

VESTIBULAR SUPPORTING CELL PROMOTERS AND USES THEREOF

Sequence Listing

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. The ASCII copy, created on April 28, 2022, is named 51471-010WO2_Sequence_Listing_4_28_22_ST25 and is 41,804 bytes in size.

Background

Vestibular dysfunction is a major public health issue that has profound consequences on quality of life. Approximately 35% of US adults age 40 years and older exhibit balance disorders and this proportion dramatically increases with age, leading to disruption of daily activities, decline in mood and cognition, and an increased prevalence of falls among the elderly. Vestibular dysfunction is often acquired, and has a variety of causes, including disease or infection, head trauma, ototoxic drugs, and aging. A common factor in the etiology of vestibular dysfunction is the damage to vestibular hair cells of the inner ear. Thus, therapies aimed at restoring hair cell function would be beneficial to patients suffering from vestibular dysfunction. Vestibular supporting cells are known to spontaneously differentiate into hair cells following damage and may, therefore, serve as a suitable therapeutic target for restoring hair cell function.

Summary

The disclosure provides compositions and methods for promoting the expression of a gene of interest, such as a gene that promotes or improves hair cell or supporting cell function, regeneration, maturation, proliferation, or survival, in specific cell types. The compositions and methods described herein relate to Solute Carrier Family 6 Member 14 (SLC6A14) promoter sequences that can be used to induce expression of a transgene in vestibular supporting cells (VSCs) of the inner ear. The SLC6A14 promoter sequences described herein may be operably linked to a transgene and may be administered to a patient to treat vestibular dysfunction (e.g., vertigo, dizziness, imbalance, bilateral vestibulopathy, bilateral vestibular hypofunction, oscillopsia, or a balance disorder). The SLC6A14 promoter sequences described herein demonstrate high cell type-specificity by driving high expression of an operably linked transgene in vestibular supporting cells with much lower expression in other inner ear cell types, such as hair cells.

In one aspect, the present disclosure refers to a nucleic acid vector comprising a Solute Carrier Family 6 Member 14 (SLC6A14) promoter having the sequence of SEQ ID NO: 1, wherein the SLC6A14 promoter is operably linked to a transgene that encodes a protein, a short hairpin RNA, an antisense oligonucleotide, a nuclease, or a microRNA.

In a first example, the disclosure provides a nucleic acid vector comprising a polynucleotide having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 1. In some examples, the polynucleotide has at least 90% sequence to SEQ ID NO: 1. In some examples, the polynucleotide has at least 95% sequence to SEQ ID NO: 1. In some examples, the polynucleotide has the sequence of SEQ ID NO: 1.

In some examples, the polynucleotide is operably linked to a transgene. In some examples, the transgene is a heterologous transgene. In some examples, the transgene encodes a protein (e.g., a therapeutic protein or a reporter protein), a short hairpin RNA (shRNA), an antisense oligonucleotide (ASO), a nuclease (e.g., CRISPR Associated Protein 9 (Cas9), Transcription Activator-

identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, sequence identity) to SEQ ID NO: 1.

In another aspect, the disclosure provides a method of expressing a transgene in a mammalian VSC by contacting the mammalian VSC with the nucleic acid vector or composition of any of the foregoing aspects and examples. In one example, the present disclosure refers to use of the nucleic acid vector disclosed herein or the composition disclosed herein in the manufacture of a medicament for expressing a transgene in a mammalian vestibular supporting cell (VSC). In some examples, the transgene is specifically expressed in VSCs (e.g., expressed in a percentage of VSCs that is at least 2-fold, 5-fold, 10-fold, 50-fold, 100-fold, 200-fold, 300-fold, 400-fold, 500-fold or greater than the percentage of one or more other inner ear cells (e.g., hair cells) in which expression is observed). In some examples, the mammalian VSC is a human VSC.

In one aspect, the present disclosure refers to the nucleic acid vector disclosed herein or the composition disclosed herein for use in therapy.

