

[0021] FIGS. 14A and 14B are graphs showing *MBD3L2* mRNA expression normalized to POLR2A (by qRT-PCR) (FIG. 14A) and apoptosis as measured by cleaved caspase-3 (FIG. 14B) determined in nine FSHD1 and three FSHD2 patient myotubes (listed in Table 2, FIG. 14B contains only two FSHD2 cell lines) following treatment with FTX-1821, FTX-839, or DMSO vehicle control.

[0022] FIG. 15 is a graph showing the time course of plasma exposure, trapezius muscle exposure and p38 target engagement (Phosphorylated p38 α :Total p38 α Ratio) in the rat following oral administration of 0.3 mg/kg FTX-1821.

[0023] FIG. 16 is a graph showing *MBD3L2* mRNA levels in A4 and C6 xenografted TA muscles.

[0024] FIG. 17 is a graph showing phosphor/total MC2 ratio in mouse trapezius muscles following treatment with vehicle control or p38 inhibitor, FTX-2865.

[0025] FIG. 18 is a graph showing *MBD3L2* mRNA levels in C6 xenografted TA muscles following treatment with vehicle control or p38 inhibitor, FTX-2865.

Summary

[0026] The present disclosure provides methods of reducing the expression a DUX4-fl mRNA, a DUX4 polypeptide, or a polypeptide encoded by a downstream target gene of DUX4, in cells, comprising contacting the cells with an agent that results in a reduction of active p38 protein in the cell, thereby reducing expression the DUX4 polypeptide or the polypeptide encoded by the downstream target gene of DUX4. These methods may be practiced using a variety of different types of agents, and for modulating a variety of different biological processes in the cell, as well as for treating subjects for diseases associated with aberrant DUX4 expression, such as FSHD.

[0026a] In one aspect, the present disclosure refers to use of an agent in the manufacture of a medicament for reducing the expression of a DUX4-fl mRNA, a DUX4 polypeptide, or a polypeptide encoded by a downstream target gene of DUX4, in a cell, wherein the agent results in a reduction of active p38 protein in the cell, thereby reducing expression of the DUX4 polypeptide or the polypeptide encoded by the downstream target gene of DUX4.

[0027] In certain examples of any of the methods disclosed herein, the cell is a muscle cell, optionally a terminally differentiated muscle cell. In some examples, the cell has an increased expression level of the DUX4-fl mRNA, the DUX4 polypeptide, or the polypeptide encoded by the downstream target gene, as compared to the expression level of the DUX4-fl mRNA, the DUX4 polypeptide, or the polypeptide encoded by the downstream target gene, in a control cell, e.g., a cell obtained from a healthy subject. In some examples, the increased expression level of the DUX4-fl mRNA, the DUX4 polypeptide, or the polypeptide encoded by the downstream target gene, is due to reduced repression at a D4Z4 locus in the cell. In certain examples, the cell is associated with facioscapulohumeral muscular

dystrophy (FSHD), e.g., it was obtained from a subject diagnosed with FSHD or is present within a subject diagnosed with FSHD. In some examples, the cell comprises a deletion of one or more macrosatellite D4Z4 repeats in the subtelomeric region of chromosome 4q35, optionally wherein the cell comprises ≤ 7 macrosatellite D4Z4 repeats in the subtelomeric region of chromosome 4q35. In some examples, the cell comprises one or more mutations in a Structural Maintenance Of Chromosomes Flexible Hinge Domain Containing 1 (SMCHD1) gene. In some examples, the cell comprises at least one non-deleted 4qA allele.

[0028] In certain examples of the methods disclosed herein, the agent inhibits the expression or activity, or reduces the amount, of the p38 protein, wherein the activity is optionally kinase activity.

[0029] In some examples, the agent inhibits the expression of the p38 protein. In particular examples, the agent binds a polynucleotide encoding the p38 protein, or binds an antisense polynucleotide thereof. In particular examples, the agent comprises or consists of a nucleic acid, optionally a DNA, RNA, gRNA, shRNA, siRNA, or antisense oligonucleotide.

[0030] In some examples, the agent inhibits the activity of the p38 protein. In particular examples, the agent binds the p38 protein. In particular examples, the agent comprises or consists of a polypeptide, optionally a protein, a peptide, a protein mimetic, a peptidomimetic, or an antibody or functional fragment thereof. In some examples, the agent comprises a small molecule, optionally a small organic molecule or a small inorganic molecule.

