in treating diabetic peripheral neuropathy with HGF-expressing nucleic acid constructs, there remains a need for additional treatments, including treatments based on administering growth factors other than HGF.

4. SUMMARY

[0009] The present invention is based on a novel finding that DNA constructs encoding a human IGF-1 isoform are effective in treating a symptom associated with neuropathy. It was further demonstrated that DNA constructs encoding Class I, Ec or Class I, Ea isoforms of IGF-1 are more effective than other DNA constructs encoding Class II, Ea or Class I, Eb isoform. The therapeutic effects become even more significant when the two types of DNA constructs, each encoding Class I, Ec or Class I, Ea isoforms of IGF-1, are administered together. Accordingly, some embodiments of the present invention are directed to a method of treating neuropathy by administering DNA constructs encoding Class I, Ec or Class I, Ea isoform of IGF-1, individually or in combination, to a subject with neuropathy.

[0010] The present invention further provides novel DNA constructs specifically designed to encode the two isoforms IGF-1 (*i.e.*, Class I, Ec and Class I, Ea) in a single vector. The DNA constructs were screened and selected based on their capability to induce high-level expression of both Class I, Ec and Class I, Ea isoforms and effectively treat symptoms associated with neuropathy *in vivo*. Their treatment effects were greater than the effects of DNA constructs encoding only one of the two isoforms, Class I, Ec or Class I, Ea, and similar to the effects

demonstrated by co-administration of two types of constructs, each encoding Class I, Ec or Class I, Ea.

[0011] Thus, the present invention provides a novel IGF-1 based therapy for treating neuropathy.

[0012] Specifically, in one aspect, the present invention provides an IGF-1 encoding DNA construct encoding human IGF-1, comprising: a first polynucleotide that has the same sequence as exons 1, 3 and 4 of the human IGF-1 gene (SEQ ID NO: 1) or a degenerate thereof; a second polynucleotide that has the same sequence as intron 4 of the human IGF-1 gene (SEQ ID NO: 2) or a fragment thereof; a third polynucleotide that has the same sequence as exons 5 and 6-1 of the human IGF-1 gene (SEQ ID NO: 3) or a degenerate thereof; a fourth polynucleotide that has the same sequence as intron 5 of the human IGF-1 gene (SEQ ID NO: 4) or a fragment thereof; and a fifth polynucleotide that has the same sequence as exon 6-2 of the human IGF-1 gene (SEQ ID NO: 5) or a degenerate thereof, wherein the first polynucleotide, the second polynucleotide, the third polynucleotide, the fourth polynucleotide and the fifth polynucleotide are linked in sequential 5' to 3' order.

[0013] In some embodiments, the second polynucleotide is a polynucleotide of SEQ ID NO: 6. In some embodiments, the second polynucleotide is a polynucleotide of SEQ ID NO: 7.

[0014] In some embodiments, the fourth polynucleotide is a polynucleotide of SEQ ID NO: 8.

[0015] In some embodiments, the IGF-1 encoding DNA construct further comprises a plasmid vector.

[0016] In some embodiments, the plasmid vector is pCK. In some embodiments, the IGF-1 encoding DNA construct is pCK-IGF-1X6 or pCK-IGF-1X10.

[0017] In some embodiments, the plasmid vector is pTx. In some embodiments, the IGF-1 encoding DNA construct is pTx-IGF-1X6 or pTx-IGF-1X10.

[0018] In other aspects, the present invention provides a pharmaceutical composition comprising the IGF-1 encoding DNA construct provided herein.

[0019] In other aspects, the present invention provides a pharmaceutical composition comprising the IGF-1 encoding DNA construct, wherein the IGF-1 encoding DNA construct encodes Class I IGF-1Ea protein comprising a polypeptide of SEQ ID NO: 14 or Class I IGF-1Ec protein comprising a polypeptide of SEQ ID NO: 16.

[0020] In some embodiments, the IGF-1 encoding DNA construct encodes both Class I IGF-1Ea protein comprising a polypeptide of SEQ ID NO: 14 and Class I IGF-1Ec protein comprising a polypeptide of SEQ ID NO: 16.

[0021] In some embodiments, the IGF-1 encoding DNA construct does not encode Class II IGF-1 Ea protein comprising a polypeptide of SEQ ID NO: 18 and Class I IGF-1Eb protein comprising a polypeptide of SEQ ID NO: 20.

[0022] In some embodiments, the IGF-1 encoding DNA construct encodes neither Class II IGF-1 Ea protein comprising a polypeptide of SEQ ID NO: 18 nor Class I IGF-1Eb protein comprising a polypeptide of SEQ ID NO: 20.

[0023] In some embodiments, the pharmaceutical composition comprises a first DNA construct encoding Class I IGF-1Ea protein comprising a polypeptide of SEQ ID NO: 14, and a second DNA construct encoding Class I IGF-1Ec protein comprising a polypeptide of SEQ ID NO: 16.

[0024] In some embodiments, the IGF-1 encoding DNA construct is pCK-IGF-1X6 or pCK-IGF-1X10. In some embodiments, the IGF-1 encoding DNA construct is pTx-IGF-1X6 or pTx-IGF-1X10.

[0025] Some aspects of the present invention provide a method of treating neuropathy, comprising the step of administering to a subject having neuropathy an effective amount of a first IGF-1 encoding DNA construct, wherein the first IGF-1 encoding DNA construct is capable of expressing at least one human IGF-1 isoform.

[0026] In some embodiments, the first IGF-1 encoding DNA construct is capable of expressing Class I IGF-1Ea protein comprising a polypeptide of SEQ ID NO: 14 or Class I IGF-1Ec protein comprising a polypeptide of SEQ ID NO: 16.

[0027] In some embodiments, the first IGF-1 encoding DNA construct is not capable of expressing Class II IGF-1 Ea protein comprising a polypeptide of SEQ ID NO: 18 or Class I IGF-1Eb protein comprising a polypeptide of SEQ ID NO: 20.

[0028] In some embodiments, the first IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 15. In some embodiments, the method further comprises the step of: administering to the subject a second IGF-1 encoding DNA construct, wherein the second IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 17.

[0029] In some embodiments, the first IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 17. In some embodiments, the method further comprises the step of: administering to the subject a second IGF-1 encoding DNA construct, wherein the second IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 15.

[0030] In some embodiments, the step of administering the first IGF-1 encoding DNA construct and the step of administering the second IGF-1 encoding DNA construct are performed concurrently.

[0031] In some embodiments, the step of administering the first IGF-1 encoding DNA construct and the step of administering the second IGF-1 encoding DNA construct are performed sequentially.

[0032] In some embodiments, the first IGF-1 encoding DNA construct encodes more than one human IGF-1 isoforms. In some embodiments, the first IGF-1 encoding DNA construct encodes Class I IGF-1Ea protein comprising a polypeptide of SEQ ID NO: 14 and Class I IGF-1 Ec protein comprising a polypeptide of SEQ ID NO: 16.

[0033] In some embodiments, the first IGF-1 encoding DNA construct comprises: a first polynucleotide that has the same sequence as exons 1, 3 and 4 of the human IGF-1 gene (SEQ ID NO: 1) or a degenerate thereof; a second polynucleotide that has the same sequence as intron 4 of the human IGF-1 gene (SEQ ID NO: 2) or a fragment thereof; a third polynucleotide that has the same sequence as exons 5 and 6-1 of the human IGF-1 gene (SEQ ID NO: 3) or a degenerate thereof; a fourth polynucleotide that has the same sequence as intron 5 of the human IGF-1 gene (SEQ ID NO: 4) or a fragment thereof; and a fifth polynucleotide that has the same sequence as exon 6-2 of the human IGF-1 gene (SEQ ID NO: 5) or a degenerate thereof, wherein the first polynucleotide, the second polynucleotide, the third polynucleotide, the fourth polynucleotide and the fifth polynucleotide are linked in sequential 5' to 3' order.

[0034] In some embodiments, the second polynucleotide is a polynucleotide of SEQ ID NO: 6. In some embodiments, the second polynucleotide is a polynucleotide of SEQ ID NO: 7.

[0035] In some embodiments, the fourth polynucleotide is a polynucleotide of SEQ ID NO: 8.

[0036] In some embodiments, the first IGF-1 encoding DNA construct comprises a plasmid vector. In some embodiments, the plasmid vector is pCK. In some embodiments, the plasmid vector is pTx.

[0037] In some embodiments, the first IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 10. In some embodiments, the first IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 9. In some embodiments, the first IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 27.

[0038] In some embodiments, the effective amount is an amount sufficient to reduce pain in the subject.

[0039] In some embodiments, the subject has neuropathic pain. In some embodiments, the subject has diabetic neuropathy.

[0040] In some embodiments, the step of administering the first IGF-1 encoding DNA construct or the second IGF-1 encoding DNA construct comprises an intramuscular injection.

[0041] In yet another aspect, the present disclosure provides an IGF-1 encoding DNA construct for use in a medical method of treating neuropathy, the method comprising the step of administering to a subject having neuropathy an effective amount of the IGF-1 encoding DNA construct, wherein the IGF-1 encoding DNA construct is capable of expressing at least one human IGF-1 isoform.

[0042] In some embodiments, the IGF-1 encoding DNA construct is capable of expressing Class I IGF-1Ea protein comprising a polypeptide of SEQ ID NO: 14 or Class I IGF-1Ec protein comprising a polypeptide of SEQ ID NO: 16. In some embodiments, the IGF-1 encoding DNA construct is not capable of expressing both Class II IGF-1 Ea protein comprising a polypeptide of SEQ ID NO: 18 and Class I IGF-1Eb protein comprising a polypeptide of SEQ ID NO: 20.

[0043] In some embodiments, the IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 15. In some embodiments, the medical method further comprises the step of: administering to the subject a second IGF-1 encoding DNA construct, wherein the second IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 17.

[0044] In some embodiments, the IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 17. In some embodiments, the medical method further comprising the step of:

administering to the subject a second IGF-1 encoding DNA construct, wherein the second IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 15.

[0045] In some embodiments, the step of administering the IGF-1 encoding DNA construct and the step of administering the second IGF-1 encoding DNA construct are performed concurrently. In some embodiments, the step of administering the IGF-1 encoding DNA construct and the step of administering the second IGF-1 encoding DNA construct are performed sequentially.

[0046] In some embodiments, the IGF-1 encoding DNA construct encodes more than one human IGF-1 isoforms. In some embodiments, the IGF-1 encoding DNA construct encodes Class I IGF-1Ea protein comprising a polypeptide of SEQ ID NO: 14 and Class I IGF-1 Ec protein comprising a polypeptide of SEQ ID NO: 16.

[0047] In some embodiments, the IGF-1 encoding DNA construct comprises: a first polynucleotide that has the same sequence as exons 1, 3 and 4 of the human IGF-1 gene (SEQ ID NO: 1) or a degenerate thereof; a second polynucleotide that has the same sequence as intron 4 of the human IGF-1 gene (SEQ ID NO: 2) or a fragment thereof; a third polynucleotide that has the same sequence as exons 5 and 6-1 of the human IGF-1 gene (SEQ ID NO: 3) or a degenerate thereof; a fourth polynucleotide that has the same sequence as intron 5 of the human IGF-1 gene (SEQ ID NO: 4) or a fragment thereof; and a fifth polynucleotide that has the same sequence as exon 6-2 of the human IGF-1 gene (SEQ ID NO: 5) or a degenerate thereof, wherein the first polynucleotide, the second polynucleotide, the third polynucleotide, the fourth polynucleotide and the fifth polynucleotide are linked in sequential 5' to 3' order.

[0048] In some embodiments, the second polynucleotide is a polynucleotide of SEQ ID NO: 6. In some embodiments, the second polynucleotide is a polynucleotide of SEQ ID NO: 7. In some embodiments, the fourth polynucleotide is a polynucleotide of SEQ ID NO: 8.

[0049] In some embodiments, the IGF-1 encoding DNA construct comprises a plasmid vector. In some embodiments, the plasmid vector is pCK. In some embodiments, the plasmid vector is pTx.

[0050] In some embodiments, the IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 10. In some embodiments, the IGF-1 encoding DNA construct comprises a

polynucleotide of SEQ ID NO: 9. In some embodiments, the IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 27

[0051] In some embodiments, the effective amount is an amount sufficient to reduce pain in the subject. In some embodiments, the subject has neuropathic pain. In some embodiments, the subject has diabetic neuropathy. In some embodiments, the step of administering the IGF-1 encoding DNA construct or the second IGF-1 encoding DNA construct comprises an intramuscular injection

5. BRIEF DESCRIPTION OF THE DRAWINGS

[0052] **FIG. 1** is a schematic representation of the human IGF-1 gene including alternative transcription initiation sites and alternative splicing sites. IGF-1 isoforms that are naturally produced from the IGF-1 gene include Class I Ec (Isoform #1); Class II Ea (Isoform #2); Class I Eb (Isoform #3); and Class I Ea (Isoform #4).

[0053] **FIG. 2A** outlines the experimental protocol for testing therapeutic efficacy of various DNA constructs encoding an IGF-1 isoform in the chronic constriction injury (CCI) model.

[0054] **FIG. 2B** is a histogram showing the frequency of paw withdrawal measured in the Sham mice or CCI mice in the experiment outlined in FIG. 2A. The CCI mice were injected with a DNA construct – (i) pCK vector, (ii) pCK-IGF-1 #1 (a construct expressing Class I Ec isoform), (iii) pCK-IGF-1 #4 (a construct expressing Class I Ea isoform), or (iv) both pCK-IGF-1 #1 and pCK-IGF-1 #4.

[0055] **FIG. 3A** outlines the experimental protocol used in Example 2 to assess *in vivo* expression of IGF-1 isoforms from various DNA constructs.

[0056] **FIG. 3B** shows results of an ELISA measuring the amount of total human IGF-1 isoforms expressed after injection of a DNA construct encoding no IGF (vector only, “pCK”); a DNA construct encoding isoform #1 (Class I Ec isoform); a DNA construct encoding isoform #4 (Class I Ea isoform); two constructs, each encoding isoforms #1 (Class I Ec isoform) or #4 (Class I Ea isoform); and a dual expression construct pCK-IGF-1X6 or pCK-IGF-1X10.

[0057] **FIG. 3C** shows the location of forward/left (L) and reverse/right (R) primers used in RT-PCR for discriminating expressions of IGF-1 isoforms #1 (Class I Ec isoform) and #4 (Class I Ea isoform).

[0058] **FIG. 3D** shows agarose gel electrophoresis of RT-PCR products, showing expression of isoforms #1 and #4 from dual expression constructs pCK-IGF-1X6 and pCK-IGF-1X10. Both pCK-IGF-1X6 and pCK-IGF-1X10 induced high-level expression of both isoforms.

[0059] **FIG. 4A** outlines the protocol used in Example 2 to assess protein expression from the IGF-1 encoding DNA constructs in 293T cells *in vitro*.

[0060] **FIG. 4B** shows western blotting results demonstrating expression of IGF-1 isoforms #1 and/or #4 after *in vitro* transfection of (i) a construct encoding isoform #1, (ii) a construct encoding isoform #4, (iii) two constructs, each encoding isoform #1 or #4, (iv) a dual expression vector pCK-IGF-1X6, or (v) a dual expression vector pCK-IGF-1X10.

[0061] **FIG. 5A** outlines the experimental protocol used in Example 3 to test efficacy of various IGF-1 encoding DNA constructs in reducing mechanical allodynia in the CCI animal model.

