RNA interference is provided for inhibition of tumor necrosis factor α (TNFα) by silencing TNFα cell surface receptor TNF receptor-1 (TNFRI) mRNA expression, or by silencing TNFα converting enzyme (TACE/ADAM17) mRNA expression. Silencing such TNFα targets, in particular, is useful for treating patients having a TNFα-related condition or at risk of developing a TNFα-related condition such as the ocular conditions dry eye, allergic conjunctivitis, or ocular inflammation, or such as dermatitis, rhinitis, or asthma, for example.

Specification includes a Sequence Listing.
FIG. 1

<table>
<thead>
<tr>
<th>siRNA</th>
<th>nM siRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-transfected</td>
<td></td>
</tr>
<tr>
<td>RISC-free</td>
<td></td>
</tr>
<tr>
<td>siTNFR1 #4</td>
<td>1</td>
</tr>
<tr>
<td>siTNFR1 #3</td>
<td>1</td>
</tr>
<tr>
<td>siTNFR1 #2</td>
<td>1</td>
</tr>
<tr>
<td>siTNFR1 #1</td>
<td>1</td>
</tr>
<tr>
<td>TNFR1</td>
<td>1</td>
</tr>
<tr>
<td>Actin</td>
<td>1</td>
</tr>
</tbody>
</table>

kDa

120 100 80 60 50 40 30
RNAI-MEDIATED INHIBITION OF TUMOR NECROSIS FACTOR ALPHA-RELATED CONDITIONS

RELATED APPLICATIONS


FIELD OF THE INVENTION

[0002] The present invention relates to the field of interfering RNA compositions for silencing tumor necrosis factor α (TNFα) by silencing the TNFα cell surface receptor TNF receptor-1 (TNFR1) mRNA, or the TNFα converting enzyme (TACE/ADAM17) mRNA. Silencing such TNFα targets is useful for treatment of patients having a TNFα-related condition or at risk of developing such a condition.

BACKGROUND OF THE INVENTION

[0003] Inflammation is generally treated with a standard anti-inflammatory regimen that includes steroids and/or non-steroidal anti-inflammatory drugs (NSAIDs). Allergic conjunctivitis, ocular inflammation, dermatitis, rhinitis, and asthma have historically been treated with a regimen of oral, intranasal or topical antiasthmatics in addition to or oral or intranasal steroids. Systemic treatment typically requires higher concentrations of the drug compound to be administered to afford an effective concentration to reach the necessary treatment site. Antihistamine compounds are known to have central nervous system activity; drowsiness and drying of mucus membranes are a common side-effect of antihistamine use. Steroids and NSAIDS have potential side effects including intraocular pressure increase, cataract, glaucoma or corneal melting.

[0004] Dry eye, also known as conjunctivitis sicca or keratoconjunctivitis sicca, is a common ophthalmological disorder involving breakdown of the pre-ocular tear film, resulting in dehydration of the exposed outer surface of the eye. To date, dry eye has been treated with topical administration of artificial tear solutions. Some of these solutions contain mucinomimetic substances to temporarily replace or replenish the mucin layer in mucin deficient patients. Use of methylprednisolone has been proposed in a short-term “pulse” treatment to treat exacerbations of dry eye. The proposed “pulse” therapy is required to avoid complications associated with traditional steroid therapy for inflammatory conditions such as increased intraocular pressure and cataract formation.

[0005] The cytokine TNFα is a target for anti-inflammatory therapy of dry eye and uveitis. In a rabbit model of lacrimal gland inflammation-induced dry eye, inhibition of corneal staining and restoration of tear breakup time has been achieved by specific modulation of ocular surface TNFα levels. Dry eye therapy resulted by inhibiting TNFα synthesis (RDP58) or by specifically neutralizing TNFα using a monoclonal antibody (REMCICA®) or a soluble receptor (ENDREL®). Each of these TNFα-directed treatments resulted in levels of efficacy obtained with topical ocular anti-inflammatory steroids.


[0007] Embodiments of the present invention address the need in the art for further agents and treatment methods for dry eye and inflammation and provide alternative therapies therefor.

SUMMARY OF THE INVENTION

[0008] Embodiments of the present invention provide highly potent and efficacious treatment, prevention or intervention of a TNFα-related condition without side effects associated with steroids or NSAIDS. In one aspect, methods of the invention include treating a subject having a TNFα-related condition or at risk of developing a TNFα-related condition by administering interfering RNAs that silence expression of TACE mRNA or TNFR1 mRNA, thus interfering with proteolytic processing of the precursor to TNFα, or interfering with binding of TNFα to its cell surface receptor, respectively, thereby attenuating activity of TNFα and preventing a cascade of events related to apoptosis and inflammation.

[0009] A TNFα-related condition includes conditions such as dry eye and TNFα-related inflammatory conditions. A TNFα-related inflammatory condition includes conditions such as ocular inflammation, allergic conjunctivitis, dermatitis, rhinitis, and asthma, for example, and includes those cellular changes resulting from the activity of TNFα that leads directly or indirectly to the TNFα-related inflammatory condition. A TNFα-related condition particularly includes TNFα-related ocular conditions such as dry eye, allergic conjunctivitis, and ocular inflammation. The interfering RNA provided herein provides for silencing the TNFα targets TACE mRNA or TNFR1 mRNA while avoiding undesirable side effects due to nonspecific agents.

[0010] A method of attenuating expression of TACE mRNA of the subject is an embodiment of the invention. The method comprises administering to the subject a composition comprising an effective amount of interfering RNA having a length of 19 to 49 nucleotides and a pharmaceutically acceptable carrier, the interfering RNA comprising a region of at least 13 contiguous nucleotides having at least 90% sequence complementarity to, or at least 90% sequence identity with, the penultimate 13 nucleotides of the 3′ end of an mRNA corresponding to any one of SEQ ID NO:3, SEQ ID NO:14-SEQ ID NO:58, and SEQ ID NO:155-SEQ ID NO:201. The expression of TACE mRNA is attenuated thereby.

[0011] A method of treating a TNFα-related condition in a subject in need thereof is an embodiment of the invention.
The method comprises administering to the subject a composition comprising an effective amount of interfering RNA having a length of 19 to 49 nucleotides, and a pharmaceutically acceptable carrier, wherein the interfering RNA comprises a region of at least 13 contiguous nucleotides having at least 90% sequence complementarity to, or at least 90% sequence identity with, the penultimate 13 nucleotides of the 3' end of an mRNA corresponding to any one of SEQ ID NO:3, SEQ ID NO:14-SEQ ID NO:58, and SEQ ID NO:155-SEQ ID NO:201. The TNFα-related condition is treated thereby.

[0012] In yet another embodiment of the invention, a method of attenuating activity of TNFα of a subject by attenuating expression of TACE mRNA or TNFR1 mRNA of the subject comprises administering to the subject a composition comprising an effective amount of interfering RNA having a length of 19 to 49 nucleotides and a pharmaceutically acceptable carrier and the interfering RNA comprises a sense nucleotide strand, an antisense nucleotide strand, and a region of at least near-perfect contiguous complementarity of at least 19 nucleotides where the antisense strand hybridizes under physiological conditions to a portion of mRNA corresponding to SEQ ID NO:1 comprising nucleotides 297, 333, 334, 335, 343, 470, 493, 547, 570, 618, 649, 755, 842, 844, 846, 860, 878, 894, 900, 909, 910, 913, 942, 970, 984, 994, 1002, 1010, 1015, 1034, 1062, 1162, 1215, 1330, 1340, 1368, 1393, 1428, 1505, 1506, 1513, 1515, 1557, 1591, 1592, 1593, 1597, 1604, 1605, 1626, 1632, 1658, 1659, 1794, 1884, 2045, 2047, 2049, 2080, 2089, 2090, 2091, 2092, or 2098. The TNFα-related condition is treated thereby.