[0031] In certain examples of any of the methods disclosed herein, the downstream target gene is RFPL2, CCNA1, SLC34A2, TPRX1, KHDC1L, ZSCAN4, PRAMEF20, TRIM49, PRAMEF4, PRAME6, PRAMEF15 or ZNF280A.

[0032] In particular examples of any of the methods disclosed herein, the expression or the activity of the p38 protein, or the amount of the p38 protein, is reduced by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%.

[0033] In a related example, the present disclosure provides a method of treating or preventing a disease or disorder associated with increased expression of a DUX4-fl mRNA, a DUX4 protein, or a polypeptide encoded by a downstream target gene of DUX4, in a subject in need thereof, comprising providing to the subject a pharmaceutical composition comprising an agent that results in a reduction in the amount of active p38 protein in one or

more tissue of the subject, thereby reducing expression of the DUX4-fl mRNA, the DUX4 protein, or the polypeptide encoding the downstream target gene in one or more tissue of the subject. In some examples, the disease or disorder is a facioscapulohumeral muscular dystrophy (FSHD), optionally FSHD1 or FSHD2. In certain examples, the subject comprises reduced repression at a D4Z4 locus. In some examples, the subject comprises a deletion of one or more macrosatellite D4Z4 repeats in the subtelomeric region of chromosome 4q35, optionally wherein the cell comprises ≤ 7 macrosatellite D4Z4 repeats in the subtelomeric region of chromosome 4q35. In some examples, the subject comprises one or more mutations in a Structural Maintenance Of Chromosomes Flexible Hinge Domain Containing 1 (SMCHD1) gene. In some examples, the subject comprises at least one non-deleted 4qA allele. In certain examples, the expression or the activity of, or the amount of, the p38 protein is reduced by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% in a muscle tissue of the subject. In some examples, the method decreases muscle degeneration in the subject. In some examples, the method reduces apoptosis of muscle cells in the subject. In some examples, the muscle tissue is terminally differentiated. In particular examples, the pharmaceutical composition is provided to the subject parenterally or orally. In certain examples, the pharmaceutical composition is provided to a muscle tissue of the subject, optionally parenterally or intramuscularly. In particular examples, the method further comprises providing to the subject a second agent or therapy for treating the disease or disorder associated with increased expression of a DUX4 protein, or a polypeptide encoded by a downstream target gene of DUX4.

[0033a] In another aspect, the present disclosure refers to use of a pharmaceutical composition comprising an agent in the manufacture of a medicament for treating or preventing a disease or disorder associated with increased expression of a DUX4-fl mRNA, a DUX4 protein, or a polypeptide encoded by a downstream target gene of DUX4, in a subject in need thereof, wherein the agent results in a reduced amount of active p38 protein in one or more tissue of the subject, thereby reducing expression of the DUX4-fl mRNA, the DUX4 protein, or the polypeptide encoding the downstream target gene in one or more tissue of the subject.

[0034] The present disclosure also provides a unit dosage form of a pharmaceutical composition comprising an agent that results in a reduction in the amount of active p38 protein in a cell, and a pharmaceutically acceptable carrier, diluent or excipient, wherein the unit dosage form is effective to reduce expression or activity of a DUX4-fl mRNA, a DUX4 polypeptide, or a polypeptide encoded by a downstream target gene of DUX4, in one or more cell or tissue in a subject to whom the unit dosage form is administered. In particular examples, the agent binds the DUX4 polypeptide or binds a polynucleotide encoding the DUX4 polypeptide. In some examples, the agent comprises or consists of a nucleic acid, optionally a DNA, RNA, gRNA, shRNA, siRNA, or antisense oligonucleotide. In some examples, the agent comprises or consists of a polypeptide, optionally a protein, a peptide, a protein mimetic, a peptidomimetic, or an antibody or functional fragment thereof. In some examples, the agent comprises a small molecule, optionally an organic molecule

or an inorganic molecule. In certain examples, the tissue is muscle tissue, optionally wherein the tissue comprises cells comprising a mutation associated with facioscapulohumeral muscular dystrophy (FSHD).

