OLIGONUCLEOTIDES FOR INDUCING PATERNAL UBE3A EXPRESSION

FIELD OF INVENTION

The present invention relates to oligonucleotides (oligomers) that are complementary to and hybridize to SNHG14 downstream of SNORD109B, leading to induction of paternal expression of Ubiquitin-protein ligase E3A (UBE3A) in an animal or human. The present invention further relates to pharmaceutical compositions and methods for treatment of Angelman syndrome.

BACKGROUND

Angelman syndrome is neuro-genetic disorder caused by deletion or inactivation of the UBE3A genes on the maternally inherited chromosome 15q11.2. The paternal copy of the UBE3A gene is subject to genomic imprinting and silencing in neurons by an endogenous antisense transcript of UBE3A, termed SNHG14 (also known as UBE3A-ATS) (Meng et al. 2012 Hum Mol Genet. Vol. 21 pp. 3001-12). Other cell types than neurons seem to express the UBE3A gene from both the maternal and paternal allele.

Angelman syndrome is characterized by severe intellectual and developmental disability, sleep disturbance, seizures, jerky movements, EEG abnormalities, frequent laughter or smiling, and profound language impairments.

WO 2012/064806 discloses a method of inducing UBE3A expression in a cell by using a topoisomerase inhibitor. The method can be used to treat Angelman syndrome. There is no disclosure of antisense oligonucleotides.

WO 2014/004572 discloses oligonucleotides with 2'-O-methoxyethyl-RNA (MOE) modifications targeting mouse UBE3A-ATS. The oligonucleotides are only tested in mice related assays. In the region downstream of MBII-52 snoRNA (also known as SNORD115) and upstream of the UBE3A pre-mRNA there is no conservation between mouse and human.Oligonucleotides targeting mouse UBE3A-ATS can therefore not be translated into oligonucleotides that will function in a human. There is no disclosure of oligonucleotides targeting human UBE3A-ATS.

OBJECTIVE OF THE INVENTION

The present invention identifies novel oligonucleotides which induce human paternal UBE3A expression in neuronal without affection expression of the paternal SNORD115, SNORD116 and SNRPN transcripts significantly.

SUMMARY

The present disclosure relates to oligonucleotides targeting a nucleic acid capable of supressing the expression of UBE3A and to treat or prevent diseases related to decreased activity of UBE3A, in particular in neuronal cells.

Accordingly, the disclosure provides oligonucleotides which comprise a contiguous nucleotide sequence of 10 to 30 nucleotides in length with at least 98% complementarity to the part of human SNHG14 long non-coding RNA corresponding to position 25278410 to 25419462 on human chromosome 15 version GRCh38.p2. This region is also resembled by SEQ ID NO: 1. The oligonucleotide can be an antisense oligonucleotide, preferably with a gapmer design. Preferably, the oligonucleotide is capable of inducing the expression of UBE3A, in particular paternal UBE3A expression in a neuron, by degradation, reduction or removal of the UBE3A suppressor, in particular by reduction of the SNHG14 long non-coding RNA transcript downstream of SNORD109B. The UBE3A re-expression is achieved, without significantly affecting the expression of SNORD115. The degradation of the target nucleic acid is preferably achieved via nuclease recruitment.

In one aspect, the disclosure provides an antisense oligonucleotide which comprises a contiguous nucleotide sequence of 10 to 30 nucleotides in length with at least 98% complementarity to position 30654-30745 of SEQ ID NO: 1 (Region A 693 in Table 1) or position 11482-11535 of SEQ ID NO: 1 (Region A321 in Table 1).

In another aspect, the disclosure provides a conjugate comprising the oligonucleotide as defined herein, and at least one conjugate moiety covalently attached to the oligonucleotide.

The disclosure provides pharmaceutical compositions comprising the oligonucleotides of the disclosure and pharmaceutically acceptable diluents, carriers, salts and/or adjuvants.

In another aspect, the disclosure provides a pharmaceutical composition comprising the oligonucleotide as defined herein or the conjugate as defined herein and a pharmaceutically acceptable diluent, solvent, carrier, salt and/or adjuvant.

The disclosure provides methods for *in vivo* or *in vitro* induction of UBE3A expression in a target cell where expression of paternal UBE3A is suppressed, by administering an oligonucleotide or composition of the disclosure in an effective amount to said cell.

In another aspect, the disclosure provides an *in vitro* method for inducing UBE3A expression in a target cell where expression of paternal UBE3A is suppressed, said method comprising administering an oligonucleotide as defined herein or the conjugate as defined herein or the pharmaceutical composition as defined herein in an effective amount to the cell.

The disclosure provides methods for treating or preventing a disease, disorder or dysfunction associated with *in vivo* activity of UBE3A comprising administering a therapeutically or prophylactically effective amount of the oligonucleotide of the disclosure to a subject suffering from or susceptible to the disease, disorder or dysfunction.

The oligonucleotide or composition of the disclosure is used for the treatment or prevention of Angelman syndrome.

In another aspect, the disclosure provides use of the oligonucleotide as defined herein or the conjugate as defined herein or the pharmaceutical composition as defined herein, for the preparation of a medicament for treatment or prevention of Angelman syndrome.

BRIEF DESCRIPTION OF FIGURES

Figure 1: The upper strand illustrates the region of the SNHG14 transcript downstream of SNORD109B (UBE3A-ATS) where the black boxes indicate the location of the tested mouse oligonucleotides. The lower strand illustrates the UBE3A coding region, where the black boxes indicate exons. Exon 1 is located around 160kb. The oligonucleotides are placed in the antisense region of Exon 9 (positioned at ~97kb), Exon 10 (positioned at ~92kb), Exon 13 (positioned at ~77kb) and the 5' end of Exon 16 (positioned at ~60kb).

Figure 2: Representation of the ability of the oligonucleotides, tested in Example 2, to induce re-expression of UBE3A in human neuronal cell cultures. Oligonucleotides complementary to the region of human SNHG14 long non-coding RNA between SNORD109B and the region upstream of the UBE3A coding region (position 1 to 55318 of SEQ ID NO: 1) are indicated with • nonoverlap. Oligonucleotides complementary to the region of human SNHG14 long non-