[0014] A method of treating a TNFα-related ocular condition in a subject in need thereof is an embodiment of the invention, the method comprising administering to the subject a composition comprising an effective amount of interfering RNA having a length of 19 to 49 nucleotides, and a pharmaceutically acceptable carrier, the interfering RNA comprising a sense nucleotide strand, an antisense nucleotide strand, and a region of at least near-perfect contiguous complementarity of at least 19 nucleotides; wherein the antisense strand hybridizes under physiological conditions to a portion of mRNA corresponding to SEQ ID NO:2 comprising nucleotides 124, 328, 387, 391, 393, 395, 406, 421, 423, 444, 447, 455, 459, 460, 467, 469, 470, 471, 475, 493, 513, 517, 531, 543, 556, 576, 587, 588, 595, 601, 602, 611, 612, 651, 664, 667, 668, 669, 677, 678, 785, 786, 788, 791, 792, 804, 813, 824, 838, 843, 877, 884, 929, 959, 960, 961, 963, 964, 965, 970, 973, 974, 1000, 1002, 1013, 1026, 1053, 1056, 1057, 1058, 1161, 1315, 1318, 1324, 1357, 1360, 1383, 1393, 1420, 1471, 1573, 1671, 2044, 2046, 2047, 2080, 2090, 2091, or 2092. The TNFα-related condition is treated thereby.

[0015] A second interfering RNA having a length of 19 to 49 nucleotides could also be administered to the subject in a further embodiment; the second interfering RNA comprising a sense nucleotide strand, an antisense nucleotide strand, and a region of at least near-perfect contiguous complementarity of at least 19 nucleotides wherein the antisense strand of the second interfering RNA hybridizes under physiological conditions to a portion of mRNA corresponding to SEQ ID NO:1 comprising a nucleotide as cited above, or where the antisense strand hybridizes under physiological conditions to a portion of mRNA corresponding to SEQ ID NO:2 beginning at nucleotide 124, 328, 387, 391, 393, 395, 406, 421, 423, 444, 447, 455, 459, 460, 467, 469, 470, 471, 475, 479, 513, 517, 531, 543, 556, 576, 587, 588, 595, 601, 602, 611, 612, 651, 664, 667, 668, 669, 677, 678, 785, 786, 788, 791, 792, 804, 813, 824, 838, 843, 877, 884, 929, 959, 960, 961, 963, 964, 965, 970, 973, 974, 1000, 1002, 1013, 1026, 1053, 1056, 1057, 1058, 1161, 1315, 1318, 1324, 1357, 1360, 1383, 1393, 1420, 1471, 1573, 1671, 2044, 2046, 2047, 2080, 2090, 2091, or 2092. The expression of TACE mRNA is attenuated in those embodiments where the antisense strand hybridizes to a portion of mRNA corresponding to SEQ ID NO:1 as cited above. The expression of TNFR1 mRNA is attenuated in those embodiments where the antisense strand hybridizes to a portion of mRNA corresponding to SEQ ID NO:2 as cited above.

[0013] A method of treating a TNFα-related condition in a subject in need thereof is an embodiment of the invention, the method comprising administering to the subject a composition comprising an effective amount of interfering RNA having a length of 19 to 49 nucleotides, and a pharmaceutically acceptable carrier, the interfering RNA comprising a sense nucleotide strand, an antisense nucleotide strand, and a region of at least near-perfect contiguous complementarity of at least 19 nucleotides; wherein the antisense strand hybridizes under physiological conditions to a portion of mRNA corresponding to SEQ ID NO:1 comprising nucleotides 297, 333, 334, 335, 343, 470, 493, 547, 570, 573, 618, 649, 755, 842, 844, 846, 860, 878, 894, 900, 909, 910, 913, 942, 970, 984, 1002, 1010, 1053, 1064, 1137, 1162, 1215, 1300, 1334, 1340, 1386, 1393, 1428, 1505, 1508, 1541, 1553, 1557, 1591, 1592, 1593, 1597, 1604, 1605, 1626, 1632, 1658, 1661, 1691, 1794, 1856, 1945, 1946, 1947, 1958, 2022, 2094, 2100, 2121, 2263, 2347, 2349, 2549, 2578, 2595, 2606, 2608, 2629, 2639, 2764, 2766, 2767, 2769, 3027, 3028, 3261, 3264, 3284, 3313, 3317, 3332, or 3337. The TNFα-related condition is treated thereby.

[0016] When a first interfering RNA targets SEQ ID NO:1, the second interfering RNA may target either SEQ ID NO:1 or SEQ ID NO:2, and conversely, when a first interfering RNA targets SEQ ID NO:2, the second interfering RNA may target either SEQ ID NO:1 or SEQ ID NO:2. In further embodiments, a third, fourth, or more interfering RNAs may be administered.
nucleotide sequence having substantial complementarity to an mRNA corresponding to the TACE gene, so that the siRNA molecule directs cleavage of the mRNA via RNA interference.

A further embodiment of the invention is a method of treating a TNFα-related ocular condition in a subject in need thereof, where the method comprises administering to the subject a composition comprising a double stranded siRNA molecule that down regulates expression of a TNFR1 gene via RNA interference, wherein each strand of the siRNA molecule is independently about 19 to about 27 nucleotides in length; and one strand of the siRNA molecule comprises a nucleotide sequence having substantial complementarity to an mRNA corresponding to the TNFR1 gene so that the siRNA molecule directs cleavage of the mRNA via RNA interference.

A method of attenuating expression of TACE mRNA of the subject, comprising administering to the subject a composition comprising an effective amount of a single-stranded interfering RNA and a pharmaceutically acceptable carrier is a further embodiment. The single-stranded interfering RNA has a length of 19 to 49 nucleotides and hybridizes under physiological conditions to a portion of mRNA corresponding to SEQ ID NO:1 comprising nucleotide 297, 333, 334, 335, 434, 470, 493, 547, 570, 573, 618, 649, 689, 755, 842, 844, 846, 860, 878, 894, 909, 909, 910, 913, 942, 970, 984, 1002, 1010, 1053, 1064, 1137, 1162, 1215, 1330, 1334, 1340, 1386, 1393, 1428, 1505, 1508, 1541, 1553, 1557, 1591, 1592, 1593, 1597, 1604, 1605, 1626, 1632, 1658, 1661, 1691, 1794, 1856, 1945, 1946, 1947, 1958, 2022, 2094, 2100, 2121, 2263, 2277, 2347, 2349, 2549, 2578, 2595, 2606, 2608, 2629, 2639, 2764, 2766, 2767, 2769, 3027, 3028, 3261, 3264, 3284, 3313, 3317, 3332, or 3337, and the interfering RNA has a region of at least near-perfect contiguous complementarity with the hybridizing portion of mRNA corresponding to SEQ ID NO:1. The expression of TACE mRNA is thereby attenuated.

The invention includes as a further embodiment a composition comprising an interfering RNA having a length of 19 to 49 nucleotides, and comprising a nucleotide sequence corresponding to any one of SEQ ID NO:3, SEQ ID NO:14-SEQ ID NO:58, and SEQ ID NO:155-SEQ ID NO:201, or a complement thereof; and a pharmaceutically acceptable carrier.

The invention includes as a further embodiment a composition comprising an interfering RNA consisting essentially of a nucleotide sequence corresponding to any one of SEQ ID NO:59-SEQ ID NO:69, SEQ ID NO:71-SEQ ID NO:92, and SEQ ID NO:94-SEQ ID NO:154, or a complement thereof; and a pharmaceutically acceptable carrier.