[0034a] In another aspect, the present disclosure refers to a unit dosage form of a pharmaceutical composition comprising an agent that results in a reduced amount of active p38 protein in a cell, and a pharmaceutically acceptable carrier, diluent or excipient, wherein the unit dosage form is effective to reduce expression or activity of a DUX4-fl mRNA, a DUX4 polypeptide, or a polypeptide encoded by a downstream target gene of DUX4, in one or more cell or tissue in a subject to whom the unit dosage form is administered.

[0035] In a further related example, the present disclosure provides a method of reducing apoptosis of a cell, e.g., a muscle cell, comprising contacting the cell with an agent that results in a reduction in the amount of active p38 protein in the cell, optionally wherein the muscle cell is terminally differentiated, thereby reducing expression of a DUX4-fl mRNA, a DUX4 protein, or a polypeptide encoded by a downstream target gene of DUX4, in the cell. In some examples, the cell has an increased expression level of the DUX4-fl mRNA, the DUX4 polypeptide, or the polypeptide encoded by the downstream target gene, as compared to the expression level of the DUX4 polypeptide, or the polypeptide encoded by the downstream target gene, in a control cell. In some examples, the increased expression level of the DUX4-fl mRNA, the DUX4 polypeptide, or the polypeptide encoded by the downstream target gene, is due to reduced repression at a D4Z4 locus in the cell. In particular examples, the cell comprises one or more mutations associated with FSHD. In certain examples, the agent inhibits the expression of the p38 protein, optionally wherein the agent binds a polynucleotide encoding the p38 protein, or an antisense polynucleotide thereof. For instance, in some examples, the agent comprises or consists of a nucleic acid, optionally a DNA, RNA, shRNA, siRNA, or antisense oligonucleotide, e.g., which targets p38. In some examples, the agent inhibits the activity of the p38 protein, optionally wherein the agent binds the p38 protein. In some examples, the agent comprises or consists of a polypeptide, optionally a protein, a peptide, a protein mimetic, a peptidomimetic, or an antibody or functional fragment thereof. In some examples, the agent comprises a small molecule, optionally a small organic molecule or a small inorganic molecule. In particular examples, the expression or the activity of the p38 protein, the DUX4 protein, or the polypeptide encoded by the DUX4 downstream gene is reduced by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%. In particular examples, the method reduces apoptosis of muscle cells in a muscle tissue at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% as compared to a control, e.g., an untreated cell.

[0035a] In another aspect, the present disclosure refers to use of an agent in the manufacture of a medicament for reducing apoptosis of a muscle cell, wherein the agent results in a reduced amount of active p38 protein in the cell, optionally wherein the muscle cell is terminally differentiated, thereby reducing expression of a DUX4-fl mRNA, a DUX4 protein, or a polypeptide encoded by a downstream target gene of DUX4, in the cell.

[0036] In certain examples of any of the methods disclosed herein, the agent reduces expression of DUX4 or the downstream target gene. In certain examples, the agent binds

a p38 protein, e.g., p38- α or p38- β , or binds a polynucleotide encoding the p38 protein, e.g., p38- α or p38- β , or an antisense polynucleotide thereof. In particular examples, the agent comprises or consists of: a nucleic acid, optionally a DNA, RNA, shRNA, siRNA, CRISPR gRNA, or antisense oligonucleotide. In particular examples, the agent comprises or consists of: a polypeptide, optionally a protein, a peptide, a protein mimetic, a peptidomimetic, or an antibody or functional fragment thereof. In particular examples, the agent comprises: a small molecule, optionally an organic molecule or an inorganic molecule. In some examples, the downstream target gene is RFPL2, CCNA1, SLC34A2, TPRX1, KHDC1L, ZSCAN4, PRAMEF20, TRIM49, PRAMEF4, PRAME6, PRAMEF15 or ZNF280A. In particular examples, the downstream target gene is MBD3L2, ZSCAN4, LEUTX, PRAMEF2, TRIM43, or KHDC1L.