[0062] **FIG. 5B** is a histogram showing the frequency of paw withdrawal measured in the Sham mice or CCI mice in the experiment outlined in FIG. 5A. The CCI mice were injected with a DNA construct – (i) pCK vector, (ii) pCK-IGF-1 #1 (a construct expressing Class I Ec isoform), (iii) pCK-IGF-1 #4 (a construct expressing Class I Ea isoform), (iv) both pCK-IGF-1 #1 and pCK-IGF-1 #4, (v) a dual expression vector pCK-IGF-1X6, or (vi) a dual expression vector pCK-IGF-1X10.

[0063] **FIG. 6A** provides transmission electron microscopic (TEM) images of the right ipsilateral sciatic nerve obtained from the Sham or the nerve crush animal model. The nerve crush animal model was injected with pCK vector or with a dual expression vector pCK-IGF-1X10.

[0064] **FIG. 6B** is a graph showing distributions of neuron diameters in the nerve crush animal treated with pCK (“Crush-pCK”) and a nerve crush animal treated with pCK-IGF-1X10 (“Crush-pCK-IGF-1X10”).

[0065] The figures depict various embodiments of the present invention for purposes of illustration only. One skilled in the art will readily recognize from the following discussion that alternative embodiments of the structures and methods illustrated herein may be employed without departing from the principles of the invention described herein.

6. DETAILED DESCRIPTION

6.1. Definitions

[0066] Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. As used herein, the following terms have the meanings ascribed to them below.

[0067] The terms “**isoform of IGF-1**,” “**human IGF-1 isoform**” or “**IGF-1 isoform**” are used interchangeably herein to refer to a polypeptide having an amino acid sequence that is at least 80% identical to the amino acid sequence of one of naturally occurring pre-pro-IGF-1 polypeptides of humans, or their allelic variant, splice variant, or deletion variant. The naturally occurring pre-pro-IGF-1 polypeptides include Class I, Ec (SEQ ID NO: 16); Class II, Ea (SEQ ID NO: 18); Class I, Eb (SEQ ID NO: 20); and Class I, Ea isoforms (SEQ ID NO: 14).

[0068] The terms “**Isoform #1**,” “**Class I, Ec isoform**,” “**Class I, IGF-1 Ec isoform**” or “**Class I, IGF-1 Ec**” are used interchangeably herein to refer to a polypeptide of SEQ ID NO: 16.

[0069] The terms “**Isoform #2**,” “**Class II, Ea isoform**,” “**Class II, IGF-1 Ea isoform**” or “**Class II, IGF-1 Ea**” are used interchangeably herein to refer to a polypeptide of SEQ ID NO: 18.

[0070] The terms “**Isoform #3**,” “**Class I, Eb isoform**,” “**Class I, IGF-1 Eb isoform**” or “**Class I, IGF-1 Eb**” are used interchangeably herein to refer to a polypeptide of SEQ ID NO: 20.

[0071] The terms “**Isoform #4**,” “**Class I, Ea isoform**,” “**Class I, IGF-1 Ea isoform**” or “**Class I, IGF-1 Ea**” are used interchangeably herein to refer to a polypeptide of SEQ ID NO: 14.

[0072] The term “**treatment**” as used herein refers to all the acts of (a) suppression a symptom of neuropathy; (b) alleviation of a symptom of neuropathy; and (c) removal of a symptom of neuropathy. In some embodiments, the composition of the present invention can treat neuropathy through the growth of neuronal cells or the suppression of neuronal cell death.

[0073] The term “**therapeutically effective dose**” or “**effective amount**” as used herein refers to a dose or an amount that produces the desired effect for which it is administered. In the context of the present methods, a therapeutically effective amount is an amount effective to treat a symptom of neuropathy.

[0074] The term “**sufficient amount**” as used herein refers to an amount sufficient to produce a desired effect.

[0075] The term “**degenerate sequence**” or “**degenerate**” as used herein refers to a nucleic acid sequence that can be translated to provide an amino acid sequence identical to that translated from the reference nucleic acid sequence.

6.2. Other interpretational conventions

[0076] Ranges recited herein are understood to be shorthand for all of the values within the range, inclusive of the recited endpoints. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50

6.3. DNA construct expressing an IGF-1 isoform

[0077] In a first aspect, DNA constructs encoding a human IGF-1 isoform is provided.

6.3.1. IGF-1 isoforms

[0078] As illustrated in FIG. 1, the human IGF-1 gene contains six exons (exons 1, 2, 3, 4, 5, and 6 (6-1 and 6-2)) spanning nearly 90kb of genomic DNA. Exons 1 and 2 are mutually exclusive leader exons, each having multiple promoter sites that are variably used. Further, the IGF-1 gene can be differentially spliced to create multiple transcript variants. Each transcript variant encodes a different pre-pro-IGF-1 protein (“IGF-1 isoform”) possessing variable signaling peptide leader sequences. Yet all the transcript isoforms give rise to the same mature 70-amino acid IGF-1 peptide that uses the same receptor after processing.

[0079] The pre-pro-IGF-1 peptides differ in their leader, or signal, sequences and in their carboxy (C) -terminus. Incorporation of exon 1 or exon 2 is mutually exclusive and one of them serves as a leader sequence of the pre-pro-IGF-1 peptide; the different leader exons create different 5'-UTRs. The pre-pro-IGF-1 polypeptides undergo posttranscriptional proteolytic cleavage to remove the leader and the E-peptide carboxy-terminus giving rise to the mature 70-amino acid IGF-1.

[0080] Transcripts containing exon 1 are referred to as Class 1 transcripts (*e.g.*, Class I, Ec; Class I, Eb; and Class I, Ea in FIG. 1) whereas those containing exon 2 are referred to as Class 2 transcripts (*e.g.*, Class II, Ea in FIG. 1). Nearly all pre-pro peptides include 27 amino acids in the signaling peptide derived from exon 3 with the remaining signal sequences derived from the inclusion of exon 1 or 2. A minority of transcripts utilize a different transcription initiation site within exon 3 generating a shorter signaling peptide of 22 amino acids. Exons 3 and 4 are invariant and encode the B, C, A, and D domains of the mature IGF-1 peptide; exon 4 encodes two thirds of the mature IGF-1 peptide. The human Eb peptide is composed of only exons 4 and 5 whereas Ec contains exons 4, 5, and 6.

[0081] Alternative splicing and mutually exclusive initiation of transcription are illustrated in FIG. 1 that result in generation of different pre-pro-IGF-1 polypeptides (*i.e.*, IGF-1 isoforms). Specifically, Class I, Ec IGF-1 isoform (SEQ ID NO: 16), comprising at least a fragment of exons 1, 3/4, 5 and 6, is generated from a transcript comprising a sequence of SEQ ID NO: 17. Class II, Ea IGF-1 isoform (SEQ ID NO:18), comprising at least a fragment of exons 2, 3/4 and 6, is generated from a transcript comprising a sequence of SEQ ID NO:19. Class I, Eb IGF-1 isoform (SEQ ID NO:20), comprising at least a fragment of exons 1, 3/4 and 5, is generated from a transcript comprising a sequence of SEQ ID NO:21. Class I, Ea IGF-1 isoform (SEQ ID NO:14), comprising at least a fragment of exons 1, 3/4 and 6 are generated from a transcript comprising a sequence of SEQ ID NO: 15.

[0082] Although the mature IGF-1 protein derived from the various transcripts does not differ, the various transcript isoforms have been suggested to have different regulatory roles. The variant forms possess different stabilities, binding partners, and activity indicating a pivotal regulatory role for the isoforms. The biological significance of the isoforms remains unclear, although it has been hypothesized that Class I isoforms with exon 1 are autocrine/paracrine forms while Class II isoforms with exon 2 are secreted endocrine forms. This is based on the finding that Class II transcripts include a typical signal peptide motif associated with efficient secretion, whereas Class I transcripts have a longer signal peptide that can possibly interfere with secretion.

[0083] Most tissues are believed to use Class I transcripts, although liver uses both forms and hepatic Class II transcripts are preferentially enhanced during development. There are many

changes in the IGF-1 transcript abundance during development. It was found that Class 1, Ea is the most abundant form during the active growth phase and Class 1, Eb is also expressed uniformly, albeit at lower levels, across the growth plate during early growth phases.

6.3.2. DNA construct expressing IGF-1 isoform

[0084] In an aspect, the present invention provides DNA constructs capable of expressing at least one isoform of human IGF-1. In some embodiments, more than one DNA constructs, each encoding a different IGF-1 isoform, are used. For example, a first construct encoding Class I, Ec isoform (Isoform #1) and a second construct encoding Class I, Ea isoform (Isoform #4) are used together. In some embodiments, a DNA construct that expresses two or more isoforms (*i.e.*, “a dual expression construct”) is used. For example, a single DNA construct encoding both Class I, Ec isoform and Class I, Ea isoform can be used.

6.3.2.1. IGF-1 encoding sequences

[0085] In some embodiment, the DNA construct contains a coding sequence of one of the IGF-1 isoforms. For example, the DNA construct can comprise a sequence encoding Class I, Ea (SEQ ID NO: 15); Class I, Eb (SEQ ID NO:21); Class I, Ec (SEQ ID NO:17); or Class II, Ea (SEQ ID NO:19).

[0086] In some embodiments, the DNA construct is a dual expression construct, a DNA construct that can express more than one IGF-1 isoforms, by comprising an expression regulatory sequence for each isoform coding sequence (CDS). In some embodiments, the construct comprises an internal ribosomal entry site (IRES) between two coding sequences, for example, in the order of (1) expression regulatory sequence – (2) coding sequence of first isoform – (3) IRES – (4) coding sequence of second isoform – (5) transcription termination sequence. IRES allows translation to start at the IRES sequence, thereby allowing expression of two protein products from a single transcript. In yet further embodiments, a plurality of constructs, each encoding a single isoform of IGF-1, are used together to induce expression of more than one isoforms of IGF-1 in the subject to whom administered.

[0087] In preferred embodiments, a DNA construct is capable of expressing two or more IGF-1 isoforms simultaneously – *e.g.*, (i) Class I, Ec isoform (Isoform #1) and Class II, Ea isoform (Isoform #2); (ii) Class I, Ec isoform (Isoform #1) and Class I, Eb isoform (Isoform #3); (iii)

Class I, Ec isoform (Isoform #1) and Class I, Ea isoform (Isoform #4); (iv) Class II, Ea isoform (Isoform #2) and Class I, Eb isoform (Isoform #3); (v) Class II, Ea isoform (Isoform #2) and Class I, Ea isoform (Isoform #4); (vi) Class I, Eb isoform (Isoform #3) and Class I, Ea isoform (Isoform #4) – by comprising an alternative splicing site.

[0088] For example, the DNA construct can comprise (i) a first sequence comprising exons 1, 3 and 4 of a human IGF-1 gene (SEQ ID NO:1) or a degenerate sequence of the first sequence; (ii) a second sequence comprising intron 4 of the human IGF-1 gene (SEQ ID NO:2) or a fragment of the second sequence; (iii) a third sequence comprising exons 5 and 6-1 of the human IGF-1 gene (SEQ ID NO:3) or a degenerate sequence of the third sequence; (iv) a fourth sequence comprising intron 5 of the human IGF-1 gene (SEQ ID NO:4) or a fragment of the second sequence; and (v) a fifth sequence comprising exon 6-2 of the human IGF-1 gene (SEQ ID NO:5) or a degenerate sequence of the fifth sequence. Introns 4 and 5 can be alternatively spliced, resulting in production of two isoforms of IGF-1 (*e.g.*, Class I, Ec and Class I, Ea).

[0089] In some embodiments, the DNA construct is tested *in vitro* and/or *in vivo* related to its capability to express one or more IGF-1 isoforms. In preferred embodiments, DNA constructs capable of expressing both Class I, Ec and Class I, Ea IGF-1 isoforms are selected.

[0090] In some embodiments, the construct comprises a full sequence of intron 4 (SEQ ID NO:2) or its fragment. In preferred embodiments, the construct comprises a fragment of intron 4 having a sequence of SEQ ID NO: 6 or SEQ ID NO: 7.

[0091] In some embodiments, the construct comprises a full sequence of intron 5 (SEQ ID NO: 4), or its fragment. In preferred embodiments, the construct comprises a fragment of intron 5 having a sequence of SEQ ID NO: 8.

[0092] Various DNA constructs comprising cDNA corresponding (i) exons 1-6 of the human IGF-1 gene and (ii) introns 4 and 5 of the human IGF-1 gene or various fragments of introns 4 and 5 are named “IGF-1X” followed by a unique number. The IGF-1X constructs tested by Applicant include, but are not limited to, IGF-1X1, IGF-1X2, IGF-1X3, IGF-1X4, IGF-1X5, IGF-1X6, IGF-1X7, IGF-1X8, IGF-1X9 and IGF-1X10. Among the tested constructs, IGF-1X6 and IGF-1X10 were identified to express both Class I, Ec and Class I, Ea IGF-1 isoforms.

[0093] In preferred embodiments, IGF-1X6 (SEQ ID NO:9) or IGF-1X10 (SEQ ID NO:10) is used. IGF-1X6 (SEQ ID NO:9) and IGF-1X10 (SEQ ID NO:10) cloned into a pCK vector are named pCK-IGF-1X6 and pCK-IGF-1X10, respectively. *E.coli* cells transformed with pCK-IGF-1X6 (“DH5 α _pCK-IGF1 X6”) were deposited under the terms of the Budapest Treaty at the Korea Collection for Type Cultures (KCTC, Korea Research Institute of Bioscience and Biotechnology (KRIBB) 181, Ipsin-gil, Jeongeup-si, Jeollabuk-do, 56212, Republic of Korea) with accession number KCTC 13539BP on May 30, 2018. *E.coli* cells transformed with pCK-IGF-1X10 (“DH5 α _pCK-IGF1 X10”) were deposited under the terms of the Budapest Treaty at the Korea Collection for Type Cultures (KCTC, Korea Research Institute of Bioscience and Biotechnology (KRIBB) 181, Ipsin-gil, Jeongeup-si, Jeollabuk-do, 56212, Republic of Korea) with accession number KCTC 13540BP on May 30, 2018.

[0094] In other preferred embodiments, IGF-1X6 (SEQ ID NO:9) and IGF-1X10 (SEQ ID NO:10) are cloned into a pTx vector. The IGF constructs are named pTx-IGF-1X6 and pTx-IGF-1X10, respectively.

[0095] IGF-1 isoforms or DNA constructs encoding IGF-1 isoforms described herein can include modifications from the wild type human IGF-1 isoforms. The modified sequences can include sequences with at least 80% identity, more preferably at least 90% identity and most preferably at least 95% identity when the modified sequences are aligned with the wild type human IGF-1 isoform sequences in the maximal manner. Methods of alignment of sequences for comparison are well-known in the art. Specifically, alignment algorithm disclosed in the NCBI Basic Local Alignment Search Tool (BLAST) of the National Center for Biological Information (NCBI, Bethesda, Md.) website and used in connection with the sequence analysis programs blastp, blastm, blastx, tblastn and tblastx can be used to determine the percent identity.

6.3.2.2. Vector

[0096] DNA constructs expressing an IGF-1 isoform of the present invention typically comprise a vector with one or more regulatory sequences (*e.g.*, a promoter or an enhancer) operatively linked to the expressed sequences. The regulatory sequence regulates expression of the isoforms of IGF-1.