Use of any of the embodiments as described herein in the preparation of a medicament for attenuating expression of TACE mRNA or of TNFR1 mRNA as a method of attenuating activity of TNFα and thereby treating a TNFα-related condition as set forth herein is also an embodiment of the present invention.

BRIEF DESCRIPTION OF THE FIGURES

In order that the manner in which the above recited and other advantages and objects of the invention are obtained, a more particular description of the invention briefly described above will be rendered by reference to specific embodiments thereof, which are illustrated, in the appended drawings. Understanding that these drawings depict only typical embodiments of the invention and are therefore not to be considered limiting of its scope, the invention will be described with additional specificity and detail through the use of the accompanying drawings in which:

FIG. 1 provides a TNFR1 western blot of GMT-3 cells transfected with TNFR1 siRNAs #1, #2, #3, and #4, and a RISC-free control siRNA, each at 10 nM, 1 nM, and 0.1 nM; a non-targeting control siRNA (NTC2) at 10 nM; and a buffer control (−siRNA). The arrows indicate the positions of the 55-kDa TNFR1 and 42-kDa actin bands.

DETAILED DESCRIPTION OF THE INVENTION

The references cited herein, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated by reference.

Those of skill in the art, in light of the present disclosure, will appreciate that obvious modifications of the embodiments disclosed herein can be made without departing from the spirit and scope of the invention. All of the embodiments disclosed herein can be made and executed without undue experimentation in light of the present disclosure. The full scope of the invention is set out in the disclosure and equivalent embodiments thereof. The specification should not be construed to unduly narrow the full scope of protection to which the present invention is entitled.

The particulars shown herein are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of various embodiments of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for the fundamental understanding of the invention. The description taken with the drawings and example making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

The following definitions and explanations are meant and intended to be controlling in any future construction unless clearly and unambiguously modified in the following examples or when application of the meaning renders any construction meaningless or essentially meaningless. In cases where the construction of the term would render it meaningless or essentially meaningless, the definition should be taken from Webster’s Dictionary, 3rd Edition.

As used herein, all percentages are percentages by weight, unless stated otherwise.

As used herein and unless otherwise indicated, the terms “a” and “an” are taken to mean “one”, “at least one” or “one or more”.

The term “dry eye,” also known as conjunctivitis sicca or keratoconjunctivitis sicca, is a common ophthalmological disorder involving breakdown of the pre-ocular tear film, resulting in dehydration of the exposed outer surface of the eye.
The term “ocular inflammation,” as used herein, includes iritis, uveitis, episcleritis, scleritis, keratitis, endophthalmitis, blepharitis, and iatrogenic inflammatory conditions, for example.

The term “allergic conjunctivitis,” as used herein, refers to inflammation of the conjunctiva which is the delicate membrane that lines the eyelids and covers the exposed surface of the sclera. The term “allergic conjunctivitis” includes, for example, atopic keratoconjunctivitis, giant papillary conjunctivitis, hay fever conjunctivitis, perennial allergic conjunctivitis, and vernal keratoconjunctivitis.

The term “dermatitis,” as used herein, refers to inflammation of the skin and includes, for example, allergic contact dermatitis, urticaria, atopic dermatitis (dry skin on the lower legs), atopic dermatitis, contact dermatitis including irritant contact dermatitis and urushiol-induced contact dermatitis, eczema, gravitational dermatitis, nummular dermatitis, otitis externa, perioral dermatitis, and seborrheic dermatitis.

The term “rhinitis,” as used herein, refers to inflammation of the mucous membranes of the nose and includes, for example, allergic rhinitis, atopic rhinitis, irritant rhinitis, eosinophilic non-allergic rhinitis, rhinitis medicamentosa, and neutrophilic rhinosinusitis.

The term “asthma,” as used herein, refers to inflammation of the air passages resulting in narrowing of the airways that transport air from the nose and mouth to the lungs and includes, for example, allergic asthma, atopic asthma, atopic bronchial IgE-mediated asthma, bronchial asthma, bronchiolitis, emphysematous asthma, essential asthma, exercise-induced asthma, extrinsic asthma caused by environmental factors, incipient asthma, intrinsic asthma caused by pathophysiologic disturbances, non-allergic asthma, non-atopic asthma, and wheezy infant syndrome.

The phrase “a region of at least 13 contiguous nucleotides having at least 90% sequence complementarity to, or at least 90% sequence identity with, the penultimate 13 nucleotides of the 3’ end of an mRNA corresponding to any one of a sequence identifier” allows a one nucleotide substitution. Two nucleotide substitutions (i.e., 11/13–85% identity/complementarity) are not included in such a phrase.

The term “percent identity” describes the percentage of contiguous nucleotides in a first nucleic acid molecule that is the same as in a set of contiguous nucleotides of the same length in a second nucleic acid molecule. The term “percent complementarity” describes the percentage of contiguous nucleotides in a first nucleic acid molecule that can base pair in the Watson-Crick sense with a set of contiguous nucleotides in a second nucleic acid molecule.

As used herein, the term “hybridization” means and refers to a process in which single-stranded nucleic acids with complementary or near-complementary base sequences interact to form hydrogen-bonded complexes called hybrids. Hybridization reactions are sensitive and selective. In vitro, the specificity of hybridization (i.e., stringency) is controlled by the concentrations of salt or formamide in prehybridization and hybridization solutions, for example, and by the hybridization temperature; such procedures are well known in the art. In particular, stringency is increased by reducing the concentration of salt, increasing the concentration of formamide, or raising the hybridization temperature.

For example, high stringency conditions could occur at about 50% formamide at 37° C. to 42° C. Reduced stringency conditions could occur at about 35% to 25% formamide at 30° C. to 35° C. Examples of stringency conditions for hybridization are provided in Sambrook, J., 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Further examples of stringent hybridization conditions include 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50° C. or 70° C. for 12-16 hours followed by washing, or hybridization at 70° C. in 1×SSC or 50° C. in 1×SSC, 50% formamide followed by washing at 70° C. in 0.3×SSC, or hybridization at 70° C. in 4×SSC or 50° C. in 4×SSC, 50% formamide followed by washing at 67° C. in 1×SSC. The temperature for hybridization is about 5-10° C. less than the melting temperature (Tm) of the hybrid where Tm is determined for hybrids between 19 and 49 base pairs in length using the following calculation: Tm° C. = 81.5 + 16.6(ln(Na+) + 40.41 (% G+C) +600/N) where N is the number of bases in the hybrid, and [Na+] is the concentration of sodium ions in the hybridization buffer.

Nucleic acid sequences cited herein are written in a 5’ to 3’ direction unless indicated otherwise. The term “nucleic acid,” as used herein, refers to either DNA or RNA or a modified form thereof comprising the purine or pyrimidine bases present in DNA (adenine “A,” cytosine “C,” guanine “G,” thymine “T”) or in RNA (adenine “A,” cytosine “C,” guanine “G,” uracil “U”). Interfering RNAs provided herein may comprise “T” bases, particularly at 3’ ends, even though “T” bases do not naturally occur in RNA. “Nucleic acid” includes the terms “oligonucleotide” and “polynucleotide” and can refer to a single-stranded molecule or a double-stranded molecule. A double-stranded molecule is formed by Watson-Crick base pairing between A and T bases, and C and G bases, and between A and U bases. The strands of a double-stranded molecule may have partial, substantial or full complementarity to each other and will form a duplex hybrid, the strength of bonding of which is dependent upon the nature and degree of complementarity of the sequence of bases.

An mRNA sequence is readily deduced from the sequence of the corresponding DNA sequence. For example, SEQ ID NO:1 provides the sense strand sequence of DNA corresponding to the mRNA for TACE. The mRNA sequence is identical to the DNA sense strand sequence with the “T” bases replaced with “U” bases. Therefore, the mRNA sequence of TACE is known from SEQ ID NO:1 and the mRNA of TNFR1 is known from SEQ ID NO:2.