[0037] In certain examples of any of the methods disclosed herein, the agent binds a p38 protein, e.g., p38- α or p38- β , or binds a polynucleotide encoding a p38 protein e.g., p38- α or p38- β . In some examples, the agent comprises or consists of: a nucleic acid, optionally a DNA, RNA, shRNA, siRNA, mRNA, CRISPR gRNA, modified mRNA, morpholino, or antisense oligonucleotide. In some examples, the mRNA or modified mRNA encodes an antibody or a functional fragment thereof. In some examples, the agent comprises or consists of: a polypeptide, optionally a protein, a peptide, a protein mimetic, a peptidomimetic, or an antibody or functional fragment thereof. In some examples, the agent comprises or consists of a gene therapy vector, e.g., a viral vector comprising a nucleic acid sequence encoding a polynucleotide or polypeptide inhibitor of p38, e.g., p38- α or p38- β , or other target. In some examples, the agent comprises or consists of a small molecule, optionally an organic molecule or an inorganic molecule. In some examples, the downstream target is RFPL2, CCNA1, SLC34A2, TPRX1, KHDC1L, ZSCAN4, PRAMEF20, TRIM49, PRAMEF4, PRAME6, PRAMEF15 or ZNF280A. In some examples, the downstream target gene is MBD3L2, ZSCAN4, LEUTX, PRAMEF2, TRIM43, or KHDC1L. In some examples, the downstream target gene is CCNA1.

Detailed Description of the Invention

[0038] The present invention is based, in part, on the discovery that inhibition of p38 kinase, e.g., p38- α , results in reduced expression of DUX4 and downstream genes regulated by DUX4. Accordingly, the invention includes methods and compositions related to using an inhibitor of p38, e.g., p38- α , (alone or in combination with another agent) to reduce the

Claims

1. Use of an agent in the manufacture of a medicament for reducing the expression of a DUX4-fl mRNA, a DUX4 polypeptide, or a polypeptide encoded by a downstream target gene of DUX4, in a cell, wherein the agent results in a reduction of active p38 protein in the cell, thereby reducing expression of the DUX4 polypeptide or the polypeptide encoded by the downstream target gene of DUX4.

2. The use of claim 1, wherein the agent inhibits the expression or activity, or reduces the amount, of the p38 protein, wherein the activity is optionally kinase activity; and/or

wherein the cell is a muscle cell, optionally a terminally differentiated muscle cell.

3. Use of an agent in the manufacture of a medicament for reducing apoptosis of a muscle cell, wherein the agent results in a reduced amount of active p38 protein in the cell, optionally wherein the muscle cell is terminally differentiated, thereby reducing expression of a DUX4-fl mRNA, a DUX4 protein, or a polypeptide encoded by a downstream target gene of DUX4, in the cell.

4. The use of claim 3, wherein the medicament reduces apoptosis of muscle cells in a muscle tissue at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%.

5. The use of any one of claims 1-4, wherein the cell:

(a) has an increased expression level of the DUX4-fl mRNA, the DUX4 polypeptide, or the polypeptide encoded by the downstream target gene, as compared to the expression level of the DUX4-fl mRNA, the DUX4 polypeptide, or the polypeptide encoded by the downstream target gene, in a control cell;

optionally wherein the increased expression level of the DUX4-fl mRNA, the DUX4 polypeptide, or the polypeptide encoded by the downstream target gene, is due to reduced repression at a D4Z4 locus in the cell;

(b) is associated with facioscapulohumeral muscular dystrophy (FSHD);

optionally wherein the cell comprises a deletion of one or more macrosatellite D4Z4 repeats in the subtelomeric region of chromosome 4q35, optionally wherein the cell comprises ≤ 7 macrosatellite D4Z4 repeats in the subtelomeric region of chromosome 4q35; and/or

(c) comprises one or more mutations in a Structural Maintenance Of Chromosomes Flexible Hinge Domain Containing 1 (SMCHD1) gene;

optionally wherein the cell comprises at least one non-deleted 4qA allele.

6. The use of any of claims 1-5, wherein the expression or the activity of the p38 protein is reduced by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%.

7. Use of a pharmaceutical composition comprising an agent in the manufacture of a medicament for treating or preventing a disease or disorder associated with increased expression of a DUX4-fl mRNA, a DUX4 protein, or a polypeptide encoded by a downstream target gene of DUX4, in a subject in need thereof, wherein the agent results in a reduced amount of active p38 protein in one or more tissue of the subject, thereby reducing expression of the DUX4-fl mRNA, the DUX4 protein, or the polypeptide encoding the downstream target gene in one or more tissue of the subject.