In another example, the disclosure provides a method of treating a subject having or at risk of developing vestibular dysfunction by administering to an inner ear of the subject an effective amount of the nucleic acid vector or composition of any of the foregoing aspects and examples. In one aspect, the present disclosure refers to use of the nucleic acid vector disclosed herein or the composition disclosed herein in the manufacture of a medicament for treating a subject having or at risk of developing vestibular dysfunction. In some examples, the vestibular dysfunction is vertigo, dizziness, imbalance, bilateral vestibulopathy (also known as bilateral vestibular hypofunction), oscillopsia, or a balance disorder. In some examples, the vestibular dysfunction is age-related vestibular dysfunction, head trauma-related vestibular dysfunction, disease or infection-related vestibular dysfunction, or ototoxic drug-induced vestibular dysfunction. In some examples, the vestibular dysfunction is associated with a genetic mutation. In some examples, the vestibular dysfunction is idiopathic vestibular dysfunction.

In another example, the disclosure provides a method of inducing or increasing vestibular hair cell regeneration in a subject in need thereof by administering to an inner ear of the subject an effective amount of the nucleic acid vector or composition of any of the foregoing aspects and examples. In one aspect, the present disclosure refers to use of the nucleic acid vector disclosed herein or the composition disclosed herein in the manufacture of a medicament for inducing or increasing vestibular hair cell regeneration in a subject in need thereof.

In another example, the disclosure provides a method of inducing or increasing VSC proliferation in a subject in need thereof by administering to an inner ear of the subject an effective amount of the nucleic acid vector or composition of any of the foregoing aspects and examples.

In another example, the disclosure provides a method of inducing or increasing vestibular hair cell proliferation in a subject in need thereof by administering to an inner ear of the subject an effective amount of the nucleic acid vector or composition of any of the foregoing aspects and examples.

In another example, the disclosure provides a method of inducing or increasing vestibular hair cell maturation in a subject in need thereof by administering to an inner ear of the subject an effective amount of the nucleic acid vector or composition of any of the foregoing aspects and examples. In some examples, the vestibular hair cell is a regenerated vestibular hair cell.

In another example, the disclosure provides a method of increasing VSC survival in a subject in need thereof by administering to an inner ear of the subject an effective amount of the nucleic acid vector or composition of any of the foregoing aspects and examples.

In another example, the disclosure provides a method of increasing vestibular hair cell survival in a subject in need thereof by administering to an inner ear of the subject an effective amount of the nucleic acid vector or composition of any of the foregoing aspects and examples.

In another example, the disclosure provides a method of inducing or increasing vestibular hair cell innervation in a subject in need thereof by administering to an inner ear of the subject an effective amount of the nucleic acid vector or composition of any of the foregoing aspects and examples.