RNA interference (RNAi) is a process by which double-stranded RNA (dsRNA) is used to silence gene expression. While not wanting to be bound by theory, RNAi begins with the cleavage of longer dsRNAs into small interfering RNAs (siRNAs) by an RNaseIII-like enzyme, dicer. siRNAs are dsRNAs that are usually about 19 to 28 nucleotides, or 20 to 25 nucleotides, or 21 to 22 nucleotides in length and often contain 2-nucleotide 3’ overhangs, and 5’ phosphate and 3’ hydroxyl termini. One strand of the siRNA is incorporated into a ribonucleoprotein complex known as the RNA-induced silencing complex (RISC). RISC uses this siRNA strand to identify mRNA molecules that are at least partially complementary to the incorporated siRNA strand, and then cleaves these target mRNAs or inhibits their translation. Therefore, the siRNA strand that is incorporated into RISC is known as the guide strand or the antisense
strand. The other siRNA strand, known as the passenger strand or the sense strand, is eliminated from the siRNA and is at least partially homologous to the target mRNA. Those of skill in the art will recognize that, in principle, either strand of an siRNA can be incorporated into RISC and function as a guide strand. However, siRNA design (e.g., decreased siRNA duplex stability at the 5' end of the antisense strand) can favor incorporation of the antisense strand into RISC.

RISC-mediated cleavage of mRNAs having a sequence at least partially complementary to the guide strand leads to a decrease in the steady state level of that mRNA and of the corresponding protein encoded by this mRNA. Alternatively, RISC can also decrease expression of the corresponding protein via translational repression without cleavage of the target mRNA. Other RNA molecules and RNA-like molecules can also interact with RISC and silence gene expression. Examples of other RNA molecules that can interact with RISC include short hairpin RNAs (shRNAs), single-stranded siRNAs, microRNAs (miRNAs), and dicer-substrate 27-mer duplexes. The term “siRNA” as used herein refers to a double-stranded interfering RNA unless otherwise noted. Examples of RNA-like molecules that can interact with RISC include RNA molecules containing one or more chemically modified nucleotides, one or more deoxyribonucleotides, and/or one or more non-phosphodiester linkages. For purposes of the present discussion, all RNA or RNA-like molecules that can interact with RISC and participate in RISC-mediated changes in gene expression will be referred to as “interfering RNAs.” siRNAs, shRNAs, miRNAs, and dicer-substrate 27-mer duplexes are, therefore, subsets of “interfering RNAs.”

Interfering RNA of embodiments of the invention appear to act in a catalytic manner for cleavage of target mRNA, i.e., interfering RNA is able to effect inhibition of target mRNA in stoichiometric amounts. As compared to antisense therapies, significantly less interfering RNA is required to provide a therapeutic effect under such cleavage conditions.

The present invention relates to the use of interfering RNA to inhibit the expression of TNFα cell surface receptor TNF receptor-1 (TNFR1), or the TNFα converting enzyme (TACE/ADAM17, designated herein “TACE”) which inhibits expression of tumor necrosis factor α (TNFα) activity. Binding of TNFα to its cell surface receptor, TNF receptor-1 (TNFR1), activates a signaling cascade which affects a variety of cellular responses including apoptosis and inflammation. TNFα itself is initially expressed as an inactive, membrane-bound precursor. Release of the active form of TNFα from the cell surface requires proteolytic processing of the precursor by TNFα converting enzyme (TACE/ADAM17), a member of the “A Disintegrin And Metalloprotease” (ADAM) family.

According to the present invention, inhibiting the expression of TNFR1 mRNA, TACE mRNA, or both TNFR1 and TACE mRNAs effectively reduces the action of TNFα. Further, interfering RNAs as set forth herein provided exogenously or expressed endogenously are particularly effective at silencing TNFR1 mRNA or TACE mRNA.

Tumor Necrosis Factor α Converting Enzyme mRNA (TACE/ADAM17): The GenBank database provides the DNA sequence for TACE as accession no. NM_003183, provided in the “Sequence Listing” as SEQ ID NO:1. SEQ ID NO:1 provides the sense strand sequence of DNA that corresponds to the mRNA encoding TACE (with the exception of “T” bases for “U” bases). The coding sequence for TACE is from nucleotides 184-2658.

Equivalents of the above cited TACE mRNA sequence are alternative splice forms, allelic forms, isoforms, or a cognate thereof. A cognate is a tumor necrosis factor α converting enzyme mRNA from another mammalian species that is homologous to SEQ ID NO:1 (i.e., an ortholog).

Tumor Necrosis Factor Receptor-1 mRNA (TNFR1): The GenBank database provides the DNA sequence for TNFR1 as accession no. NM_001065, provided in the “Sequence Listing” as SEQ ID NO:2. SEQ ID NO:2 provides the sense strand sequence of DNA that corresponds to the mRNA encoding TNFR1 (with the exception of “T” bases for “U” bases). The coding sequence for TNFR1 is from nucleotides 282-1649.

Equivalents of the above cited TNFR1 mRNA sequence are alternative splice forms, allelic forms, isoforms, or a cognate thereof. A cognate is a tumor necrosis factor receptor-1 mRNA from another mammalian species that is homologous to SEQ ID NO:2 (i.e., an ortholog).

Attenuating expression of an mRNA: The phrase, “attenuating expression of an mRNA,” as used herein, means administering or expressing an amount of interfering RNA (e.g., an siRNA) to reduce translation of the target mRNA into protein, either through mRNA cleavage or through direct inhibition of translation. The reduction in expression of the target mRNA or the corresponding protein, is commonly referred to as “knock-down” and is reported relative to levels present following administration or expression of a non-targeting control RNA (e.g., a non-targeting control siRNA). Knock-down of expression of an amount including and between 50% and 100% is contemplated by embodiments herein. However, it is not necessary that such knock-down levels be achieved for purposes of the present invention. In one embodiment, a single interfering RNA targeting TACE mRNA or TNFR1 mRNA is administered. In other embodiments, two or more interfering RNAs targeting TACE mRNA or TNFR1 mRNA are administered. In further embodiments, interfering RNAs targeting each of TACE mRNA and TNFR1 mRNA are administered in combination or in a time interval so as to have overlapping effects.

Knock-down is commonly assessed by measuring the mRNA levels using quantitative polymerase chain reaction (qPCR) amplification or by measuring protein levels by western blot or enzyme-linked immunosorbent assay (ELISA). Analyzing the protein level provides an assessment of both mRNA cleavage as well as translation inhibition. Further techniques for measuring knock-down include RNA solution hybridization, nuclease protection, northern hybridization, gene expression monitoring with a microarray, antibody binding, radioimmunoassay, and fluorescence activated cell analysis.

Inhibition of TACE or TNFR1 may also be determined in vitro by evaluating target mRNA levels or target protein levels in, for example, human corneal epithelial cells following transfection of TACE- or TNFR1-interfering RNA as described infra.

Inhibition of TNFα activity due to inhibition of TACE mRNA expression or of TNFR1 mRNA expression is also inferred in a human or mammal by observing an improvement in a TNFα-related condition symptom such as
An siRNA of the invention for targeting a corresponding mRNA sequence of SEQ ID NO:3 and having 21-nucleotide strands and a 2-nucleotide 3' overhang is:

\[
5'\text{-GCUCUCAGACUGAAUUU-3',}
\]

\[
3'\text{-UUCCAGAUGUGCAUAUAA-5'}.\]

Each "N" residue can be any nucleotide (A, C, G, U, or modified nucleotide. The 3' end can have a number of "N" residues between and including 1, 2, 3, 4, 5, and 6. The "N" residues on either strand can be the same residue (e.g., UU, AA, CC, GG, or TT) or they can be different (e.g., AC, AG, AU, CA, CG, CU, GA, GC, GU, UA, UC, or UG). The 3' overhangs can be the same or they can be different. In one embodiment, both strands have a 3'UU overhang.