[0097] It is preferred that the polynucleotide encoding one or more isoforms of IGF-1 isoforms is operatively linked to a promoter in an expression construct. The term “operatively linked” refers to functional linkage between a nucleic acid expression control sequence (such as a promoter, signal sequence, or array of transcription factor binding sites) and a second nucleic acid sequence, wherein the expression control sequence affects transcription and/or translation of the nucleic acid corresponding to the second sequence.

[0098] In typical embodiments, the promoter linked to the polynucleotide is operable in, preferably, animal, more preferably, mammalian cells, to control transcription of the polynucleotide, including the promoters derived from the genome of mammalian cells or from mammalian viruses, for example, CMV (cytomegalovirus) promoter, the adenovirus late promoter, the vaccinia virus 7.5K promoter, SV40 promoter, HSV tk promoter, RSV promoter, EFl alpha promoter, metallothionein promoter, beta-actin promoter, human IL- 2 gene promoter, human IFN gene promoter, human IL-4 gene promoter, human lymphotoxin gene promoter and human GM-CSF gene promoter, but not limited to. More preferably, the promoter useful in this invention is a promoter derived from the IE (immediately early) gene of human CMV (hCMV) or EFl alpha promoter, most preferably hCMV IE gene-derived promoter/enhancer and 5'-UTR (untranslated region) comprising the overall sequence of exon 1 and exon 2 sequence spanning a sequence immediately before the ATG start codon.

[0099] The expression cassette used in this invention can comprise a polyadenylation sequence, for example, including bovine growth hormone terminator (Gimmi, E. R., *et al.*, Nucleic Acids Res. 17:6983-6998 (1989)), SV40- derived polyadenylation sequence (Schek, N, *et al.*, Mol. Cell Biol. 12:5386-5393 (1992)), HIV-1 polyA (Klasens, B. I. F., *et al.*, Nucleic Acids Res. 26:1870-1876 (1998)), β -globin polyA (Gil, A., *et al.*, Cell 49:399-406 (1987)), HSV TK polyA (Cole, C. N. and T. P. Stacy, Mol. Cell. 5 Biol. 5: 2104-2113 (1985)) or polyoma virus polyA (Batt, D. Band G. G. Carmichael, Mol. Cell. Biol. 15:4783-4790 (1995)), but not limited thereto.

6.3.2.3. Non-viral Vector

[0100] In some embodiments, the DNA construct is a non-viral vector capable of expressing one or more isoforms of IGF-1.

[0101] In typical embodiments, the non-viral vector is a plasmid. In currently preferred embodiments, the plasmid is pCK, pCP, pVAX1, pTx or pCY. In particularly preferred embodiments, the plasmid is pCK, details of which can be found in WO 2000/040737 and Lee *et al.*, *Biochem. Biophys. Res. Comm.* 272:230–235 (2000), both of which are incorporated herein by reference in their entireties. *E. coli* transformed with pCK (Top10-pCK) was deposited at the Korean Culture Center of Microorganisms (KCCM) under the terms of the Budapest Treaty on March 21, 2003 (Accession NO: KCCM-10476). *E. coli* transformed with pCK-VEGF165 (*i.e.*, pCK vector with VEGF coding sequence – Top10-pCK/VEGF165') was deposited at the Korean Culture Center of Microorganisms (KCCM) under the terms of the Budapest Treaty on December 27, 1999 (Accession NO: KCCM-10179).

[0102] The pCK vector is constructed such that the expression of a gene, *e.g.*, an IGF-1 gene, is regulated under enhancer/promoter of the human cytomegalovirus (HCMV), as disclosed in detail in Lee *et al.*, *Biochem. Biophys. Res. Commun.* 272: 230 (2000); WO 2000/040737, both of which are incorporated by reference in their entirety. pCK vector has been used for clinical trials on human body, and its safety and efficacy were confirmed (Henry *et al.*, *Gene Ther.* 18:788 (2011)).

[0103] In preferred embodiments, the pCK plasmid contains a coding sequence for Class I, Ec IGF-1 isoform and/or Class I, Ea IGF-1 isoform. In particularly preferred embodiments, the pCK plasmid contains IGF-1X6 (*i.e.*, pCK-IGF-1X6) or IGF-1 X10 (*i.e.*, pCK-IGF-1X10).

[0104] In other preferred embodiments, the plasmid is pTx (SEQ ID NO: 26), a plasmid vector derived from pCK. pTx was generated by two sequential rounds of mutagenesis of pCK. The first deletion mutagenesis campaign was conducted to remove the unnecessary sequence between Kanamycin resistance gene and ColE1 of pCK. Specifically, deletion mutagenesis PCR was performed using a first primer pair (SEQ ID NOs: 22 and 23). The deletion of 228 base pairs between Kanamycin resistance and ColE1 was confirmed by sequencing the plasmid. The second deletion mutagenesis campaign was then performed using a second primer pair (SEQ ID NOs: 24 and 25), to optimize the size of HCMV intron sequence. HCMV intron sequence (421 base pairs) between IE1 exon 1 and exon 2 was deleted and the deletion was confirmed by sequencing.

[0105] In particular embodiments, the pTx plasmid contains IGF-1X6 (*i.e.*, pTx-IGF-1X6) or IGF-1 X10 (*i.e.*, pTx-IGF-1X10). For example, pTx-1X10 (SEQ ID NOs: 27) was generated by ligating IGF-1X10 in pTx digested with ClaI enzyme at 5' and SalI enzyme at 3'.

6.3.2.4. Viral Vector

[0106] In other embodiments, various viral vectors known in the art can be used to deliver and express one or more IGF-1 isoforms of the present invention. For example, vectors developed using retroviruses, lentiviruses, adenoviruses, or adeno-associated viruses can be used for some embodiments of the present invention.

(a) Retrovirus

[0107] Retroviruses capable of carrying relatively large exogenous genes have been used as viral gene delivery vectors in the senses that they integrate their genome into a host genome and have broad host spectrum.

[0108] In order to construct a retroviral vector, the polynucleotide of the invention (*e.g.*, a coding sequence of one or more IGF-1 isoforms) is inserted into the viral genome in the place of certain viral sequences to produce a replication-defective virus. To produce virions, a packaging cell line containing the *gag*, *pol* and *env* genes but without the LTR (long terminal repeat) and W components is constructed (Mann *et al.*, Cell, 33:153-159 (1983)). When a recombinant plasmid containing the polynucleotide of the invention, LTR and W is introduced into this cell line, the W sequence allows the RNA transcript of the recombinant plasmid to be packaged into viral particles, which are then secreted into the culture media (Nicolas and Rubinstein "Retroviral vectors," In: Vectors: A survey of molecular cloning vectors and their uses, Rodriguez and Denhardt (eds.), Stoneham: Butterworth, 494-513(1988)) The media containing the recombinant retroviruses is then collected, optionally concentrated and used for gene delivery.

[0109] A successful gene transfer using the second generation retroviral vector has been reported. Kasahara *et al.* (Science, 266 :1373-1376 (1994)) prepared variants of moloney murine leukemia virus in which the EPO (erythropoietin) sequence is inserted in the place of the envelope region, consequently, producing chimeric proteins having novel binding properties. Likely, the present gene delivery system can be constructed in accordance with the construction strategies for the second-generation retroviral vector.

(b) **Lentiviruses**

[0110] Lentiviruses can be also used in some embodiments of the present invention. Lentiviruses are a subclass of Retroviruses. However, Lentivirus can integrate into the genome of non-dividing cells, while Retroviruses can infect only dividing cells.

[0111] Lentiviral vectors are usually produced from packaging cell line, commonly HEK293, transformed with several plasmids. The plasmids include (1) packaging plasmids encoding the virion proteins such as capsid and the reverse transcriptase, (2) a plasmid comprising an exogenous gene (*e.g.*, a coding sequence of one or more IGF-1 isoforms) to be delivered to the target.

[0112] When the virus enters the cell, the viral genome in the form of RNA is reverse-transcribed to produce DNA, which is then inserted into the genome by the viral integrase enzyme. Thus, the exogenous delivered with the Lentiviral vector can remain in the genome and is passed on to the progeny of the cell when it divides.

(c) **Adenovirus**

[0113] Adenovirus has been usually employed as a gene delivery system because of its mid-sized genome, ease of manipulation, high titer, wide target-cell range, and high infectivity. Both ends of the viral genome contains 100-200 bp ITRs (inverted terminal repeats), which are *cis*-elements necessary for viral DNA replication and packaging. The El region (ElA and ElB) encodes proteins responsible for the regulation of transcription of the viral genome and a few cellular genes. The expression of the E2 region (E2A and E2B) results in the synthesis of the proteins for viral DNA replication.

[0114] Of adenoviral vectors developed so far, the replication incompetent adenovirus having the deleted El region is usually used. The deleted E3 region in adenoviral vectors may provide an insertion site for transgenes (Thimmappaya, B. *et al.*, Cell, 31:543-551(1982); and Riordan, J. R. *et al.*, Science, 245:1066- 1073 (1989)). Therefore, it is preferred that the decorin-encoding nucleotide sequence is inserted into either the deleted El region (ElA region and/or ElB 5 region, preferably, ElB region) or the deleted E3 region. The polynucleotide of the invention may be inserted into the deleted E4 region. The term “deletion” with reference to viral genome sequences encompasses whole deletion and partial deletion as well. In nature, adenovirus can

package approximately 105% of the wildtype genome, providing capacity for about 2 extra kb of DNA (Ghosh-Choudhury *et al.*, EMBO J. 6:1733- 1 739 (1987)). In this regard, the foreign sequences described above inserted into adenovirus may be further inserted into adenoviral wild-type genome.

[0115] The adenovirus may be of any of the known serotypes or subgroups A-F. Adenovirus type 5 of subgroup C is the most preferred starting material for constructing the adenoviral gene delivery system of this invention. A great deal of biochemical and genetic information about adenovirus type 5 is known. The foreign genes delivered by the adenoviral gene delivery system are episomal, and genotoxicity to host cells. Therefore, gene therapy using the adenoviral gene delivery system may be considerably safe.

(d) **Adeno-associated virus (AAV)**

[0116] Adeno-associated viruses are capable of infecting non-dividing cells and various types of cells, making them useful in constructing the gene delivery system of this invention. Detailed descriptions for use and preparation of AAV vectors are found in U.S. Pat. Nos. 10,308,958; 10,301,650; 10,301,648; 10,266,846; 10,265,417; 10,208,107; 10,167,454; 10,155,931; 10,149,873; 10,144,770; 10,138,295; 10,137,176; 10,113,182; 10,041,090; 9,890,365; 9,790,472; 9,770,011; 9,738,688; 9,737,618; 9,719,106; 9,677,089; 9,617,561; 9,597,363; 9,593,346; 9,587,250; 9,567,607; 9,493,788; 9,382,551; 9,359,618; 9,217,159; 9,206,238; 9,163,260; 9,133,483; 8,962,332, the disclosures of which are incorporated herein by reference in their entireties, and 5,139,941 and 4,797,368, the disclosures of which are incorporated herein by reference in their entireties.

[0117] Research results for AAV as gene delivery systems are disclosed in LaFace *et al.*, Viology, 162: 483486 (1988), Zhou *et al.*, Exp. Hematol. (NY), 21:928-933(1993), Walsh *et al.*, J. Clin. Invest., 94:1440-1448(1994) and Flotte *et al.*, Gene Therapy, 2:29-37(1995). Typically, a recombinant AAV virus is made by cotransfecting a plasmid containing the gene of interest (*i.e.*, nucleotide sequence of interest to be delivered, *e.g.*, a coding sequence of an IGF-1 isoform) flanked by the two AAV terminal repeats (McLaughlin *et al.*, 1988; Samulski *et al.*, 1989) and an expression plasmid containing the wild type AAV coding sequences without the terminal repeats (McCarty *et al.*, J. Viral., 65:2936-2945 (1991)).

(e) **Other viral vectors**

[0118] Other viral vectors may be employed as a gene delivery system in the present invention. Vectors derived from viruses such as vaccinia virus (Puhlmann M. *et al.*, Human Gene Therapy 10:649-657(1999); Ridgeway, "Mammalian expression vectors," In: Vectors: A survey of molecular cloning vectors and their uses. Rodriguez and Denhardt, eds. Stoneham: Butterworth, 467-492 (1988); Baichwal and Sugden, "Vectors for gene transfer derived from animal DNA viruses: Transient and stable expression of transferred genes," In: Kucherlapati R, ed. Gene transfer. New York: Plenum Press, 117-148 (1986) and Coupar *et al.*, Gene, 68:1-10(1988)), lentivirus (Wang G. *et al.*, J. Clin. Invest. 104 (11): RS 5-62 (1999)) and herpes simplex virus (Chamber R., *et al.*, Proc. Natl. 10 15 Acad. Sci USA 92:1411-1415(1995)) may be used in the present delivery systems for transferring the polynucleotide of the invention into cells.

6.4. Pharmaceutical composition comprising IGF-1 encoding DNA construct

[0119] In another aspect, a pharmaceutical composition comprising the IGF-1 encoding DNA construct is provided.

6.4.1. Pharmacological compositions and unit dosage forms adapted for injection

[0120] For intravenous, intramuscular, intradermal, or subcutaneous injection, the DNA construct can be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilizers, buffers, antioxidants and/or other additives, as required.

[0121] In some embodiments, the pharmaceutical composition comprises a DNA construct encoding one IGF-1 isoform. For example, the DNA construct can express Class I, Ec isoform (Isoform #1); Class II, Ea isoform (Isoform #2); Class I, Eb isoform (Isoform #3); or Class I, Ea isoform (Isoform #4).

[0122] In some embodiments, the pharmaceutical composition comprises more than one DNA constructs, each encoding one IGF-1 isoform. For example, the pharmaceutical composition can comprise (i) a first DNA construct encoding Class I, Ec isoform (Isoform #1) and a second DNA

construct encoding Class II, Ea isoform (Isoform #2); (ii) a first DNA construct encoding Class I, Ec isoform (Isoform #1) and a second DNA construct encoding Class I, Eb isoform (Isoform #3); (iii) a first DNA construct encoding Class I, Ec isoform (Isoform #1) and a second DNA construct encoding Class I, Ea isoform (Isoform #4); (iv) a first DNA construct encoding Class II, Ea isoform (Isoform #2) and a second DNA construct encoding Class I, Eb isoform (Isoform #3); (v) a first DNA construct encoding Class II, Ea isoform (Isoform #2) and a second DNA construct encoding Class I, Ea isoform (Isoform #4); (vi) a first DNA construct encoding Class I, Eb isoform (Isoform #3) and a second DNA construct encoding Class I, Ea isoform (Isoform #4).

[0123] In some embodiments, the pharmaceutical composition comprises a dual expression construct, a DNA construct that can express more than one IGF-1 isoforms. For example, the pharmaceutical composition can comprise a dual expression construct that can express (i) Class I, Ec isoform (Isoform #1) and Class II, Ea isoform (Isoform #2); (ii) Class I, Ec isoform (Isoform #1) and Class I, Eb isoform (Isoform #3); (iii) Class I, Ec isoform (Isoform #1) and Class I, Ea isoform (Isoform #4); (iv) Class II, Ea isoform (Isoform #2) and Class I, Eb isoform (Isoform #3); (v) Class II, Ea isoform (Isoform #2) and Class I, Ea isoform (Isoform #4); (vi) Class I, Eb isoform (Isoform #3) and Class I, Ea isoform (Isoform #4).

