

COMPOSITIONS AND METHODS FOR MODULATING UTRN EXPRESSION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit under 35 U.S.C. §119(e) of U.S. Provisional Application No. 61/647,886, entitled "COMPOSITIONS AND METHODS FOR MODULATING UTRN EXPRESSION", filed May 16, 2012, which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] The invention relates to oligonucleotide based compositions, as well as methods of using oligonucleotide based compositions for treating disease.

BACKGROUND OF THE INVENTION

[0003] Muscular dystrophy (MD) is a group of inherited diseases characterized by damage to muscle fibers and includes Duchenne's muscular dystrophy, Becker's muscular dystrophy, and myotonic dystrophy. Duchenne's muscular dystrophy (DMD) is a recessive X-linked form of muscular dystrophy resulting from mutation in the dystrophin gene. Dystrophin is a structural component of muscle tissue that stabilized the dystroglycan complex and is important for connecting the cytoskeleton of muscle fibers to the basal lamina. When dystrophin is mutated, excess calcium penetrates the cell membrane of muscle cells, causing mitochondrial damage from an influx of water. This mitochondrial damage results in increased oxidative stress and cell death, leading to necrosis of muscle fibers. The destruction of muscle fibers causes the symptoms of DMD, including gradual muscular degeneration, gait ataxia, difficulty breathing, and eventually, death. Becker's Muscular dystrophy (BMD) is a less severe form of muscular dystrophy characterized by progressive muscle weakness in the legs and pelvis. BMD is also caused by a mutation in dystrophin, but unlike DMD, some functional dystrophin is present. Myotonic dystrophy is an autosomal dominant disease characterized by wasting of the muscles, cataracts, heart conduction defects, endocrine changes, and myotonia. Utrophin, encoded by the UTRN gene, is a component of the cytoskeleton located at the neuromuscular synapse and myotendinous junctions, and is involved in membrane maintenance and acetylcholine receptor clustering. Utrophin has homology with dystrophin, especially in the actin binding domain, and can partially compensate for a lack of dystrophin in mice.

SUMMARY OF THE INVENTION

[0004] Aspects of the invention disclosed herein provide methods and compositions that are useful for overexpression of Utrophin (UTRN) for the treatment and/or prevention of diseases associated with reduced expression of UTRN, or for which enhanced expression of UTRN would be beneficial (e.g., muscular dystrophies, including Duchenne muscular dystrophy (DMD), Becker Muscular Dystrophy (BMD), and myotonic dystrophy). In certain aspects, the invention provides methods and compositions that are useful for upregulating UTRN in a cell. In some embodiments, the methods and compositions are useful for the treatment and/or prevention (e.g., reducing the risk or delaying the onset) of muscular dystrophies, including DMD, BMD, and myotonic dystrophy. In some embodiments, single stranded oligonucleotides

are provided that target a PRC2-associated region of a UTRN gene (e.g., human UTRN) and thereby cause upregulation of the gene. In some embodiments, single stranded oligonucleotides are provided that target a PRC2-associated region of the gene encoding UTRN. In some embodiments, these single stranded oligonucleotides activate or enhance expression of UTRN by relieving or preventing PRC2 mediated repression of UTRN.

[0005] Further aspects of the invention provide methods for selecting oligonucleotides for activating or enhancing expression of UTRN. In some embodiments, methods are provided for selecting a set of oligonucleotides that is enriched in candidates (e.g., compared with a random selection of oligonucleotides) for activating or enhancing expression of UTRN. Accordingly, the methods may be used to establish sets of clinical candidates that are enriched in oligonucleotides that activate or enhance expression of UTRN. Such libraries may be utilized, for example, to identify lead oligonucleotides for developing therapeutics to treat UTRN. Furthermore, in some embodiments, oligonucleotide chemistries are provided that are useful for controlling the pharmacokinetics, biodistribution, bioavailability and/or efficacy of the single stranded oligonucleotides for activating expression of UTRN.

[0006] According to some aspects of the invention single stranded oligonucleotides are provided that have a region of complementarity that is complementary with (e.g., at least 8 consecutive nucleotides of) a PRC2-associated region of a UTRN gene, e.g., a PRC2-associated region of the nucleotide sequence set forth as SEQ ID NO: 1 or 2. In some embodiments, the oligonucleotide has at least one of the following features: a) a sequence that is 5'X-Y-Z, in which X is any nucleotide and in which X is at the 5' end of the oligonucleotide, Y is a nucleotide sequence of 6 nucleotides in length that is not a human seed sequence of a microRNA, and Z is a nucleotide sequence of 1 to 23 nucleotides in length; b) a sequence that does not comprise three or more consecutive guanosine nucleotides; c) a sequence that has less than a threshold level of sequence identity with every sequence of nucleotides, of equivalent length to the second nucleotide sequence, that are between 50 kilobases upstream of a 5'-end of an off-target gene and 50 kilobases downstream of a 3'-end of the off-target gene; d) a sequence that is complementary to a PRC2-associated region that encodes an RNA that forms a secondary structure comprising at least two single stranded loops; and e) a sequence that has greater than 60% G-C content. In some embodiments, the single stranded oligonucleotide has at least two of features a), b), c), d), and e), each independently selected. In some embodiments, the single stranded oligonucleotide has at least three of features a), b), c), d), and e), each independently selected. In some embodiments, the single stranded oligonucleotide has at least four of features a), b), c), d), and e), each independently selected. In some embodiments, the single stranded oligonucleotide has each of features a), b), c), d), and e). In certain embodiments, the oligonucleotide has the sequence 5'X-Y-Z, in which the oligonucleotide is 8-50 nucleotides in length.

[0007] According to some aspects of the invention, single stranded oligonucleotides are provided that have a sequence X-Y-Z, in which X is any nucleotide, Y is a nucleotide sequence of 6 nucleotides in length that is not a seed sequence of a human microRNA, and Z is a nucleotide sequence of 1 to 23 nucleotides in length, in which the single stranded oligonucleotide is complementary with a PRC2-associated region