An siRNA of the invention for targeting a corresponding mRNA sequence of SEQ ID NO:3 and having 21-nucleotide strands and a 3'UU overhang on each strand is:

\[
5'\text{-GCUCUCAGACUGAAUUU-3',}
\]

\[
3'\text{-UUCCAGAUGUGCAUAUAA-5'}.\]

The interfering RNA may also have a 5' overhang of nucleotides or it may have blunt ends. An siRNA of the invention for targeting a corresponding mRNA sequence of SEQ ID NO:3 and having 19-nucleotide strands and blunt ends is:

\[
5'\text{-GCUCUCAGACUGAAU-3',}
\]

\[
3'\text{-CUAGAGUCUGCAUAUAA-5'}.\]

The interfering RNA may also have a 5' overhang of nucleotides or it may have blunt ends. An siRNA of the invention for targeting a corresponding mRNA sequence of SEQ ID NO:3 and having 19-nucleotide strands and blunt ends is:

\[
5'\text{-GCUCUCAGACUGAAU-3',}
\]

\[
3'\text{-CUAGAGUCUGCAUAUAA-5'}.\]

Techniques for selecting target sequences for siRNAs are provided by Tuschi, T. et al., "The siRNA User Guide," revised May 6, 2004, available on the Rockefeller University web site; by Technical Bulletin #506, "siRNA Design Guidelines," Ambion Inc. at Ambion’s web site; and by other web-based design tools at, for example, the Invitrogen, Dharmacco, Integrated DNA Technologies, Gen-Script, or Qiagen web sites. Initial search parameters can include G/C contents between 35% and 55% and siRNA lengths between 19 and 27 nucleotides. The target sequence may be located in the coding region or in the 5' or 3' untranslated regions of the mRNAs.

An embodiment of a 19-nucleotide DNA target sequence for TACE mRNA is present at nucleotides 297 to 315 of SEQ ID NO:1:

\[
5'\text{-GCTTCGACACTGATATT-3'}.\]

N is a nucleotide A, T, C, G, U, or a modified form known by one of ordinary skill in the art. The number of nucleotides N in the loop is a number between and including 3 to 23, or 5 to 15, or 7 to 13, or 4 to 9, or 9 to 11, or the number of nucleotides N is 9. Some of the nucleotides in the loop can be involved in base-pair interactions with other nucleotides in the loop. Examples of oligonucleotide sequences that can be used to form the loop include 5'-UUCAAGAGA-3' (Brummelkamp, T. R. et al. (2002) Science 296: 550) and...
TABLE 1 - continued

<table>
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<th>TACE Target Sequence</th>
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<tr>
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<td>CTATGACAGAGCTGTTAA</td>
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</table>

A dicer-substrate 27-mer duplex of the invention for targeting a corresponding mRNA sequence of SEQ ID NO:11 is:

SEQ ID NO: 11
5'-GCTTCAGACTACGATATTCTTCTCT-3'.

A dicer-substrate 27-mer duplex from 19-21 nucleotide target sequences, such as provided herein, is further discussed by the Integrated DNA Technologies (IDT) website and by Kim, D.-H. et al., (February, 2005) Nature Biotechnology 23:2, 222-226.

When interfering RNAs are produced by chemical synthesis, phosphorylation at the 5' position of the nucleotide at the 5' end of one or both strands (when present) can enhance siRNA efficacy and specificity of the bound RISC complex but is not required since phosphorylation can occur intracellularly.

Table 1 lists examples of TACE DNA target sequences of SEQ ID NO:1 from which siRNAs of the present invention are designed in a manner as set forth above. TACE encodes tumor necrosis factor α converting enzyme, as noted above.

| TABLE 1 |
| TACE Target Sequences for siRNAs |
|----------------------|-------------------------------------------------------|------------|
| # of Starting Nucleotide with reference to SEQ ID NO: 1 | SEQ ID NO: |
|----------------------|-------------------------------------------------------|------------|
| GCTTCAGACTACGATATT  | 297                                                   | 3          |
| CCAGCAGCATTGCTGAGA  | 333                                                   | 14         |
| CAGCAGCTTGGCTGAGA   | 334                                                   | 15         |
| AGAGACCTACGTAGAAA   | 335                                                   | 16         |
| AGAGATCTACAGACTCAA  | 355                                                   | 17         |
| GAAAGCCGATACCTGATA  | 493                                                   | 18         |
| CTATGACAGAGCTGTTAA  | 842                                                   | 19         |

5'-UUUGUGUAG-3' (Castanotto, D. et al. (2002) RNA 8:1454). It will be recognized by one of skill in the art that the resulting single chain oligonucleotide forms a stem-loop or hairpin structure comprising a double-stranded region capable of interacting with the RNAi machinery.
**Table 1 - continued**

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<thead>
<tr>
<th>TACE Target Sequence</th>
<th># of Starting Nucleotide with reference to SEQ ID NO: 1</th>
<th>SEQ ID NO:</th>
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<td>GGRATCTACCAAGTTGTA</td>
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<tr>
<td>TCGTGGTCGATGATCTAA</td>
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**Table 2**

<table>
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<th>TNFR1 Target Sequence</th>
<th># of Starting Nucleotide with reference to SEQ ID NO: 2</th>
<th>SEQ ID NO:</th>
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</table>

Table 2 lists examples of TNFR1 DNA target sequences of SEQ ID NO: 2 from which siRNAs of the present invention are designed in a manner as set forth above. TNFR1 encodes tumor necrosis factor α receptor-1, as noted above.

**Table 2**

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<th>TNFR1 Target Sequence</th>
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<th>TNFR1 Target Sequence</th>
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<th>SEQ ID NO:</th>
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<tr>
<td>AGGAATGGGTCAAGTGGA</td>
<td>601</td>
<td>123</td>
</tr>
<tr>
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<td>602</td>
<td>124</td>
</tr>
<tr>
<td>GTGTCAGGGCTCAAGAAGA</td>
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<td>125</td>
</tr>
<tr>
<td>GAAGGACAGTACCAGCGCA</td>
<td>664</td>
<td>126</td>
</tr>
<tr>
<td>CACATACGCTTCTTCTCATA</td>
<td>785</td>
<td>127</td>
</tr>
<tr>
<td>CATGACAGGCTCTCTCTA</td>
<td>786</td>
<td>128</td>
</tr>
<tr>
<td>TGACAGGTTCTCTTCTAAG</td>
<td>798</td>
<td>129</td>
</tr>
<tr>
<td>AGGGTTCTCTTCTAAGAAGAA</td>
<td>791</td>
<td>130</td>
</tr>
<tr>
<td>GGTTCCTTCTCTAAGAAGA</td>
<td>792</td>
<td>131</td>
</tr>
<tr>
<td>CTGATGAACTGTTAAGAAGAA</td>
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<td>GAATGGCTGCTCTTCTGAGTA</td>
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<tr>
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<td>134</td>
</tr>
<tr>
<td>AGAAGGCTGCTCTGACAC</td>
<td>830</td>
<td>135</td>
</tr>
<tr>
<td>TTGAGATGTCTTGAAGGAC</td>
<td>877</td>
<td>136</td>
</tr>
<tr>
<td>TTTAAGGCGACTGAGAC</td>
<td>884</td>
<td>137</td>
</tr>
<tr>
<td>GTCATTCTTTCTGCTCTT</td>
<td>929</td>
<td>138</td>
</tr>
<tr>
<td>CCTCCTCTTCTTGAGTTA</td>
<td>959</td>
<td>139</td>
</tr>
</tbody>
</table>
TABLE 2—continued