[0124] In some embodiments, the pharmaceutical composition comprises a dual expression construct, pCK-IGF-1X6 or pCK-IGF-1X10. In some embodiments, the pharmaceutical composition comprises a dual expression construct, pTx-IGF-1X6 or pTx-IGF-1X10. In some embodiments, the pharmaceutical composition comprises two dual expression constructs, for example, including both pCK-IGF-1X6 and pCK-IGF-1X10. In some embodiments, the pharmaceutical composition comprises two dual expression constructs, for example, including both pTx-IGF-1X6 and pTx-IGF-1X10.

[0125] In some embodiments, the pharmaceutical composition further comprises another therapeutic agent. For example, the pharmaceutical composition can further comprise another therapeutic agent effective in treating neuropathy.

[0126] In various embodiments, the DNA construct is present in the liquid composition at a concentration of 0.01 mg/ml, 0.05 mg/ml, 0.1 mg/ml, 0.25 mg/ml, 0.45 mg/ml, 0.5 mg/ml, or 1 mg/ml. In some embodiments, the unit dosage form is a vial containing 2 ml of the pharmaceutical composition at a concentration of 0.01 mg/ml, 0.1 mg/ml, 0.5 mg/ml, or 1mg/ml.

In some embodiments, the unit dosage form is a vial containing 1 ml of the pharmaceutical composition at a concentration of 0.01 mg/ml, 0.1 mg/ml, 0.5 mg/ml, or 1mg/ml. In some embodiments, the unit dosage form is a vial containing less than 1 ml of the pharmaceutical composition at a concentration of 0.01 mg/ml, 0.1 mg/ml, 0.5 mg/ml, or 1mg/ml.

[0127] In some embodiments, the unit dosage form is a vial, ampule, bottle, or pre-filled syringe. In some embodiments, the unit dosage form contains 0.01 mg, 0.1 mg, 0.2 mg, 0.25 mg, 0.5 mg, 1 mg, 2.5 mg, 5 mg, 8mg, 10 mg, 12.5 mg, 16 mg, 24 mg, 25 mg, 50 mg, 75 mg, 100 mg, 150 mg, or 200 mg of the DNA construct of the present invention.

[0128] In some embodiments, the pharmaceutical composition in the unit dosage form is in liquid form. In various embodiments, the unit dosage form contains between 0.1 ml and 50 ml of the pharmaceutical composition. In some embodiments, the unit dosage form contains 0.25 ml, 0.5 ml, 1 ml, 2.5 ml, 5 ml, 7.5 ml, 10 ml, 25 ml, or 50 ml of pharmaceutical composition.

[0129] In particular embodiments, the unit dosage form is a vial containing 0.5ml, 1 ml, 1.5 ml or 2 ml of the pharmaceutical composition at unit dosage form embodiments suitable for subcutaneous, intradermal, or intramuscular administration include preloaded syringes, auto-injectors, and auto-inject pens, each containing a predetermined amount of the pharmaceutical composition described hereinabove.

[0130] In various embodiments, the unit dosage form is a preloaded syringe, comprising a syringe and a predetermined amount of the pharmaceutical composition. In certain preloaded syringe embodiments, the syringe is adapted for subcutaneous administration. In certain embodiments, the syringe is suitable for self-administration. In particular embodiments, the preloaded syringe is a single-use syringe.

[0131] In various embodiments, the preloaded syringe contains about 0.1 mL to about 0.5 mL of the pharmaceutical composition. In certain embodiments, the syringe contains about 0.5 mL of the pharmaceutical composition. In specific embodiments, the syringe contains about 1.0 mL of the pharmaceutical composition. In particular embodiments, the syringe contains about 2.0 mL of the pharmaceutical composition.

[0132] In certain embodiments, the unit dosage form is an auto-inject pen. The auto-inject pen comprises an auto-inject pen containing a pharmaceutical composition as described herein. In

some embodiments, the auto-inject pen delivers a predetermined volume of pharmaceutical composition. In other embodiments, the auto-inject pen is configured to deliver a volume of pharmaceutical composition set by the user.

[0133] In various embodiments, the auto-inject pen contains about 0.1 mL to about 5.0 mL of the pharmaceutical composition. In specific embodiments, the auto-inject pen contains about 0.5 mL of the pharmaceutical composition. In particular embodiments, the auto-inject pen contains about 1.0 mL of the pharmaceutical composition. In other embodiments, the auto-inject pen contains about 5.0 mL of the pharmaceutical composition.

6.4.2. Lyophilized DNA formulations

[0134] In some embodiments, DNA constructs of the present inventions are formulated as a lyophilized composition. In specific embodiments, DNA constructs are lyophilized as disclosed in U.S. Patent No. 8,389, 492, incorporated by reference in its entirety herein.

[0135] In some embodiments, DNA constructs are formulated with certain excipients, *e.g.*, a carbohydrate and a salt, prior to lyophilization. Stability of the DNA construct to be utilized as a diagnostic or therapeutic agent can be increased by formulating the DNA construct prior to lyophilization with an aqueous solution comprising a stabilizing amount of carbohydrate.

[0136] The carbohydrate can be a mono-, oligo-, or polysaccharide, such as sucrose, glucose, lactose, trehalose, arabinose, pentose, ribose, xylose, galactose, hexose, idose, mannose, talose, heptose, fructose, gluconic acid, sorbitol, mannitol, methyl α -glucopyranoside, maltose, isoascorbic acid, ascorbic acid, lactone, sorbose, glucaric acid, erythrose, threose, allose, altrose, gulose, erythrulose, ribulose, xylulose, psicose, tagatose, glucuronic acid, galacturonic acid, mannuronic acid, glucosamine, galactosamine, neuraminic acid, arabinans, fructans, fucans, galactans, galacturonans, glucans, mannans, xylans, levan, fucoidan, carrageenan, galactocarolose, pectins, pectic acids, amylose, pullulan, glycogen, amylopectin, cellulose, dextran, cyclodextrin, pustulan, chitin, agarose, keratin, chondroitin, dermatan, hyaluronic acid, alginic acid, xantham gum, or starch.

[0137] In one series of embodiments, the carbohydrate is mannitol or sucrose.

[0138] The carbohydrate solution prior to lyophilization can correspond to carbohydrate in water alone, or a buffer can be included. Examples of such buffers include PBS, HEPES, TRIS or

TRIS/EDTA. Typically the carbohydrate solution is combined with the DNA construct to a final concentration of about 0.05% to about 30% sucrose, typically 0.1% to about 15% sucrose, such as 0.2% to about 5%, 10% or 15% sucrose, preferably between about 0.5% to 10% sucrose, 1% to 5% sucrose, 1% to 3% sucrose, and most preferably about 1.1% sucrose.

[0139] DNA formulation of the invention can also include a salt, *e.g.*, NaCl or KCl. In some embodiments, the salt is NaCl. In some embodiments, the salt of the DNA formulation is in an amount selected from the group consisting of between about 0.001% to about 10%, between about 0.1% and 5%, between about 0.1% and 4%, between about 0.5% and 2%, between about 0.8% and 1.5%, between about 0.8% and 1.2% w/v. In certain embodiments, the salt of the DNA formulation is in an amount of about 0.9% w/v.

[0140] The final concentration of the DNA construct in liquid compositions reconstituted from lyophilized formulations can be from about 1 ng/mL to about 30 mg/mL. For example, the final concentration can be about 1 ng/mL, about 5 ng/mL, about 10 ng/mL, about 50 ng/mL, about 100 ng/mL, about 200 ng/mL, about 500 ng/mL, about 1 µg/mL, about 5 µg/mL, about 10 µg/mL, about 50 µg/mL, about 100 µg/mL, about 200 µg/mL, about 400 µg/mL, about 500 µg/mL, about 600 µg/mL, about 800 µg/mL, about 1 mg/mL, about 2 mg/mL, about 2.5 mg/mL, about 3 mg/mL, about 3.5 mg/mL, about 4 mg/mL, about 4.5 mg/mL, about 5 mg/mL, about 5.5 mg/mL, about 6 mg/mL, about 7 mg/mL, about 8 mg/mL, about 9 mg/mL, about 10 mg/mL, about 20 mg/mL, or about 30 mg/mL. In certain embodiments of the invention, the final concentration of the DNA construct is from about 100 µg/mL to about 2.5 mg/mL. In particular embodiments of the invention, the final concentration of the DNA construct is from about 0.5 mg/mL to 1 mg/mL.

[0141] The DNA formulation of the invention is lyophilized under standard conditions known in the art. A method for lyophilization of the DNA formulation of the invention may comprise (a) loading a container (*e.g.*, a vial), with a DNA formulation (*e.g.*, a DNA formulation comprising a plasmid DNA), a salt and a carbohydrate, where the plasmid DNA comprises an IGF-1 gene, or variant thereof, into a lyophilizer, wherein the lyophilizer has a starting temperature of about 5° C to about -50° C; (b) cooling the DNA formulation to subzero temperatures (*e.g.*, -10° C. to -50° C); and (c) substantially drying the DNA formulation. The conditions for lyophilization, *e.g.*, temperature and duration, of the DNA formulation of the invention can be adjusted by a

person of ordinary skill in the art taking into consideration factors that affect lyophilization parameters, *e.g.*, the type of lyophilization machine used, the amount of DNA used, and the size of the container used.

[0142] The container holding the lyophilized DNA formulation may then be sealed and stored for an extended period of time at various temperatures (*e.g.*, room temperature to about -180°C , preferably about $2-8^{\circ}\text{C}$ to about -80°C , more preferably about -20°C to about -80°C , and most preferably about -20°C). In certain aspects, the lyophilized DNA formulations are preferably stable within a range of from about $2-8^{\circ}\text{C}$ to about -80°C for a period of at least 6 months without losing significant activity. Stable storage plasmid DNA formulation can also correspond to storage of plasmid DNA in a stable form for long periods of time before use as such for research or plasmid-based therapy. Storage time may be as long as several months, 1 year, 5 years, 10 years, 15 years, or up to 20 years. Preferably the preparation is stable for a period of at least about 3 years.

6.5. Methods of treating neuropathy

[0143] In another aspect, methods are presented for treating neuropathy by administering the DNA construct encoding a human IGF-1 isoform.

6.5.1. Administration of a DNA construct encoding an IGF-1 isoform

[0144] The methods comprise administering a therapeutically effective amount of a DNA construct that expresses a human IGF-1 isoform to a subject. The subject can be a human or an animal with neuropathy.

[0145] In some embodiments, the method comprises administration of a DNA construct encoding a single human IGF-1 isoform. In some embodiments, the method comprises administration of a first DNA construct encoding a first human IGF-1 isoform or administration of a second DNA construct encoding a second human IGF-1 isoform. In some embodiments, the method comprises administration of a first DNA construct encoding a first human IGF-1 isoform and administration of a second DNA construct encoding a second human IGF-1 isoform. In some embodiments, the first IGF-1 isoform is Class I, Ec (Isoform #1) and the second IGF-1 isoform is Class I, Ea (Isoform #4). In some embodiments, the first IGF-1 isoform is Class I, Ea

(Isoform #4) and the second IGF-1 isoform is Class I, Ec (Isoform #1). The first and second DNA construct can be administrated together or sequentially.

[0146] In some embodiments, the method comprises administration of a DNA construct encoding more than one human IGF-1 isoforms, dual expression constructs. In some embodiments, the DNA construct can express both Class I, Ec (Isoform #1) and Class I, Ea isoforms (Isoform #4). In some embodiments, the DNA construct further expresses Class I, Eb or Class II, Ea. In some embodiments, the DNA construct cannot express Class I, Eb or Class II, Ea. In some embodiments, the DNA construct can express Class I, Ec (Isoform #1) and Class I, Ea isoforms (Isoform #4), but cannot express any other IGF-1 isoforms.

[0147] In some embodiments, the method comprises administration of a DNA construct encoding human IGF-1, comprising: a first polynucleotide that has the same sequence as exons 1, 3 and 4 of the human IGF-1 gene (SEQ ID NO: 1) or a degenerate thereof; a second polynucleotide that has the same sequence as intron 4 of the human IGF-1 gene (SEQ ID NO: 2) or a fragment thereof; a third polynucleotide that has the same sequence as exons 5 and 6-1 of the human IGF-1 gene (SEQ ID NO: 3) or a degenerate thereof; a fourth polynucleotide that has the same sequence as intron 5 of the human IGF-1 gene (SEQ ID NO: 4) or a fragment thereof; and a fifth polynucleotide that has the same sequence as exon 6-2 of the human IGF-1 gene (SEQ ID NO: 5) or a degenerate thereof, wherein the first polynucleotide, the second polynucleotide, the third polynucleotide, the fourth polynucleotide and the fifth polynucleotide are linked in sequential 5' to 3' order.

[0148] In some embodiments, the method comprises administration of pCK-IGF-1X6 or pCK-IGF-1X10. In some embodiments, the method comprises administration of pTx-IGF-1X6 or pTx-IGF-1X10.

[0149] In some embodiments, the method comprises administration of more than one of the DNA constructs provided herein.

6.5.1.1. Delivery methods

[0150] Various delivery methods can be used to administer the DNA construct expressing one or more isoforms of IGF-1.

6.5.1.1.1. Injection

[0151] In some embodiments, the DNA construct is administered by injection of a liquid pharmaceutical composition.

[0152] In currently preferred embodiments, the DNA construct is administered by intramuscular injection. Typically, the DNA construct is administered by intramuscular injection close to the site of nerve damage, site of pain or patient-perceived site of pain, or site of other symptom of the neuropathic disease. In some embodiments, the DNA constructs are administered to the muscles of hands, feet, legs, or arms of the subject.

[0153] In some embodiments, the construct is injected subcutaneously or intradermally.

[0154] In some embodiments, the DNA construct is administered by intravascular delivery. In certain embodiments, the construct is injected by retrograde intravenous injection.

6.5.1.1.2. Electroporation

[0155] Transformation efficiency of plasmid DNA into cells *in vivo* can in some instances be improved by performing injection followed by electroporation. Thus, in some embodiments, the DNA construct is administered by injection followed by electroporation. In particular embodiments, electroporation is administered using the TriGrid™ Delivery System (Ichor Medical Systems, Inc., San Diego, USA).

6.5.1.1.3. Sonoporation

[0156] In some embodiments, sonoporation is used to enhance transformation efficiency of the DNA construct of the present invention. Sonoporation utilizes ultrasound wave to temporarily permeabilize the cell membrane to allow cellular uptake of DNA. DNA constructs can be incorporated within microbubbles and administered into systemic circulation, followed by external application of ultrasound. The ultrasound induces cavitation of the microbubble within the target tissue to result in release and transfection of the constructs.

6.5.1.1.4. Magnetofection

[0157] In some embodiments, magnetofection is used to enhance transformation efficiency of a DNA construct of the present invention. The construct is administered after being coupled to a magnetic nanoparticle. Application of high gradient external magnets cause the complex to be

captured and held at the target. The DNA construct can be released by enzymatic cleavage of cross linking molecule, charge interaction or degradation of the matrix.

