COMPOSITIONS AND METHODS FOR INHIBITING EXPRESSION OF TMPRSS6 GENE

Cross-Reference to Related Applications

This application of United States provisional application 61/468,830, filed March 29, 2011, and United States provisional application 61/568,942, filed December 9, 2011. These prior applications are incorporated by reference herein in their entirety.

Field of the Invention

The invention relates to the specific inhibition of the expression of the TMPRSS6 gene.

Background of the Invention

TMPRSS6 (Transmembrane Protease, Serine 6) encodes a type II serine protease and is expressed mainly in the liver. TMPRSS6 influences iron levels in the liver by binding and proteolytically degrading the hepcidin activator and BMP co-receptor HJV (hemojuvelin), which causes down-regulation of hepcidin levels.

TMPRSS6 consists of a short N-terminal intracytoplasmic tail, a type II transmembrane domain, a stem region composed of two extracellular CUB (complement factor C1s/C1r, urchin embryonic growth factor and BMP (bone morphogenetic protein)) domains, three LDLR (low-density-lipoprotein receptor class A) domains, and a C-terminal trypsin-like serine protease domain. There are also consensus sites for N-glycosylation in the extracellular domain, and a potential phosphorylation site in the intracytoplasmic tail region.

Summary of the Invention

Described herein are compositions and methods that effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of the TMPRSS6 gene, such as in a cell or mammal. Also described are compositions and methods for treating pathological conditions and diseases caused by the expression of a TMPRSS6 gene, such as a disorder characterized by iron overabundance (*e.g.*, thalassemia, *e.g.*, a β-thalassemia intermedia or an α-thalassemia). Also described are compositions and methods for

decreasing or preventing iron absorption or mobilization, thereby ameliorating iron overload in certain pathological conditions. The methods and compositions described herein are generally useful for the treatment of hemochromatosis (iron build-up in the body).

In a first aspect, there is provided a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of TMPRSS6, wherein said dsRNA comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity to a TMPRSS6 transcript which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the antisense sequence of SEQ ID NO: 420.

In a second aspect, there is provided a cell containing the dsRNA as defined above.

In a third aspect, there is provided a pharmaceutical composition for inhibiting expression of a TMPRSS6 gene comprising the dsRNA as defined above.

In a fourth aspect, there is provided an *in vitro* method of inhibiting TMPRSS6 expression in a cell, the method comprising: (a) introducing into the cell the dsRNA as defined above; and (b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a TMPRSS6 gene, thereby inhibiting expression of the TMPRSS6 gene in the cell.

In a fifth aspect, there is provided the dsRNA as defined above or a pharmaceutical composition as defined above for use in treating a disorder mediated by TMPRSS6 expression.

In a sixth aspect, there is provided a vector encoding at least one strand of dsRNA, wherein said dsRNA comprises a region of complementarity to the sequence of SEQ ID NO: 419, wherein said dsRNA is 30 base pairs or less in length, and wherein said dsRNA targets said mRNA for cleavage.

In a seventh aspect, there is provided a cell comprising the vector as defined above.

As used herein, the term "iRNA" refers to an agent that contains RNA as that term is defined herein, and which mediates the targeted cleavage of an RNA transcript via an RNA-induced silencing complex (RISC) pathway. In one embodiment, an iRNA as described herein inhibits the expression of TMPRSS6 in a cell or mammal. Alternatively, in another embodiment, the iRNA up-regulates the expression of TMPRSS6 in a cell or mammal.

The iRNAs included in the compositions featured herein encompass a double-stranded RNA (dsRNA) having an RNA strand (the antisense strand) having a region that is 30 nucleotides or less, generally 19-24 nucleotides in length, that is substantially complementary to at least part of an mRNA transcript of a TMPRSS6 gene. In one embodiment, the dsRNA comprises a region of at least 15 contiguous nucleotides.

In one embodiment, an iRNA for inhibiting expression of a TMPRSS6 gene includes at least two sequences that are complementary to each other. The iRNA includes a sense strand having a first sequence and an antisense strand having a second sequence. The antisense strand includes a nucleotide sequence that is substantially complementary to at least part of an mRNA encoding TMPRSS6, and the region of complementarity is 30 nucleotides or less, and at least 15 nucleotides in length. Generally, the iRNA is 19 to 24, *e.g.*, 19 to 21 nucleotides in length. In some embodiments the iRNA is from about 15 to about 25 nucleotides in length, and in other embodiments the iRNA is from about 25 to about 30 nucleotides in length. The iRNA, upon contacting with a cell expressing TMPRSS6, inhibits the expression of a TMPRSS6 gene by at least 10%, at least 20%, at least 25%, at least 30%, at least 35% or at least 40% or more, such as when assayed by a method as described herein. In one embodiment, the TMPRSS6 iRNA is formulated in a stable nucleic acid lipid particle (SNALP).

In one embodiment, an iRNA featured herein includes a first sequence of a dsRNA that is selected from the group consisting of the sense sequences of Tables 2, 3 or 4 and a second sequence that is selected from the group consisting of the corresponding antisense sequences of Tables 2, 3 or 4. The iRNA molecules featured herein can include naturally