<table>
<thead>
<tr>
<th>TNFR1 Target Sequence</th>
<th># of Starting Nucleotide with reference to SEQ ID NO: 2</th>
<th>SEQ ID NO:</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCCTCTCATTTTTTTAAT</td>
<td>961</td>
<td>140</td>
</tr>
<tr>
<td>CTCCTCTATTGTTAATG</td>
<td>963</td>
<td>141</td>
</tr>
<tr>
<td>TCCTCTATTGTTTAAAGT</td>
<td>964</td>
<td>142</td>
</tr>
<tr>
<td>CTCTCTATTGTTTAAATG</td>
<td>965</td>
<td>143</td>
</tr>
<tr>
<td>TCCAAGCTCTACCTATG</td>
<td>1002</td>
<td>144</td>
</tr>
<tr>
<td>CTCCTATTGTTGCGGAAAT</td>
<td>1013</td>
<td>145</td>
</tr>
<tr>
<td>GGAAATCGACACCTGAAA</td>
<td>1026</td>
<td>146</td>
</tr>
<tr>
<td>TGAGAAGCTACTACTAGA</td>
<td>1058</td>
<td>147</td>
</tr>
<tr>
<td>ACCTCCAAGCTCCACCTATA</td>
<td>1161</td>
<td>148</td>
</tr>
<tr>
<td>CCAACACGCGCCAGGCCT</td>
<td>1315</td>
<td>149</td>
</tr>
<tr>
<td>AGCCCGTGCTGGGAGACGTT</td>
<td>1360</td>
<td>150</td>
</tr>
<tr>
<td>GAAAGCTTTGCTGGCGCGG</td>
<td>1393</td>
<td>151</td>
</tr>
<tr>
<td>TGGCGGACCCAGGAGATCA</td>
<td>1420</td>
<td>152</td>
</tr>
<tr>
<td>TGGCGGAGCGAATAACGAT</td>
<td>1471</td>
<td>153</td>
</tr>
<tr>
<td>TGGCGTCCCTGGGAGCAT</td>
<td>1573</td>
<td>154</td>
</tr>
</tbody>
</table>

[0069] As cited in the examples above, one of skill in the art is able to use the target sequence information provided in Tables 1 or 2 to design interfering RNAs having a length shorter or longer than the sequences provided in the tables and by referring to the sequence position in SEQ ID NO:1 or SEQ ID NO:2 and adding or deleting nucleotides complementary or near complementary to SEQ ID NO:1 or SEQ ID NO:2, respectively.

[0070] The target RNA cleavage reaction guided by siRNAs and other forms of interfering RNA is highly sequence specific. In general, a sense nucleotide strand identical in sequence to a portion of the target mRNA and an antisense nucleotide strand exactly complementary to a portion of the target mRNA are siRNA embodiments for inhibition of mRNAs cited herein. However, 100% sequence complementarity between the antisense siRNA strand and the target mRNA, or between the antisense siRNA strand and the sense siRNA strand, is not required to practice the present invention. Thus, for example, the invention allows for sequence variations that might be expected due to genetic mutation, strain polymorphism, or evolutionary divergence.

[0071] In one embodiment of the invention, the antisense strand of the siRNA has at least near-perfect contiguous complementarity of at least 19 nucleotides with the target mRNA. “Near-perfect,” as used herein, means the antisense strand of the siRNA is “substantially complementary to,” and the sense strand of the siRNA is “substantially identical” to at least a portion of the target mRNA. “Identity,” as known by one of ordinary skill in the art, is the degree of sequence relatedness between nucleotide sequences as determined by matching the order and identity of nucleotides between the sequences. In one embodiment, the antisense strand of an siRNA having 80% and between 80% up to 100% complementarity, for example, 85%, 90% or 95% complementarity, to the target mRNA sequence are considered near-perfect complementarity and may be used in the present invention. “Perfect” contiguous complementarity is standard Watson-Crick base pairing of adjacent base pairs. “At least near-perfect” contiguous complementarity includes “perfect” complementarity as used herein. Computer methods for determining identity or complementarity are designed to identify the greatest degree of matching of nucleotide sequences, for example, BLASTN (Altschul, S. F., et al. (1990) J. Mol. Biol. 215:403-410).

[0072] The relationship between a target mRNA (sense strand) and one strand of an siRNA (the sense strand) is that of identity. The sense strand of an siRNA is also called a passenger strand, if present. The relationship between a target mRNA (sense strand) and the other strand of an siRNA (the antisense strand) is that of complementarity. The antisense strand of an siRNA is also called a guide strand.

[0073] The penultimate base in a nucleic acid sequence that is written in a 5' to 3' direction is the next to the last base, i.e., the base next to the 3' base. The penultimate 13 bases of a nucleic acid sequence written in a 5' to 3' direction are the last 13 bases of a sequence next to the 3' base and not including the 3' base. Similarly, the penultimate 14, 15, 16, 17, or 18 bases of a nucleic acid sequence written in a 5' to 3' direction are the last 14, 15, 16, 17, or 18 bases of a sequence, respectively, next to the 3' base and not including the 3' base.

[0074] In an embodiment of the invention, the region of contiguous nucleotides is a region of at least 13 contiguous nucleotides having at least 90% sequence complementarity to, or at least 90% sequence identity with, the penultimate 13 nucleotides of the 3' end of an mRNA corresponding to the sequence identified by each sequence identifier.

[0075] In one embodiment of the invention, the region of contiguous nucleotides is a region of at least 14 contiguous nucleotides having at least 85% sequence complementarity to, or at least 85% sequence identity with, the penultimate 14 nucleotides of the 3' end of an mRNA corresponding to the sequence identified by each sequence identifier. Two nucleotide substitutions (i.e., 12/14 ~ 86% identity/complementarity) are included in such a phrase.

[0076] In a further embodiment of the invention, the region of contiguous nucleotides is a region of at least 15, 16, 17, or 18 contiguous nucleotides having at least 80% sequence complementarity to, or at least 80% sequence identity with, the penultimate 14 nucleotides of the 3' end of an mRNA corresponding to the sequence of the sequence identifier. Three nucleotide substitutions are included in such a phrase.

[0077] The target sequence in the mRNAs corresponding to SEQ ID NO:1 or SEQ ID NO:2 may be in the 5' or 3' untranslated regions of the mRNA as well as in the coding region of the mRNA.

[0078] One or both of the strands of double-stranded interfering RNA may have a 3' overhang of from 1 to 6 nucleotides, which may be ribonucleotides or deoxyribonucleotides or a mixture thereof. The nucleotides of the overhang are not base-paired. In one embodiment of the invention, the interfering RNA comprises a 3' overhang of TT or UU. In another embodiment of the invention, the
interfering RNA comprises at least one blunt end. The termini usually have a 5' phosphate group or a 3' hydroxyl group. In other embodiments, the antisense strand has a 5' phosphate group, and the sense strand has a 5' hydroxyl group. In still other embodiments, the termini are further modified by covalent addition of other molecules or functional groups.

[0079] The sense and antisense strands of the double-stranded siRNA may be in a duplex formation of two single strands as described above or may be a single molecule where the regions of complementarity are base-paired and are covalently linked by a hairpin loop so as to form a single strand. It is believed that the hairpin is cleaved intracellularly by a protein termed dicer to form an interfering RNA of two individual base-paired RNA molecules.