6.5.1.1.5. Liposome

[0158] In some embodiments, DNA constructs of the present invention can be delivered by liposomes. Liposomes are formed spontaneously when phospholipids are suspended in an excess of aqueous medium. Liposome-mediated DNA delivery has been successful as described in Dos Santos Rodrigues *et al.*, *Int. J. Pharm.* 566:717-730 (2019); Rasoulianboroujeni *et al.*, *Mater Sci Eng C Mater Biol Appl.* 75:191-197 (2017); Xiong *et al.*, *Pharmazie* 66(3):158-164 (2011); Nicolau and Sene, *Biochim. Biophys. Acta*, 721:185-190(1982) and Nicolau *et al.*, *Methods Enzymol.*, 149:157-176 (1987). Example of commercially accessible reagents for transfecting animal cells using liposomes includes Lipofectamine (Gibco BRL). Liposomes entrapping DNA constructs of the invention interact with cells by mechanism such as endocytosis, adsorption and fusion and then transfer the sequences into cells.

6.5.1.1.6. Transfection

[0159] When a viral vector is used to deliver a DNA construct encoding IGF-1, the construct may be delivered into cells by various viral infection methods known in the art. Infection of host cells using viral vectors are described in the above-mentioned cited documents.

[0160] Preferably, the pharmaceutical composition of this invention may be administered parenterally. For non-oral administration, intravenous injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, or local injection may be employed. For example, the pharmaceutical composition may be injected by retrograde intravenous injection.

[0161] Preferably, the pharmaceutical composition of the present invention may be administered into the muscle. In some embodiments, the administration is targeted to the muscle affected by neuropathy (*e.g.*, neuropathic pain or other symptoms).

6.5.1.2. Dose

[0162] The DNA construct can be administered in a therapeutically effective dose. In the methods described herein, the therapeutically effective dose is a dose effective to treat neuropathy in the subject.

[0163] In some embodiments of the methods described herein, the DNA construct is administered at a total dose of 1 µg to 200mg, 1mg to 200mg, 1mg to 100mg, 1mg to 50mg, 1mg to 20mg, 5mg to 10mg, 16 mg, 8 mg, or 4 mg.

[0164] In typical embodiments, the total dose is divided into a plurality of individual injection doses. In some embodiments, the total dose is divided into a plurality of equal injection doses. In some embodiments, the total dose is divided into unequal injection doses.

[0165] In various divided dose embodiments, the total dose is administered to 4, 8, 16, 24, 32 or 64 different injection sites.

[0166] In some embodiments, the dose per injection is between 0.1 and 20 mg, between 1 and 10 mg, between 2 and 8 mg, or between 3 and 8 mg. In certain embodiments, the dose per injection is 0.1 mg, 0.15 mg, 0.2 mg, 0.25 mg, 0.3 mg, 0.35 mg, 0.4 mg, 0.45 mg, 0.5 mg, 1 mg, 2 mg, 4 mg, 8 mg, 16 mg, or 32 mg.

[0167] The total dose can be administered during one visit or over two or more visits.

[0168] In typical divided dose embodiments, all of the plurality of injection doses are administered within 1 hour of one another. In some embodiments, all of the plurality of injection doses are administered within 1.5, 2, 2.5 or 3 hours of one another.

[0169] In various embodiments of the methods, a total dose of DNA construct, whether administered as a single unitary dose or divided into plurality of injection doses, is administered only once to the subject.

[0170] In some embodiments, administration of a total dose of DNA construct into a plurality of injection sites over one, two, three or four visits can comprise a single cycle. In particular, administration of 64 mg, 32 mg, 16 mg, 8 mg, or 4 mg of DNA construct into a plurality of injection sites over two visits can comprise a single cycle. The two visits can be 3, 5, 7, 14, 21 or 28 days apart.

[0171] In some embodiments, the cycle can be repeated. The cycle can be repeated twice, three times, four times, five times, six times, or more.

[0172] In some embodiments, the cycle can be repeated 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more months after the previous cycle.

[0173] In some embodiments, the total dose administered in the subsequent cycle is same as the total dose administered in the prior cycle. In some embodiments, the total dose administered in the subsequent cycle is different from the total dose administered in the prior cycle.

[0174] In currently preferred embodiments, the DNA construct is administered at a dose of 8 mg per affected limb, equally divided into a plurality of intramuscular injections and plurality of visits, wherein each of the plurality of injections in any single visit is performed at a separate injection site. In certain embodiments, the DNA construct is administered at a dose of 8 mg per affected limb, equally divided into a first dose of 4 mg per limb on day 0 and a second dose of 4 mg per limb on day 14, wherein each of the first and second dose is equally divided into a plurality of injection doses. In some embodiments, the administration of the DNA construct at a dose of 8 mg per affect limb constitutes one cycle. The cycle can be repeated once, twice, three times or more.

[0175] The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of neuropathy being treated. In typical embodiments, the DNA construct is administered in an amount effective to reduce symptoms of neuropathy, for example, neuropathic pain. In some embodiments, the amount is effective to reduce the symptom of neuropathy within 1 week of administration. In some embodiments, the amount is effective to reduce the symptom of neuropathy within 2 weeks, 3 weeks, or 4 weeks of administration.

[0176] In some embodiments, two different types of constructs are administered together to induce expression of two isoforms of IGF-1, *e.g.*, a first construct encoding Class I, Ec isoform and a second construct encoding Class I, Ea isoform. In some embodiments, a dual expression construct that encodes both Class I, Ec isoform and Class I, Ea isoform is delivered to induce expression of both isoforms.

[0177] According to the conventional techniques known to those skilled in the art, the pharmaceutical composition may be formulated with pharmaceutically acceptable carrier and/or vehicle as described above, finally providing several forms a unit dose form and a multi-dose form. Non-limiting examples of the formulations include, but not limited to, a solution, a suspension or an emulsion in oil or aqueous medium, an extract, an elixir, a powder, a granule, a tablet and a capsule, and may further comprise a dispersion agent or a stabilizer.

6.5.1.3. Variations

[0178] *In vivo* and/or *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the condition, and should be decided according to the judgment of the practitioner and each subject's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[0179] The DNA construct can be administered alone or in combination with other treatments, either simultaneously or sequentially.

6.5.2. Patients with neuropathy

[0180] In the methods described herein, the patients selected for treatment have neuropathy. The patients can have peripheral neuropathy, cranial neuropathy, autonomic neuropathy or focal neuropathy. The neuropathy can be caused by diseases, injuries, infections or vitamin deficiency states. For example, the neuropathy can be caused by diabetes, vitamin deficiencies, autoimmune diseases, genetic or inherited disorders, amyloidosis, uremia, toxins or poisons, trauma or injury, tumors, or can be idiopathic. In some embodiments, the patients have diabetic peripheral neuropathy.

[0181] The patients can have one or more symptoms associated with neuropathy, such as pain (neuropathic pain), other sensory defects (*e.g.*, loss of feeling, numbness, tingling, *etc.*), motor defects (*e.g.*, weakness, loss of reflexes, loss of muscle mass, cramping, loss of dexterity, *etc.*), and autonomic dysfunction (*e.g.*, nausea, vomiting, impotence, dizziness, constipation, diarrhea, *etc.*).

[0182] The patients can be treated by one or more treatment methods known in the art in addition to the treatment method provided herein.

[0183] Treatment methods of the present invention can be used to treat a human patient or an animal with neuropathy.

6.6. Examples

[0184] 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 make and use the present invention, and are

not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations can be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); nt, nucleotide(s); and the like.

[0185] The practice of the present invention will employ, unless otherwise indicated, conventional methods of protein chemistry, biochemistry, recombinant DNA techniques and pharmacology, within the skill of the art.

6.6.1. Example 1: DNA constructs encoding an IGF-1 isoform reduce mechanical allodynia in a murine CCI neuropathy model

[0186] DNA constructs encoding individual human IGF-1 isoforms were constructed in the expression plasmid pCK using standard molecular cloning techniques.

[0187] Specifically, four polynucleotides (SEQ ID NOS: 15, 17, 19 and 21) were obtained by customized DNA synthesis process provides by Bioneer (Korea). These polynucleotides were synthesized with 5' linker, Cla I and 3' linker, Sal I. pCK vector and the polynucleotides were restricted with Cla I and Sal I. Plasmid #1 encoding Class I, Ec (Isoform #1) was generated by inserting a polynucleotide of SEQ ID NO: 17, which is a coding sequence of Class I, Ec isoform and comprises at least a part of exons 1, 3/4, 5 and 6 of IGF-1 gene, into the cloning site in pCK vector. Plasmid #2 encoding Class II, Ea (Isoform #2) was generated by inserting a polynucleotide of SEQ ID NO: 19, which is a coding sequence of Class II, Ea isoform and comprises at least a part of exons 2, 3/4 and 6 of IGF-1 gene, into the cloning site in pCK vector. Plasmid #3 encoding Class I, Eb (Isoform #3) was generated by inserting a polynucleotide of SEQ ID NO: 21, which is a coding sequence of Class I, Eb isoform and comprises at least a part of exons 1, 3/4 and 5 of IGF-1 gene, into the cloning site in pCK vector. Plasmid #4 encoding Class I, Ea (Isoform #4) was generated by inserting a polynucleotide of SEQ ID NO: 15 and comprises at least a part of exons 1, 3/4 and 6, into the cloning site in pCK vector.

[0188] Expression of each IGF-1 isoform from each plasmid was tested and confirmed both *in vitro* and *in vivo*.

[0189] Therapeutic effects of the DNA constructs were then tested in the CCI mice, which is a widely accepted model for studying neuropathy. The CCI mouse model generated by the chronic constriction injury (CCI) of the sciatic nerve which is known to initiate several processes that result in chronic nerve injury in the periphery. The CCI mice are also known to have neuropathic pain. CCI surgery was performed on adult male ICR mice (with 24-26 g of body weight), following surgical and experimental procedures approved by the Institutional Animal Care and Use committee at Seoul National University.

[0190] Specifically, blunt dissections about 1 cm long were made under anesthesia to expose the right sciatic nerve, which usually lies between the *gluteus* and *biceps femoris* muscles. Once the sciatic nerve proximal to the trifurcation site was exposed, it was given loose ligatures three times with 0.5 mm spacing using 6-0 silk (Ethicon) sutures. The ligatures were slightly tightened until there was a noticeable twitch of the right hind limb. Sham-operated mice were given the same dissections in the right thigh, but did not receive ligatures in the sciatic nerve.

[0191] As schematized in FIG. 2A, a total of 200 µg of plasmid DNA was intramuscularly injected on the day of CCI surgery. The injected plasmid DNA was either 200 µg pCK (vector without insert; “pCK”) or 200 µg of pCK-IGF-1 – (i) 200 µg of plasmid#1 (“IGF-1#1” encoding Class I, Ec IGF-1); (ii) 200 µg of plasmid#4 (“IGF-1#4” encoding Class I, Ea IGF-1) or (iii) 100 µg of plasmid#1 (“IGF-1#1” encoding Class I, Ec IGF-1) and 100 µg of plasmid#4 (“IGF-1#4” encoding Class I, Ea IGF-1).

[0192] One week after CCI surgery, the development of mechanical allodynia was assessed using a Von Frey’s filament test. Briefly, animals were placed individually in a cylinder on top of a metal mesh floor for adaptation. The mechanical sensitivity of mice was assessed by stimulating the hind paw using constant thickness of the filament (0.16 g). The test was repeated weekly thereafter.

[0193] **FIG. 2B** is a histogram summarizing the frequency (%) of paw withdrawal measured in the CCI experiment described in **FIG. 2A**. The result demonstrates (i) that injection of IGF-1 #1, expressing IGF-1 Class I Ec provided measurable reduction in paw withdrawal frequency as compared to vector alone (pCK); (ii) that injection of a IGF-1 #4, expressing IGF-1 Class I Ea,

also provided measurable reduction in paw withdrawal frequency; and (iii) that injection of DNA constructs expressing IGF-1 Class I Ec (IGF-1 #1) and Class I Ea (IGF-1 #4) together provided greater, statistically significant, decreases in frequency of paw withdrawal frequency as compared to vector alone (pCK).

[0194] All values are presented as mean $\pm$ standard error mean (SEM) from three independent experiments. Differences between values were determined by one-way ANOVA followed by Tukey's post-hoc test or Bonferroni's multiple comparison test.

6.6.2. Example 2: DNA constructs capable of expressing both IGF-1 Class I Ec and Class I Ea isoforms

[0195] Since a DNA construct expressing IGF-1 Class I Ec (IGF-1 #1) and another DNA construct expressing Class I Ea (IGF-1 #4) together had greater, statistically more significant, effects on mechanical allodynia in the behavioral experiments described above, we constructed several plasmids designed to simultaneously express both the IGF-1 Class I Ec (IGF-1 #1) and Class I Ea (IGF-1 #4) through alternative splicing of the RNA transcript. In particular, DNA constructs were generated to comprise sequences for exons 1, 3/4, 5 and 6 and introns of IGF-1 gene or their fragments. Several DNA constructs including different variations were generated and tested for their capability to express both IGF-1 Class I Ec isoform and Class I Ea isoform.

[0196] Each plasmid was constructed using pCK as the plasmid backbone to contain an insert operably linked to the pCK expression control sequences. The insert was created by concatenating (1) a first polynucleotide encoding human IGF-1 exons 1, 3, and 4 (SEQ ID NO:1); (2) a second polynucleotide, either the IGF-1 intron 4 (SEQ ID NO: 2) or a fragment thereof; (3) a third polynucleotide encoding exons 5 and 6-1 (SEQ ID NO: 3); (4) a fourth polynucleotide, either intron 5 (SEQ ID NO: 4) or a fragment thereof; and (5) a fifth polynucleotide encoding exon 6-2 (of SEQ ID NO: 5), in which the first polynucleotide, the second polynucleotide, the third polynucleotide, the fourth polynucleotide and the fifth polynucleotide were linked in sequential 5' to 3' order. The plasmids differed in the size of the fragment of intron 4 and/or intron 5. Specifically, SEQ ID NO: 6 provides the nucleotide sequence of the intron 4 fragment used in vector pCK-IGF-1X6, and SEQ ID NO: 7 provides the nucleotide sequence of the intron 4 fragment used in vector pCK-IGF-1-X10. SEQ ID NO: 8

provides the nucleotide sequence of the intron 5 fragment used in vector pCK-IGF-1X6 and pCK-IGF-1X10.

[0197] To test expression of Isoform 1 (Class I, Ec) and Isoform 4 (Class I, Ea) from the various constructs *in vivo*, 9 week old male C57BL/6 male mice were injected with 50 µg plasmid in their T.A. (tibialis anterior) muscle. Their T.A. skeletal muscles were obtained 5 days after the injection. The skeletal muscles were then homogenized in a lysis buffer containing protease inhibitor, phosphatase inhibitor cocktail (Roche Diagnostic Ltd.), and PMSF (Sigma) using polypropylene pestles (Bel-Art Scienceware). The samples were centrifuged at 12,000 rpm for 15 minutes at 4°C, and the supernatants containing total protein were subjected to human IGF-1 ELISA (R&D Systems) as set forth in the manufacturer's protocol. The level of IGF-1 detected was normalized to the total amount of protein extracts from the tissue, as measured by BCA protein assay kit (Thermo, IL, USA). The experimental procedure is summarized in FIG. 3A.