Claims

1. A nucleic acid vector comprising a Solute Carrier Family 6 Member 14 (SLC6A14) promoter having the sequence of SEQ ID NO: 1, wherein the SLC6A14 promoter is operably linked to a transgene that encodes a protein, a short hairpin RNA, an antisense oligonucleotide, a nuclease, or a microRNA.
2. The nucleic acid vector of claim 1, wherein the transgene is a heterologous transgene.
3. The nucleic acid vector of claim 1, wherein the transgene encodes a protein.
4. The nucleic acid vector of claim 3, wherein the protein is Atonal BHLH Transcription Factor 1 (Atoh1), Spalt Like Transcription Factor 2 (Sall2), Calmodulin Binding Transcription Activator 1 (Camta1), Hes Related Family BHLH Transcription Factor With YRPW Motif 2 (Hey2), Gata Binding Protein 2 (Gata2), Hes Related Family BHLH Transcription Factor With YRPW Motif 1 (Hey1), Ceramide Synthase 2 (Lass2), SRY-Box 10 (Sox10), GATA Binding Protein 3 (Gata3), Cut Like Homeobox 1 (Cux1), Nuclear Receptor Subfamily 2 Group F Member (Nr2f1), Hes Related Family BHLH Transcription Factor (Hes1), RAR Related Orphan Receptor B (Rorb), Jun Proto-Oncogene AP-1 Transcription Factor Subunit (Jun), Zinc Finger Protein 667 (Zfp667), LIM Homeobox 3 (Lhx3), Nescient Helix-Loop-Helix 1 (Nhlh1), MAX Dimerization Protein 4 (Mxd4), Zinc Finger MIZ-Type Containing 1 (Zmiz1), Myelin Transcription Factor 1 (Myt1), Signal Transducer And Activator Of Transcription 3 (Stat3), BarH Like Homeobox 1 (Barhl1), Thymocyte Selection Associated High Mobility Group Box (Tox), Prospero Homeobox 1 (Prox1), Nuclear Factor I A (Nfia), Thyroid Hormone Receptor Beta (Thrb), MYCL Proto-Oncogene BHLH Transcription Factor (Mycl1), Lysine Demethylase 5A (Kdm5a), CAMP Responsive Element Binding Protein 3 Like 4 (Creb3l4), ETS Variant 1 (Etv1), Paternally Expressed 3 (Peg3), BTB Domain And CNC Homolog 2 (Bach2), ISL LIM Homeobox 1 (Isl1), Zinc Finger And BTB Domain Containing 38 (Zbtb38), Limb Bud And Heart Development (Lbh), Tubby Bipartite Transcription Factor (Tub), Ubiquitin C (Hmg20), RE1 Silencing Transcription Factor (Rest), Zinc Finger Protein 827 (Zfp827), AF4/FMR2 Family Member 3 (Aff3), PBX/Knotted 1 Homeobox 2 (Pknx2), AT-Rich Interaction Domain 3B (Arid3b), MLX Interacting Protein (Mlxip), Zinc Finger Protein (Zfp532), IKAROS Family Zinc Finger 2 (Ikzf2), Spalt Like Transcription Factor 1 (Sall1), SIX Homeobox 2 (Six2), Spalt Like Transcription Factor 3 (Sall3), Lin-28 Homolog B (Lin28b), Regulatory Factor X7 (Rfx7), Brain Derived Neurotrophic Factor (Bdnf), Growth Factor Independent 1 Transcriptional Repressor (Gfi1), POU Class 4 Homeobox 3 (Pou4f3), MYC Proto-Oncogene BHLH Transcription Factor (Myc), β -catenin (Ctnnb1), SRY-Box 2 (Sox2), SRY-Box 4 (Sox4), SRY-Box 11 (Sox11), or TEA Domain Transcription Factor 2 (Tead2).
5. The nucleic acid vector of claim 4, wherein the protein is Atoh1.
6. The nucleic acid vector of any one of claims 1-5, wherein the nucleic acid vector additionally comprises a first inverted terminal repeat 5' of the SLC6A14 promoter; and, 3' of the transgene and in 5' to 3' order, an optional posttranscriptional regulatory element, a polyadenylation signal, and a second inverted terminal repeat.

7. The nucleic acid vector of any one of claims 1-6, wherein the nucleic acid vector is a plasmid.
8. The nucleic acid vector of any one of claims 1-6, wherein the nucleic acid vector is an adeno-associated virus (AAV) vector.
9. The nucleic acid vector of claim 8, wherein the AAV vector has an AAV8 capsid.
10. A pharmaceutical composition comprising the nucleic acid vector of any one of claims 1-9 and a pharmaceutically acceptable carrier, diluent, or excipient.
11. A method of expressing a transgene in a mammalian vestibular supporting cell (VSC), the method comprising contacting the VSC with the nucleic acid vector of any one of claims 1-9 or the composition of claim 10.
12. The method of claim 11, wherein the mammalian VSC is a human VSC.
13. Use of the nucleic acid vector of any one of claims 1-9 or the composition of claim 10 in the manufacture of a medicament for treating a subject having or at risk of developing vestibular dysfunction.
14. Use of the nucleic acid vector of any one of claims 1-9 or the composition of claim 10 in the manufacture of a medicament for inducing or increasing vestibular hair cell regeneration in a subject in need thereof.
15. Use of the nucleic acid vector of any one of claims 1-9 or the composition of claim 10 in the manufacture of a medicament for treating a subject having or at risk of developing bilateral vestibulopathy.
16. The nucleic acid vector of any one of claims 1-9 or the composition of claim 10 for use in therapy.