[0080] Interfering RNAs may differ from naturally-occurring RNA by the addition, deletion, substitution or modification of one or more nucleotides. Non-nucleotide material may be bound to the interfering RNA, either at the 5' end, the 3' end, or internally. Such modifications are commonly designed to increase the nuclear resistance of the interfering RNAs, to improve cellular uptake, to enhance cellular targeting, to assist in tracing the interfering RNA, to further improve stability, or to reduce the potential for activation of the interferon pathway. For example, interfering RNAs may comprise a purine nucleotide at the ends of overhangs. Conjugation of cholesterol to the 3' end of the sense strand or the 5' end of the antisense strand may be made in a manner consistent with those described herein. Examples of such conjugations include cholesterol conjugates, cholesterol conjugates having a biotin moiety, or cholesterol conjugates having another appropriate biotin moiety. Chemically synthesized interfering RNAs, produced from protected ribonucleoside phosphoramidites using a conventional DNA/RNA synthesizer, may be obtained from commercial suppliers such as Ambion Inc. (Austin, Tex.), Invitrogen (Carlsbad, Calif.), or Dharmacon (Lafayette, Colo.). Interfering RNAs are purified by extraction with a solvent or resin, precipitation, electrophoresis, chromatography, or a combination thereof, for example. Alternatively, interfering RNA may be used with little if any purification to avoid losses due to sample processing.

[0085] Interfering RNAs can also be expressed endogenously from plasmid or viral expression vectors or from minimal expression cassettes, for example, PCR generated fragments comprising one or more promoters and an appropriate template or templates for the interfering RNA. Examples of commercially available plasmid-based expression vectors for shRNA include members of the pSilencer series (Ambion, Austin, Tex.) and pCPG-siRNA (InvivoGen, San Diego, Calif.). Viral vectors for expression of interfering RNA may be derived from a variety of viruses including adenovirus, adeno-associated virus, lentivirus (e.g., HIV, FIV, and EIAV), and herpes viruses. Examples of commercially available viral vectors for shRNA expression include pSilencer adenovirus (Ambion, Austin, Tex.) and pLent1/ BLOCK-iTM-DEST (Invitrogen, Carlsbad, Calif.). Selection of viral vectors, methods for expressing the interfering RNA from the vector and methods of delivering the viral vector are within the ordinary skill of one in the art. Examples of kits for production of PCR-generated shRNA expression cassettes include Silencer Express (Ambion, Austin, Tex.) and siXpress (Mirus, Madison, Wis.). A first interfering RNA may be administered via in vivo expression from a first expression vector capable of expressing the first interfering RNA and a second interfering RNA may be administered via in vivo expression from a second expression vector capable of expressing the second interfering RNA, or both interfering RNAs may be administered via in vivo expression from a single expression vector capable of expressing both interfering RNAs.

[0086] Interfering RNAs may be expressed from a variety of eukaryotic promoters known to those of ordinary skill in the art, including pol III promoters, such as the U6 or H1 promoters, or pol II promoters, such as the cytomegalovirus promoter. Those of skill in the art will recognize that these promoters can also be adapted to allow inducible expression of the interfering RNA.

[0087] Hybridization under Physiological Conditions: In certain embodiments of the present invention, an antisense strand of an interfering RNA hybridizes with an mRNA in vivo as part of the RISC complex.

[0088] For example, high stringency conditions could occur at about 50% formamide at 37° C. to 42° C. Reduced stringency conditions could occur at about 35% to 25% formamide at 30° C. to 35° C. Examples of stringency conditions for hybridization are provided in Sambrook, J., 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Further examples of stringent hybridization conditions include 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50° C. or 70° C. for 12-16 hours followed by washing, or hybridization at 70° C. in 1xSSC or 50° C. in 1xSSC, 50% formamide followed by washing at 70° C. in 0.3xSSC, or hybridization at 70° C. in 4xSSC or 50° C. in 4xSSC, 50% formamide followed by washing at 67° C. in 1xSSC. The temperature for hybridization is about 5-10° C. less than the
melting temperature ($T_m$) of the hybrid where $T_m$ is determined for hybrids between 19 and 49 base pairs in length using the following calculation: $T_m = 81.5 + 16.6 \log [\text{Na}^+] + 0.41 \times (\% \text{G+C}) - (600/N)$ where $N$ is the number of bases in the hybrid, and $[\text{Na}^+]$ is the concentration of sodium ions in the hybridization buffer.

[0089] The above-described in vitro hybridization assay provides a method of predicting whether binding between a candidate siRNA and a target will have specificity. However, in the context of the RISC complex, specific cleavage of a target can also occur with an antisense strand that does not demonstrate high stringency for hybridization in vitro.

[0090] Single-stranded interfering RNA: As cited above, interfering RNAs ultimately function as single strands. Single-stranded (ss) interfering RNA has been found to effect mRNA silencing, albeit less efficiently than double-stranded RNA. Therefore, embodiments of the present invention also provide for administration of a ss interfering RNA that hybridizes under physiological conditions to a portion of SEQ ID NO:1 or SEQ ID NO:2 and has a region of at least near-perfect contiguous complementarity of at least 19 nucleotides with the hybridizing portion of SEQ ID NO:1 or SEQ ID NO:2, respectively. The ss interfering RNA of Table 1 or Table 2 has a length of 19 to 49 nucleotides as for the ds interfering RNA cited above. The ss interfering RNA has a 5’ phosphate or is phosphorylated in situ or in vivo at the 5’ position. The term “5’ phosphorylated” is used to describe, for example, polynucleotides or oligonucleotides having a phosphate group attached via ester linkage to the C5 hydroxyl of the sugar (e.g., ribose, deoxyribose, or an analog of same) at the 5’ end of the polynucleotide or oligonucleotide.

[0091] SS interfering RNAs are synthesized chemically or by in vitro transcription and expressed endogenously from vectors or expression cassettes as for ds interfering RNAs. 5’ Phosphate groups may be added via a kinase, or a 5’ phosphate may be the result of nuclease cleavage of an RNA. Delivery is as for ds interfering RNAs. In one embodiment, ss interfering RNAs having protected ends and nuclease resistant modifications are administered for silencing. SS interfering RNAs may be dried for storage or dissolved in an aqueous solution. The solution may contain buffers or salts to inhibit annealing or for stabilization.

[0092] Hairpin interfering RNA: A hairpin interfering RNA is a single molecule (e.g., a single oligonucleotide chain) that comprises both the sense and antisense strands of an interfering RNA in a stem-loop or hairpin structure (e.g., a shRNA). For example, shRNAs can be expressed from DNA vectors in which the DNA oligonucleotides encoding a sense interfering RNA strand are linked to the DNA oligonucleotides encoding the reverse complementary antisense interfering RNA strand by a spacer. If needed for the chosen expression vector, 3’ terminal T’s and nucleotides forming restriction sites may be added. The resulting RNA transcript folds back onto itself to form a stem-loop structure.

[0093] Mode of administration: Interfering RNA may be delivered via aerosol, buccal, dermal, intradermal, inhalation, intramuscular, intranasal, intraocular, intrapulmonary, intravenous, intraperitoneal, nasal, ocular, oral, parenteral, patch, subcutaneous, sublingual, topical, or transdermal administration, for example.

[0094] Administration may be directly to the eye by ocular tissue administration such as periorcular, conjunctival, subtenon, intracameral, intravitreal, intracocular, subconjunctival, retrobulbar, intracanalicul, or suprachoroidal administration; by injection, by direct application to the eye using a catheter or other placement device such as a retinal pellet, intraocular insert, suppository or an implant comprising a porous, non-porous, or gelatinous material; by topical ocular drops or ointments; or by a slow release device in the cul-de-sac or implanted adjacent to the sclera (transscleral) or within the eye. Intracameral injection may be through the cornea into the anterior chamber to allow the agent to reach the trabecular meshwork. Intracameral injection may be into the venous collector channels draining Schlemm’s canal or into Schlemm’s canal.