[0198] As shown in FIG. 3B, the total expression level of human IGF-1 protein in mouse T.A muscle was determined by ELISA. Regardless of whether the mouse received 50 µg of construct expressing a single isoform ("1" (Class I, Ec) or "4" (Class I, Ea)), 25 µg of a first construct expressing isoform #1 (Class I, Ec) plus 25 µg of a second construct expressing isoform #4 (Class I, Ea) ("1+4"), or 50 µg of either construct expressing both isoforms, pCK-IGF-1X6 ("X6") or pCK-IGF-1X10 ("X10"), the total expression levels of human IGF-1 protein were similar.

[0199] We used RT-PCR to determine whether the constructs expressed mature transcripts for both isoform #1 and isoform #4 simultaneously. RT-PCR reactions were performed with a forward/left primer (L) that binds to exon 3/4 and a reverse/right primer (R) that binds to exon 6. As further explained in FIG. 3C, the RT-PCR of a transcript for Isoform #1 (Class I, Ec) would generate two amplicons – 178 bp amplicon and 259 bp amplicon, whereas the RT-PCR of a transcript for Isoform #4 (Class I, Ea) would generate a single amplicon of 129 bp.

[0200] For RT-PCR, skeletal muscles were collected, mechanically homogenized using polypropylene pestles (Bel-Art Scienceware), and extracted in RNAiso plus (Takara). Quantification of RNA was done by using a nanodrop instrument. Equal amounts of RNA were used to synthesize cDNA using Reverse Transcriptase XL (AMV) (Takara), and PCR was

performed using the forward (TGA TCT AAG GAG GCT GGA (SEQ ID NO: 28)) and reverse (CTA CTT GCG TTC TTC AAA TG (SEQ ID NO: 29)) primers indicated in FIG. 3C.

[0201] As illustrated in FIG. 3D, pCK-IGF-1X6 and pCK-IGF-1X10 expressed mature transcripts for both isoform #1 (178bp and 259bp bands) and isoform #4 (129bp band). Expression of mature transcripts for both isoform #1 and isoform #4 were not detected from constructs other than the pCK-IGF-1X6 and pCK-IGF-1X10, which data are not provided herein.

[0202] In order to confirm that the two isoform transcripts were both effectively translated into protein, we transfected 293T cells with pCK-IGF-1-X6 or pCK-IGF-1-X10, as illustrated in FIG. 4A. For immunoblotting, cells were prepared 2 days (48 hours) after the transfection of plasmid DNA, followed by lysis using RIPA buffer with protease and phosphatase inhibitor cocktail (Roche Diagnostic Ltd.). Equal amounts of protein were separated on 10% SDS-polyacrylamide gel, and transferred to a western membrane (PVDF). The membrane were blocked with 1% BSA (Invitrogen-Gibco) in TBST (20 mM Tris-HCl, pH 7.4, 0.9% NaCl, and 0.1% Tween20) for 1 hour and probed with primary antibodies diluted in blocking solution at 4°C overnight. Primary antibodies used to examine the level of IGF-1 isoform 1 and isoform 4 were provided by Abclon (Korea), and those for IGF-1 and β -actin were purchased from Abcam (UK) and Sigma-Aldrich (US). After washing with TBST, membranes were incubated with HRP-conjugated goat anti-mouse or rabbit IgG secondary antibody (Sigma) at room temperature for 1 hour. The blots were then washed three times with TBST, and the protein bands were visualized with the enhanced chemiluminescence system (Millipore). β -actin was used as a loading control.

[0203] Western blotting data shown in FIG. 4B confirm that pCK-IGF-1X6 and pCK-IGF-1X10 plasmids express both isoforms of IGF-1 at the protein level.

6.6.3. Example 3: DNA constructs that simultaneously express IGF-1 Class I Ec isoform and Class I Ea isoform reduce mechanical allodynia in a murine CCI neuropathy model

[0204] Using the protocol described in Example 1 and schematized in FIG. 5A, we tested the ability of our DNA constructs to reduce mechanical allodynia in a mouse CCI model of neuropathy.

[0205] FIG. 5B is a histogram showing the frequency of paw withdrawal, demonstrating statistically significant reductions in mechanical allodynia after simultaneous intramuscular injection of constructs encoding IGF-1 isoform (IGF-1 #1, IGF-1 #4, IGF-1 #1 + #4, IGF-1X6, and IGF-1X10). In particular, the effects on mechanical allodynia was even better when the mice were administered constructs separately encoding IGF-1 isoforms #1 and #4, or injected with the dual expression construct pCK-IGF-1X10.

6.6.4. Example 4: DNA constructs that simultaneously express IGF-1 Class I Ec isoform and Class I Ea isoform induce axon growth in a mouse nerve crush model

[0206] Therapeutic effects of the DNA constructs were further tested in a nerve crush model. The nerve crush model was generated with adult male C57BL/6 mice (with 24-26 g of body weight) following surgical and experimental procedures approved by the Institutional Animal Care and Use committee at Seoul National University. To induce sciatic nerve crush, the right sciatic nerve of each mice was exposed by an incision and the nerve was crushed with fine hemostatic forceps for 15 seconds. The incision was then sutured using 5-0 black silk suture. Total amounts of 200 µg plasmid DNA (200 µg of pCK, or 200 µg of pCK-IGF-1X10) were intramuscularly administered on the day of nerve crush to the muscles nearby the sciatic nerve.

[0207] Four weeks (28 days) after nerve crush and plasmid DNA injection, mice were fixed by perfusion with 2% paraformaldehyde + 2% glutaraldehyde in 0.1M PBS. Right ipsilateral sciatic nerve was then prepared and further incubated with fixation solution for 4 hours. Fixed tissues were treated with 1% osmium tetroxide in 0.1 M PBS, followed by *en bloc* staining using 2% aqueous uranyl acetate overnight. The next day, tissues were then dehydrated by a serial passage in 30 % to 100 % ethanol for 10 minutes, and dehydrated tissues were then embedded in resin compound. After the embedding process, samples were polymerized with Spurr resin and incubated in dry oven. Samples were then sectioned and visualized using transmission electron microscopy (TEM).

[0208] TEM images of the samples from a Sham animal, pCK-treated animal, and pCK-IGF-1X10 treated animal are provided in FIG. 6A. The images clearly demonstrate that nerve crush animals have significant nerve damages compared to Sham animals, with significantly smaller

axon diameters. The reduction in axon diameters was inhibited by administration of pCK-IGF-1X10, but not by administration of pCK.

[0209] pCK-IGF-1X10's effects on axon growth were quantified by measuring neuron diameters. Distributions of neuron diameters in the nerve crush animal treated with pCK ("Crush-pCK") and a nerve crush animal treated with pCK-IGF-1X10 ("Crush-X10") are provided in the graph of FIG. 6B. The graph clarifies that the animal treated with pCK-IGF-1X10 had neurons having larger diameters. This again confirms that pCK-IGF-1X10 can treat nerve damages by inducing axon growth.

7. INCORPORATION BY REFERENCE

[0210] All publications, patents, patent applications and other documents cited in this application are hereby incorporated by reference in their entireties for all purposes to the same extent as if each individual publication, patent, patent application or other document were individually indicated to be incorporated by reference for all purposes.

8. EQUIVALENTS

[0211] While various specific embodiments have been illustrated and described, the above specification is not restrictive. It will be appreciated that various changes can be made without departing from the spirit and scope of the invention(s). Many variations will become apparent to those skilled in the art upon review of this specification.

WHAT IS CLAIMED IS:

1. An IGF-1 encoding DNA construct, comprising:
 - a first polynucleotide that has the same sequence as exons 1, 3 and 4 of the human IGF-1 gene (SEQ ID NO: 1) or a degenerate thereof;
 - a second polynucleotide that has the same sequence as intron 4 of the human IGF-1 gene (SEQ ID NO: 2) or a fragment thereof;
 - a third polynucleotide that has the same sequence as exons 5 and 6-1 of the human IGF-1 gene (SEQ ID NO: 3) or a degenerate thereof;
 - a fourth polynucleotide that has the same sequence as intron 5 of the human IGF-1 gene (SEQ ID NO: 4) or a fragment thereof; and
 - a fifth polynucleotide that has the same sequence as exon 6-2 of the human IGF-1 gene (SEQ ID NO: 5) or a degenerate thereof;wherein the first polynucleotide, the second polynucleotide, the third polynucleotide, the fourth polynucleotide and the fifth polynucleotide are linked in sequential 5' to 3' order.
2. The IGF-1 encoding DNA construct of claim 1, wherein the second polynucleotide is a polynucleotide of SEQ ID NO: 6.
3. The IGF-1 encoding DNA construct of claim 1, wherein the second polynucleotide is a polynucleotide of SEQ ID NO: 7.
4. The IGF-1 encoding DNA construct of claim 1, wherein the fourth polynucleotide is a polynucleotide of SEQ ID NO: 8.
5. The IGF-1 encoding DNA construct of any of claims 1-4, further comprising a plasmid vector.
6. The IGF-1 encoding DNA construct of claim 5, wherein the plasmid vector is pCK.
7. The IGF-1 encoding DNA construct of claim 6, selected from the group consisting of pCK-IGF-1X6 and pCK-IGF-1X10.

8. The IGF-1 encoding DNA construct of claim 5, wherein the plasmid vector is pTx.
9. The IGF-1 encoding DNA construct of claim 8, selected from the group consisting of pTx-IGF-1X6 and pTx-IGF-1X10.
10. A pharmaceutical composition comprising the IGF-1 encoding DNA construct of any one of claims 1-9.
11. A pharmaceutical composition comprising an IGF-1 encoding DNA construct, wherein the IGF-1 encoding DNA construct encodes Class I IGF-1Ea protein comprising a polypeptide of SEQ ID NO: 14 or Class I IGF-1Ec protein comprising a polypeptide of SEQ ID NO: 16.
12. The pharmaceutical composition of claim 11, wherein the IGF-1 encoding DNA construct encodes both Class I IGF-1Ea protein comprising a polypeptide of SEQ ID NO: 14 and Class I IGF-1Ec protein comprising a polypeptide of SEQ ID NO: 16.
13. The pharmaceutical composition of any of claims 11-12, wherein the IGF-1 encoding DNA construct is not capable of expressing both Class II IGF-1 Ea protein comprising a polypeptide of SEQ ID NO: 18 and Class I IGF-1Eb protein comprising a polypeptide of SEQ ID NO: 20.
14. The pharmaceutical composition of any of claims 11-12, wherein the IGF-1 encoding DNA construct is capable of expressing neither Class II IGF-1 Ea protein comprising a polypeptide of SEQ ID NO: 18 nor Class I IGF-1Eb protein comprising a polypeptide of SEQ ID NO: 20.
15. The pharmaceutical composition of claim 11, comprising:
 - a first DNA construct encoding Class I IGF-1Ea protein comprising a polypeptide of SEQ ID NO: 14, and
 - a second DNA construct encoding Class I IGF-1Ec protein comprising a polypeptide of SEQ ID NO: 16.

16. The pharmaceutical composition of claim 12, wherein the IGF-1 encoding DNA construct is pCK-IGF-1X6 or pCK-IGF-1X10.

17. The pharmaceutical composition of claim 12, wherein the IGF-1 encoding DNA construct is pTx-IGF-1X6 or pTx-IGF-1X10.

18. A method of treating neuropathy, comprising the step of:
administering to a subject having neuropathy an effective amount of a first IGF-1 encoding DNA construct, wherein the first IGF-1 encoding DNA construct is capable of expressing at least one human IGF-1 isoform.

19. The method of claim 18, wherein the first IGF-1 encoding DNA construct is capable of expressing Class I IGF-1Ea protein comprising a polypeptide of SEQ ID NO: 14 or Class I IGF-1Ec protein comprising a polypeptide of SEQ ID NO: 16.

20. The method of any of claims 18-19, wherein the first IGF-1 encoding DNA construct is not capable of expressing both Class II IGF-1 Ea protein comprising a polypeptide of SEQ ID NO: 18 and Class I IGF-1Eb protein comprising a polypeptide of SEQ ID NO: 20

21. The method of any one of claims 18-20, wherein the first IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 15.

22. The method of claim 21, further comprising the step of:
administering to the subject a second IGF-1 encoding DNA construct,
wherein the second IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 17.

23. The method of any of claims 18-20, wherein the first IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 17.

24. The method of claim 23, further comprising the step of:
administering to the subject a second IGF-1 encoding DNA construct,
wherein the second IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 15.

25. The method of claim 22 or claim 24, wherein the step of administering the first IGF-1 encoding DNA construct and the step of administering the second IGF-1 encoding DNA construct are performed concurrently.

26. The method of claim 22 or claim 24, wherein the step of administering the first IGF-1 encoding DNA construct and the step of administering the second IGF-1 encoding DNA construct are performed sequentially.

27. The method of claim 18, wherein the first IGF-1 encoding DNA construct encodes more than one human IGF-1 isoforms.

28. The method of claim 27, wherein the first IGF-1 encoding DNA construct encodes Class I IGF-1Ea protein comprising a polypeptide of SEQ ID NO: 14 and Class I IGF-1 Ec protein comprising a polypeptide of SEQ ID NO: 16.

29. The method of claim 27 or claim 28, wherein the first IGF-1 encoding DNA construct comprises:

a first polynucleotide that has the same sequence as exons 1, 3 and 4 of the human IGF-1 gene (SEQ ID NO: 1) or a degenerate thereof;

a second polynucleotide that has the same sequence as intron 4 of the human IGF-1 gene (SEQ ID NO: 2) or a fragment thereof;

a third polynucleotide that has the same sequence as exons 5 and 6-1 of the human IGF-1 gene (SEQ ID NO: 3) or a degenerate thereof;

a fourth polynucleotide that has the same sequence as intron 5 of the human IGF-1 gene (SEQ ID NO: 4) or a fragment thereof; and

a fifth polynucleotide that has the same sequence as exon 6-2 of the human IGF-1 gene (SEQ ID NO: 5) or a degenerate thereof,

wherein the first polynucleotide, the second polynucleotide, the third polynucleotide, the fourth polynucleotide and the fifth polynucleotide are linked in sequential 5' to 3' order.

30. The method of claim 29, wherein the second polynucleotide is a polynucleotide of SEQ ID NO: 6.

31. The method of claim 29, wherein the second polynucleotide is a polynucleotide of SEQ ID NO: 7.

32. The method of claim 29, wherein the fourth polynucleotide is a polynucleotide of SEQ ID NO: 8.

33. The method of any one of claims 18-32, wherein the first IGF-1 encoding DNA construct comprises a plasmid vector.

34. The method of claim 33, wherein the plasmid vector is pCK.

35. The method of claim 33, wherein the plasmid vector is pTx.

36. The method of any one of claims 29-34, wherein the first IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 10.

37. The method of any one of claims 29-34, wherein the first IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 9

38. The method of any one of claims 29-34, wherein the first IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 27.

39. The method of any one of claims 18-37, wherein the effective amount is an amount sufficient to reduce pain in the subject.

40. The method of any one of claims 18-39, wherein the subject has neuropathic pain.

41. The method of any one of claims 18-40, wherein the subject has diabetic neuropathy.

42. The method of any one of claims 18-41, wherein the step of administering the first IGF-1 encoding DNA construct or the second IGF-1 encoding DNA construct comprises an intramuscular injection.

43. An IGF-1 encoding DNA construct for use in a medical method of treating neuropathy, the medical method comprising the step of administering to a subject having

neuropathy an effective amount of the IGF-1 encoding DNA construct, wherein the IGF-1 encoding DNA construct is capable of expressing at least one human IGF-1 isoform.