8. The use of claim 7, wherein the disease or disorder is a facioscapulohumeral muscular dystrophy (FSHD), optionally FSHD1 or FSHD2.

9. The use of claim 7 or claim 8, wherein the subject:

(a) comprises reduced repression at a D4Z4 locus;

(b) comprises a deletion of one or more macrosatellite D4Z4 repeats in the subtelomeric region of chromosome 4q35, optionally wherein the cell comprises ≤ 7 macrosatellite D4Z4 repeats in the subtelomeric region of chromosome 4q35; and/or

(c) comprises one or more mutations in a Structural Maintenance Of Chromosomes Flexible Hinge Domain Containing 1 (SMCHD1) gene,

optionally wherein the subject comprises at least one non-deleted 4qA allele.

10. The use of any of claims 7-9, wherein the expression or the activity of the p38 protein is reduced by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% in a muscle tissue of the subject.

11. The use of any of claims 7-10, wherein the medicament:

(a) inhibits the expression or activity of the p38 protein in a muscle tissue of the subject;

(b) decreases muscle degeneration in the subject; and/or

(c) reduces apoptosis of muscle cells in the subject,

optionally wherein the muscle tissue is terminally differentiated.

12. The use of any of claims 7-11, wherein the medicament is to be provided to the subject parenterally, or

wherein the medicament is to be provided to a muscle tissue of the subject.

13. The use of any of claims 7-12, wherein a second agent for treating the disease or disorder associated with increased expression of a DUX4 protein, or a polypeptide encoded by a downstream target gene of DUX4 is to be further provided to the subject.

14. The use of any of claims 1-13, wherein the agent:

(a) inhibits the expression of the p38 protein; optionally wherein the agent binds a polynucleotide encoding the p38 protein, or an antisense polynucleotide thereof; and/or

(b) comprises or consists of a nucleic acid, optionally a DNA, RNA, gRNA, shRNA, siRNA, or antisense oligonucleotide.

15. The use of any of claims 1-13, wherein the agent inhibits the activity of the p38 protein; optionally wherein the agent binds the p38 protein.

16. The use of any of claims 1-13 and 15, wherein the agent:

(a) comprises or consists of a polypeptide, optionally a protein, a peptide, a protein mimetic, a peptidomimetic, or an antibody or functional fragment thereof; or

(b) comprises a small molecule, optionally a small organic molecule or a small inorganic molecule.

17. The use of any of claims 1-16, wherein the downstream target gene is RFPL2, CCNA1, SLC34A2, TPRX1, KHDC1L, ZSCAN4, PRAMEF20, TRIM49, PRAMEF4, PRAME6, PRAMEF15 or ZNF280A.

18. A unit dosage form of a pharmaceutical composition comprising an agent that results in a reduced amount of active p38 protein in a cell, and a pharmaceutically acceptable carrier, diluent or excipient, wherein the unit dosage form is effective to reduce expression or activity of a DUX4-fl mRNA, a DUX4 polypeptide, or a polypeptide encoded by a downstream target gene of DUX4, in one or more cell or tissue in a subject to whom the unit dosage form is administered.

19. The unit dosage form of claim 18, wherein the agent binds the DUX4 polypeptide or binds a polynucleotide encoding the DUX4 polypeptide;

optionally wherein the agent:

(a) comprises or consists of a nucleic acid, optionally a DNA, RNA, gRNA, shRNA, siRNA, or antisense oligonucleotide;

(b) comprises or consists of a polypeptide, optionally a protein, a peptide, a protein mimetic, a peptidomimetic, or an antibody or functional fragment thereof; or

(c) comprises a small molecule, optionally an organic molecule or an inorganic molecule.

20. The unit dosage form of any of claims 18-19, wherein the downstream target gene is RFPL2, CCNA1, SLC34A2, TPRX1, KHDC1L, ZSCAN4, PRAMEF20, TRIM49, PRAMEF4, PRAME6, PRAMEF15 or ZNF280A;

and/or wherein the tissue is muscle tissue, optionally wherein the tissue comprises cells comprising a mutation associated with facioscapulohumeral muscular dystrophy (FSHD).