44. The IGF-1 encoding DNA construct of claim 43, wherein the IGF-1 encoding DNA construct is capable of expressing Class I IGF-1Ea protein comprising a polypeptide of SEQ ID NO: 14 or Class I IGF-1Ec protein comprising a polypeptide of SEQ ID NO: 16.

45. The IGF-1 encoding DNA construct of any of claims 43-44, wherein the IGF-1 encoding DNA construct is not capable of expressing both Class II IGF-1 Ea protein comprising a polypeptide of SEQ ID NO: 18 and Class I IGF-1Eb protein comprising a polypeptide of SEQ ID NO: 20.

46. The IGF-1 encoding DNA construct of any one of claims 43-45, wherein the IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 15.

47. The IGF-1 encoding DNA construct of claim 46, wherein the medical method further comprises the step of: administering to the subject a second IGF-1 encoding DNA construct, wherein the second IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 17.

48. The IGF-1 encoding DNA construct of any of claims 43-45, wherein the IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 17.

49. The IGF-1 encoding DNA construct of claim 48, wherein the medical method further comprises the step of: administering to the subject a second IGF-1 encoding DNA construct, wherein the second IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 15.

50. The IGF-1 encoding DNA construct of claim 47 or claim 49, wherein the step of administering the IGF-1 encoding DNA construct and the step of administering the second IGF-1 encoding DNA construct are performed concurrently.

51. The IGF-1 encoding DNA construct of claim 47 or claim 49, wherein the step of administering the IGF-1 encoding DNA construct and the step of administering the second IGF-1 encoding DNA construct are performed sequentially.

52. The IGF-1 encoding DNA construct of claim 43, wherein the IGF-1 encoding DNA construct encodes more than one human IGF-1 isoforms.

53. The IGF-1 encoding DNA construct of claim 52, wherein the IGF-1 encoding DNA construct encodes Class I IGF-1Ea protein comprising a polypeptide of SEQ ID NO: 14 and Class I IGF-1 Ec protein comprising a polypeptide of SEQ ID NO: 16.

54. The IGF-1 encoding DNA construct of claim 52 or claim 53, wherein the IGF-1 encoding DNA construct comprises:

a first polynucleotide that has the same sequence as exons 1, 3 and 4 of the human IGF-1 gene (SEQ ID NO: 1) or a degenerate thereof;

a second polynucleotide that has the same sequence as intron 4 of the human IGF-1 gene (SEQ ID NO: 2) or a fragment thereof;

a third polynucleotide that has the same sequence as exons 5 and 6-1 of the human IGF-1 gene (SEQ ID NO: 3) or a degenerate thereof;

a fourth polynucleotide that has the same sequence as intron 5 of the human IGF-1 gene (SEQ ID NO: 4) or a fragment thereof; and

a fifth polynucleotide that has the same sequence as exon 6-2 of the human IGF-1 gene (SEQ ID NO: 5) or a degenerate thereof;

wherein the first polynucleotide, the second polynucleotide, the third polynucleotide, the fourth polynucleotide and the fifth polynucleotide are linked in sequential 5' to 3' order.

55. The IGF-1 encoding DNA construct of claim 54, wherein the second polynucleotide is a polynucleotide of SEQ ID NO: 6.

56. The IGF-1 encoding DNA construct of claim 54, wherein the second polynucleotide is a polynucleotide of SEQ ID NO: 7.

57. The IGF-1 encoding DNA construct of claim 54, wherein the fourth polynucleotide is a polynucleotide of SEQ ID NO: 8.

58. The IGF-1 encoding DNA construct of any one of claims 43-57, wherein the IGF-1 encoding DNA construct comprises a plasmid vector.

59. The IGF-1 encoding DNA construct of claim 58, wherein the plasmid vector is pCK.

60. The IGF-1 encoding DNA construct of claim 58, wherein the plasmid vector is pTx.

61. The IGF-1 encoding DNA construct of any one of claims 43-60, wherein the IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 10.

62. The IGF-1 encoding DNA construct of any one of claims 43-60, wherein the IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 9.

63. The IGF-1 encoding DNA construct of any one of claims 43-60, wherein the IGF-1 encoding DNA construct comprises a polynucleotide of SEQ ID NO: 27

64. The IGF-1 encoding DNA construct of any one of claims 43-63, wherein the effective amount is an amount sufficient to reduce pain in the subject.

65. The IGF-1 encoding DNA construct of any one of claims 43-64, wherein the subject has neuropathic pain.

66. The IGF-1 encoding DNA construct of any one of claims 43-65, wherein the subject has diabetic neuropathy.

67. The IGF-1 encoding DNA construct of claims 43-66, wherein the step of administering the IGF-1 encoding DNA construct or the second IGF-1 encoding DNA construct comprises an intramuscular injection.

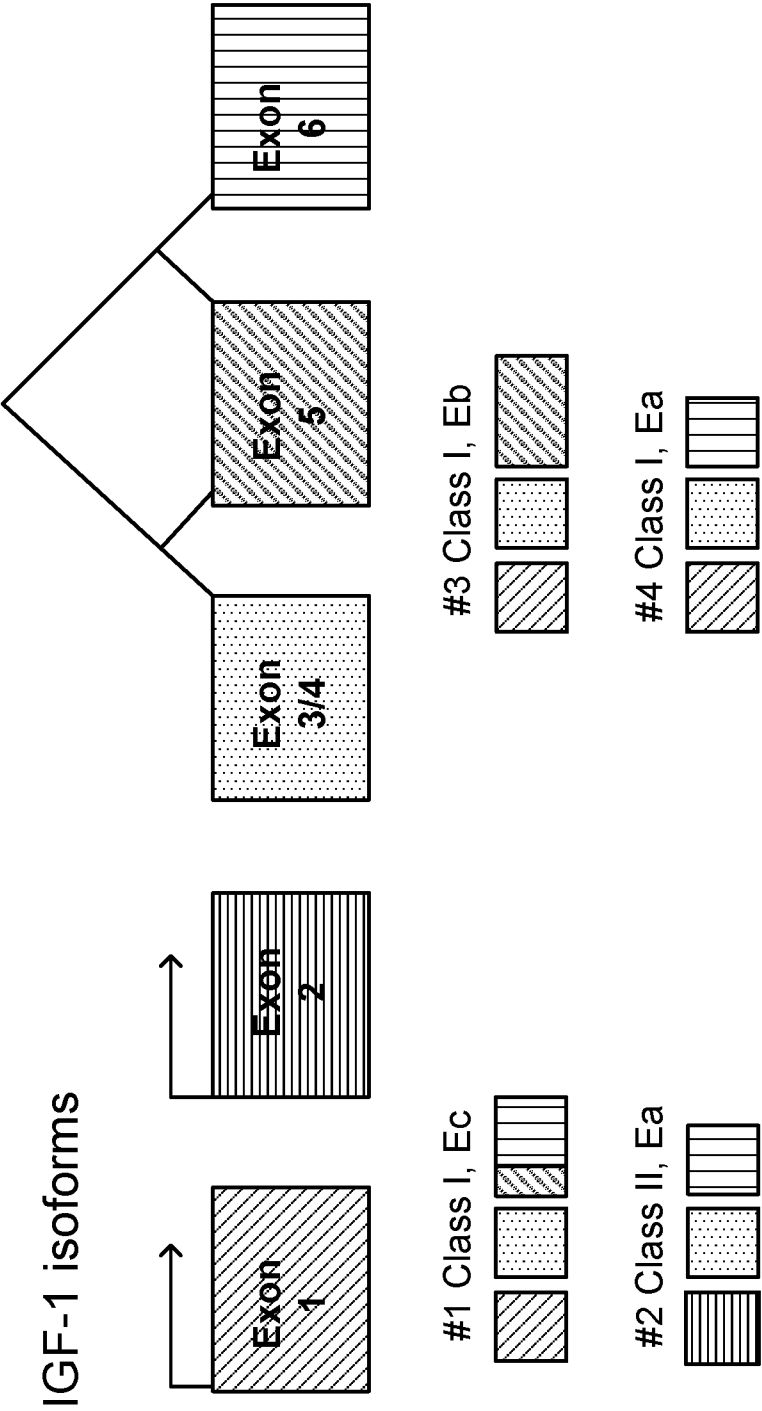


FIG 1

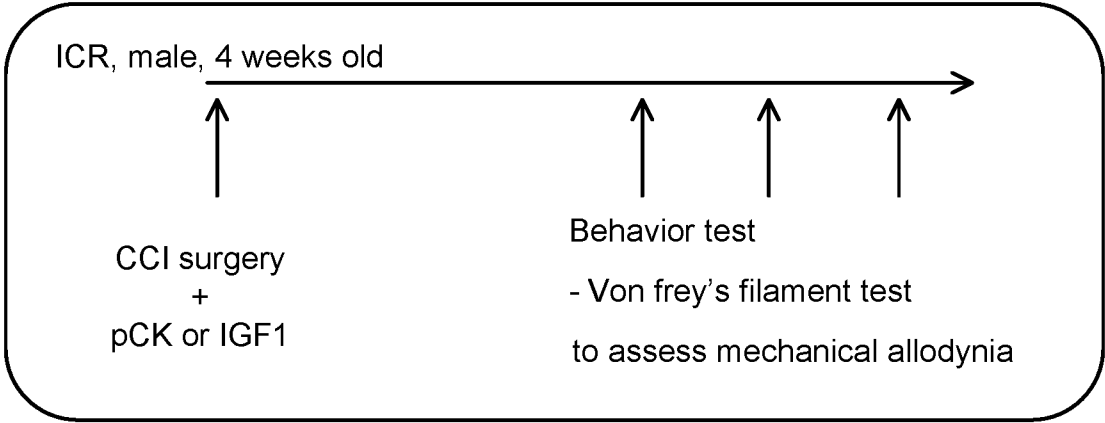


FIG 2A

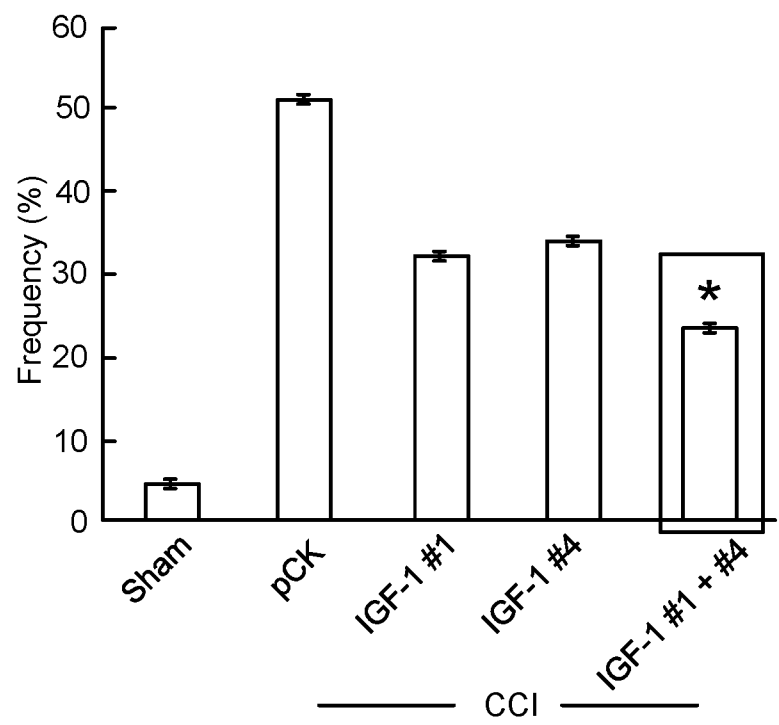


FIG 2B

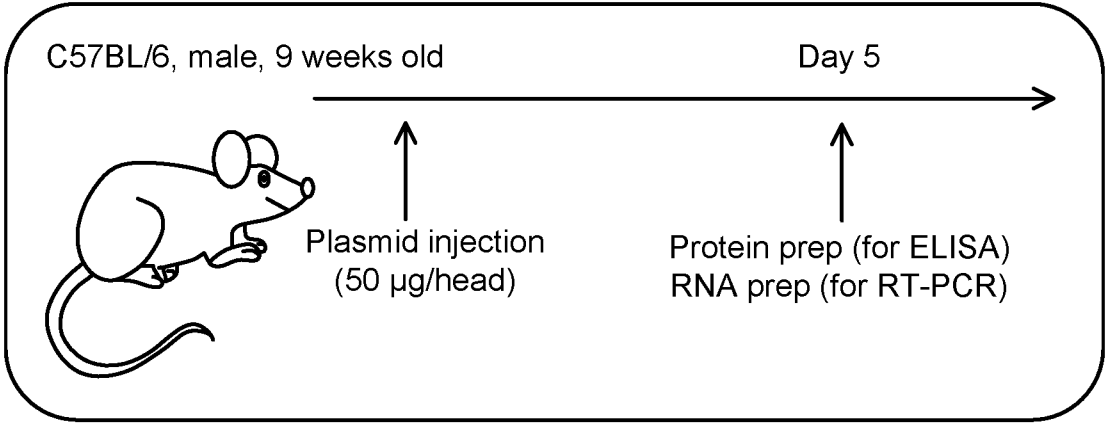


FIG 3A

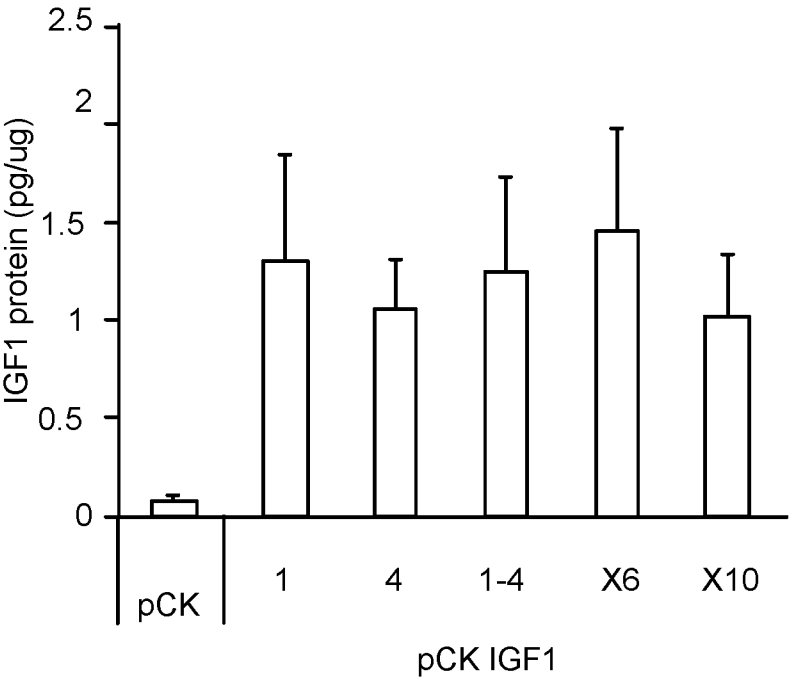


FIG 3B

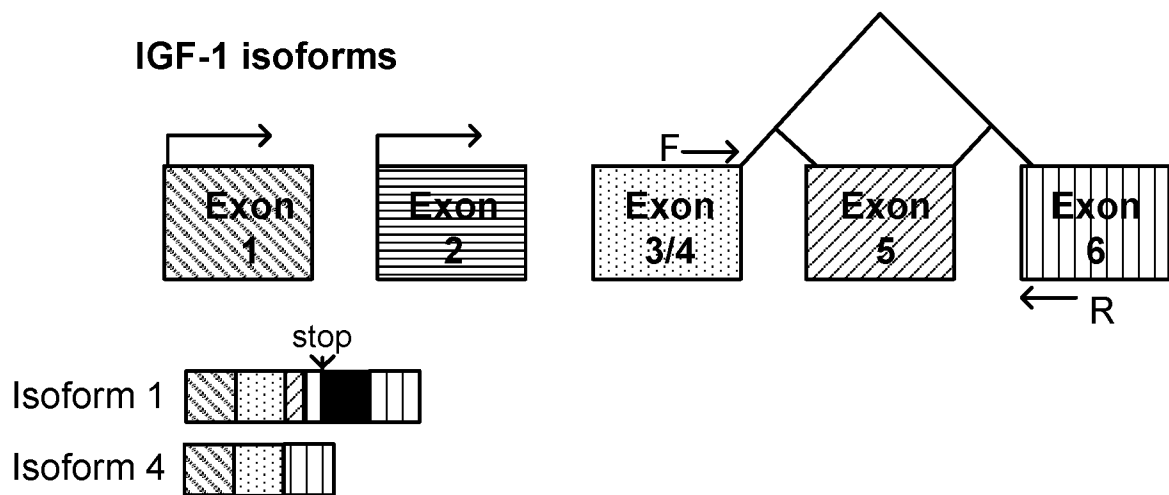
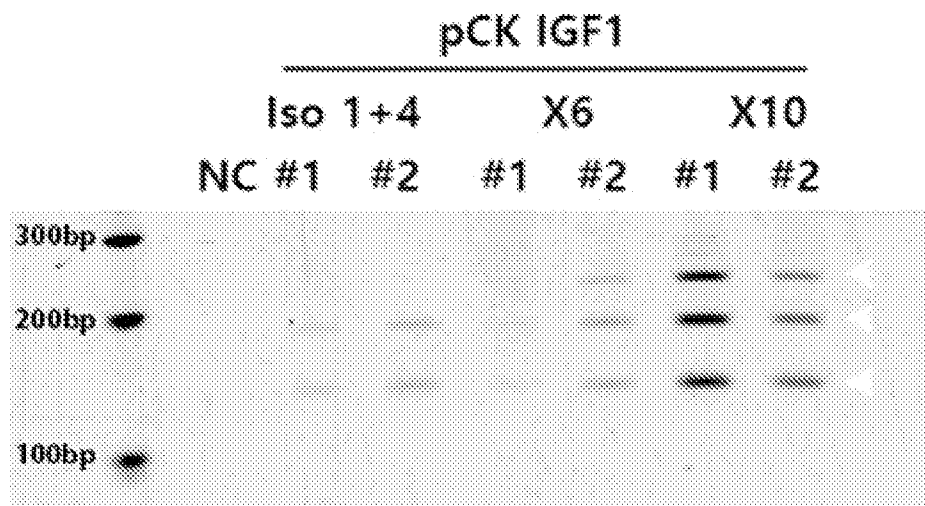


FIG 3C



Amplicon size

- Isoform 1: 178bp, 259bp
- Isoform 4: 129bp

FIG 3D

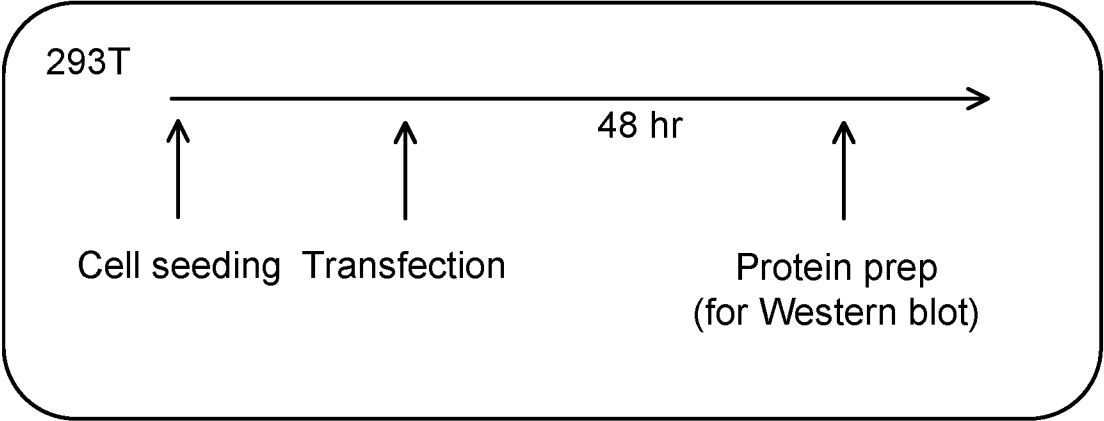


FIG 4A

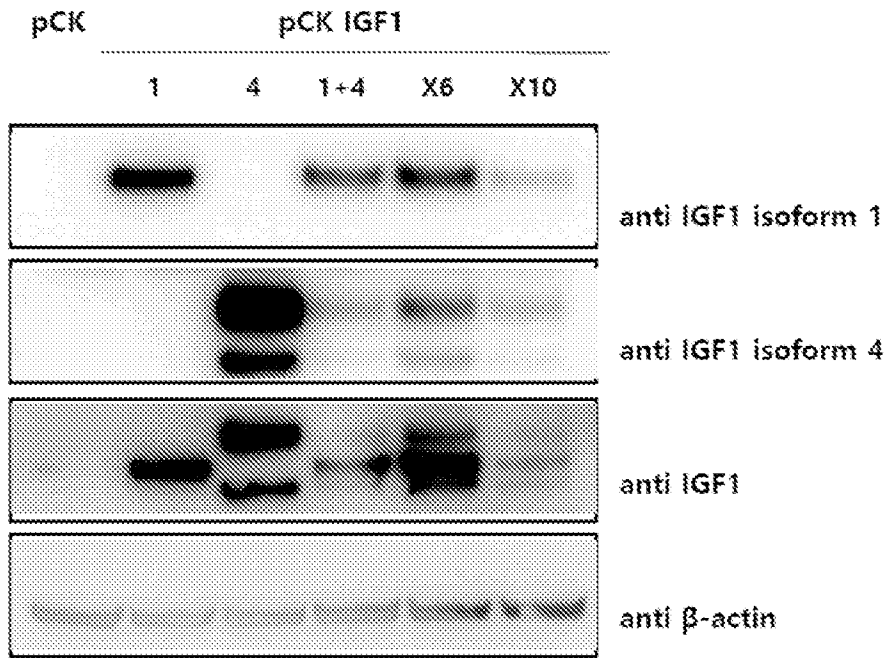


FIG 4B

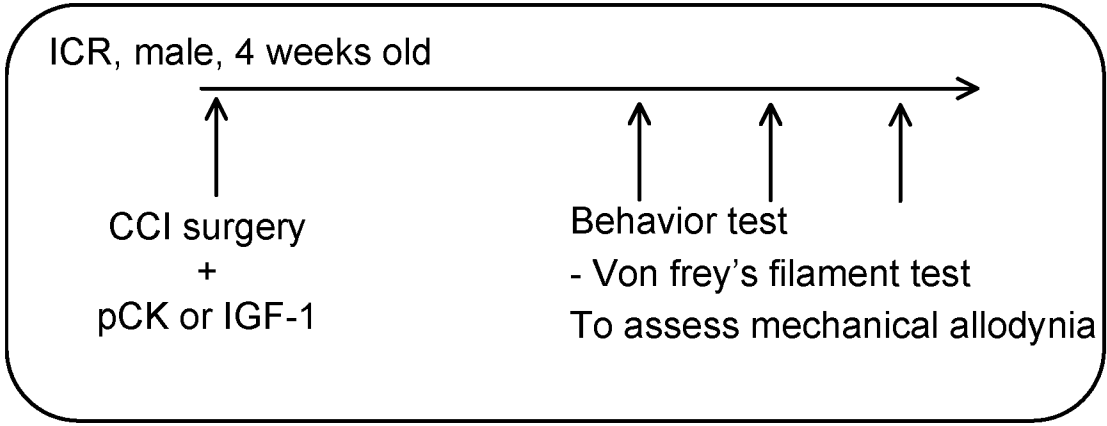


FIG 5A

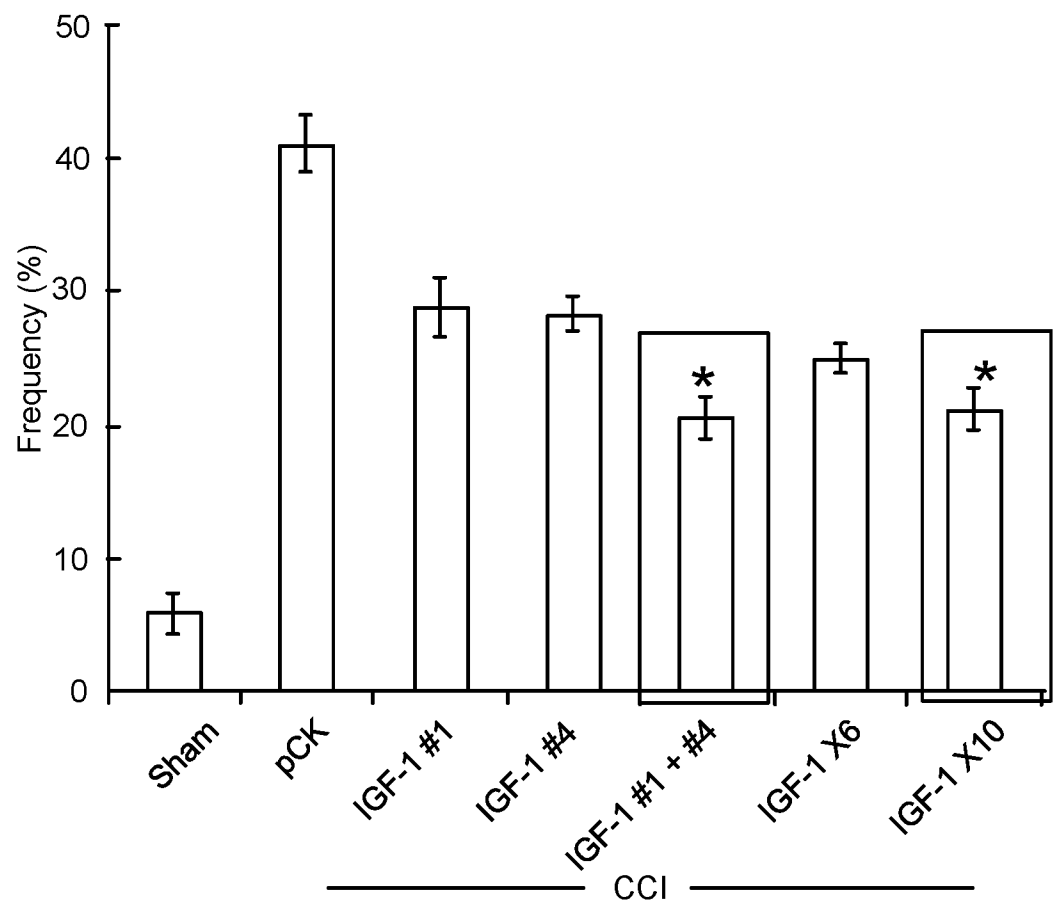


FIG 5B

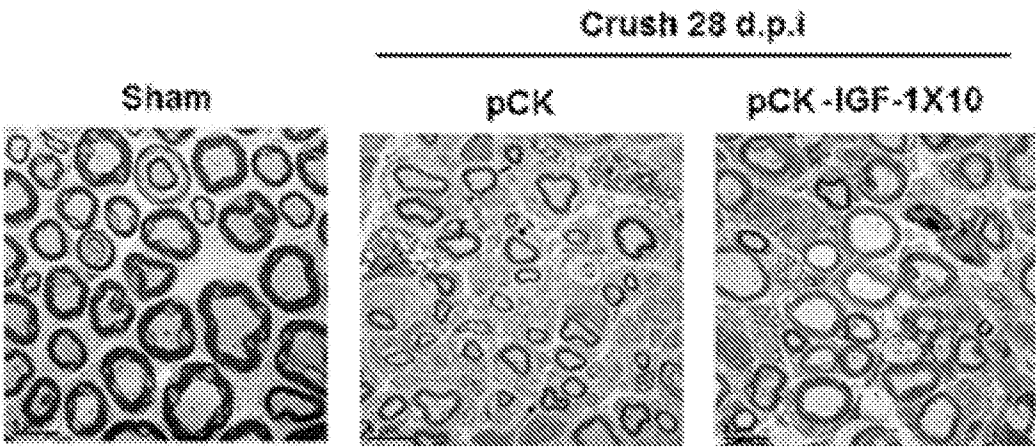


FIG 6A

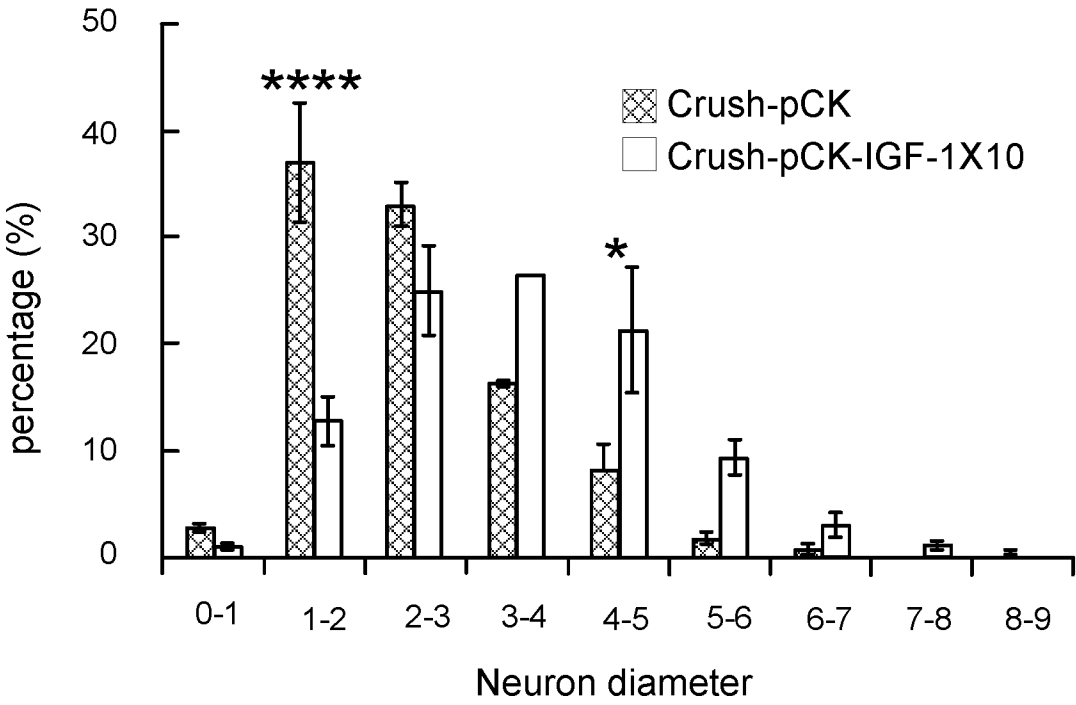


FIG 6B