[0095] Administration may be directly to the ear via, for example, topical otic drops or ointments, slow release devices in the ear or implanted adjacent to the ear. Local administration includes otic intramuscular, intratympanic cavity and intracochlear injection routes of administration. Furthermore, agents can be administered to the inner ear by placement of a gelfoam, or similar absorbent and adherent product, soaked with the interfering RNA against the window membrane of the middle/inner ear or adjacent structure.

[0096] Administration may be directly to the lungs, via, for example, an aerosolized preparation, and by inhalation via an inhaler or a nebulizer, for example.

[0097] Further modes of administration include tablets, pills, and capsules, all of which are capable of formulation by one of ordinary skill in the art.

[0098] Subject: A subject in need of treatment for a TNFα-related condition or at risk for developing a TNFα-related condition is a human or other mammal having a TNFα-related inflammatory condition or having dry eye or at risk of developing a TNFα-related inflammatory condition or dry eye. A TNFα-related inflammatory condition includes, for example, allergic conjunctivitis, ocular inflammation, dermatitis, rhinitis, or asthma associated with undesired or inappropriate activity of TNFα as cited herein.

[0099] Ocular structures associated with a TNFα-related condition may include the eye, retina, choroid, lens, cornea, trabecular meshwork, iris, optic nerve, optic nerve head, sclera, aqueous chamber, vitreous chamber, ciliary body, or posterior segment, for example.

[0100] Otic structures associated with such disorders may include the inner ear, middle ear, outer ear, tympanic cavity or membrane, cochlea, or Eustachian tube, for example.

[0101] Pulmonary structures associated with such disorders may include the nose, mouth, pharynx, larynx, bronchial tubes, trachea, carina (the ridge separating the opening of the right and left main bronchi), and lungs, particularly the lower lungs, such as bronchioli and alveoli.

[0102] A subject may also be an otic cell, a lung cell, an ocular cell, cell culture, organ or an ex vivo organ or tissue.

[0103] Formulations and Dosage: Pharmaceutical formulations comprise interfering RNAs, or salts thereof, of the invention up to 99% by weight mixed with a physiologically acceptable carrier medium such as water, buffer, saline, glycine, hyaluronic acid, manniitol, and the like.

[0104] Interfering RNAs of the present invention are administered as solutions, suspensions, or emulsions. The following are examples of possible formulations embodied by this invention.
<table>
<thead>
<tr>
<th>Amount in weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interfering RNA</td>
</tr>
<tr>
<td>Hydroxypropylmethylcellulose</td>
</tr>
<tr>
<td>Sodium chloride</td>
</tr>
<tr>
<td>Benzalkonium Chloride</td>
</tr>
<tr>
<td>EDTA</td>
</tr>
<tr>
<td>NaOH/HCl</td>
</tr>
<tr>
<td>Purified water (RNase-free)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Amount in weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interfering RNA</td>
</tr>
<tr>
<td>Phosphate Buffered Saline</td>
</tr>
<tr>
<td>Benzalkonium Chloride</td>
</tr>
<tr>
<td>Polysorbate 80</td>
</tr>
<tr>
<td>Purified water (RNase-free)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Amount in weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interfering RNA</td>
</tr>
<tr>
<td>Monobasic sodium phosphate</td>
</tr>
<tr>
<td>Dibasic sodium phosphate (anhydrous)</td>
</tr>
<tr>
<td>Sodium chloride</td>
</tr>
<tr>
<td>Disodium EDTA</td>
</tr>
<tr>
<td>Cremophor EL</td>
</tr>
<tr>
<td>Benznalcohol chloride</td>
</tr>
<tr>
<td>HCl or NaOH</td>
</tr>
<tr>
<td>Purified water (RNase-free)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Amount in weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interfering RNA</td>
</tr>
<tr>
<td>Phosphate Buffered Saline</td>
</tr>
<tr>
<td>Hydroxypropyl-β-cyclodextrin</td>
</tr>
<tr>
<td>Purified water (RNase-free)</td>
</tr>
</tbody>
</table>

[0107] An effective amount of a formulation may depend on factors such as the age, race, and sex of the subject, the severity of the TNFα-related condition, the rate of target gene transcript/protein turnover, the interfering RNA potency, and the interfering RNA stability, for example. In one embodiment, the interfering RNA is delivered topically to a target organ and reaches TACE mRNA- or TNFR1 mRNA-containing tissue at a therapeutic dose thereby ameliorating a TNFα-related process.

[0108] Acceptable carriers: An acceptable carrier refers to those carriers that cause at most, little to no ocular irritation, provide suitable preservation if needed, and deliver one or more interfering RNAs of the present invention in a homogenous dosage. An acceptable carrier for administration of interfering RNA of embodiments of the present invention include the cationic lipid-based transfection reagents TransIT®-TKO (Mirus Corporation, Madison, Wis.), LIPOFECTAMINE®, Lipofectamine, OLI-GOFECTAMINETM (Invitrogen, Carlsbad, Calif.), or DHARMAFECTTM (Dharmacon, Lafayette, Colo.); polycations such as polyethyleneimine; cationic peptides such as Tat, polyarginine, or Penetratin (Anp peptide); or liposomes. Liposomes are formed from standard vesicle-forming lipids and a sterol, such as cholesterol, and may include a targeting molecule such as a monoclonal antibody having binding affinity for endothelial cell surface antigens, for example. Further, the liposomes may be PEGylated liposomes.

[0109] The interfering RNAs may be delivered in solution, in suspension, or in biodegradable or non-biodegradable delivery devices. The interfering RNAs can be delivered alone as components of defined, covalent conjugates. The interfering RNAs can also be complexed with cationic lipids, cationic peptides, or cationic polymers; complexed with proteins, fusion proteins, or protein domains with nucleic acid binding properties (e.g., protamine); or encapsulated in nanoparticles. Tissue- or cell-specific delivery can be accomplished by the inclusion of an appropriate targeting moiety such as an antibody or antibody fragment.

[0110] For ophthalmic, ocic, or pulmonary delivery, an interfering RNA may be combined with ophthalmologically, optically, or pulmonary acceptable preservatives, co-solvents, surfactants, viscosity enhancers, penetration enhancers, buffers, sodium chloride, or water to form an aqueous, sterile suspension or solution. Solution formulations may be prepared by dissolving the interfering RNA in a physiologically acceptable isotonic aqueous buffer. Further, the solutions may include an acceptable surfactant to assist in dissolving the inhibitor. Viscosity building agents, such as hydroxyethyl cellulose, hydroxyethyl cellulose, methylcellulose, polyvinylpyrrolidone, or the like may be added to the compositions of the present invention to improve the retention of the compound.

[0111] In order to prepare a sterile ointment formulation, the interfering RNA is combined with a preservative in an appropriate vehicle, such as mineral oil, liquid lanolin, or white petrolatum. Sterile gel formulations may be prepared by suspending the interfering RNA in a hydrophilic base formulated from the combination of, for example, CARBOPOL®-940 (BF Goodrich, Charlotte, N.C.), or the like, according to methods known in the art. VISCOCR® (Akorn Laboratories, Inc., Fort Worth, Tex.) may be used for intraocular injection, for example. Other compositions of the present invention may contain penetration enhancing agents such as cremephor and TWEEN® 80 (polyoxyethylene...
sorbitan monolaureate, Sigma Aldrich, St. Louis, Mo.), in the event the interfering RNA is less penetrating in the organ or tissue of interest.

[0112] Kits: Embodiments of the present invention provide a kit that includes reagents for attenuating the expression of an mRNA as cited herein in a cell. The kit contains an siRNA or an shRNA expression vector. For siRNAs and non-viral shRNA expression vectors the kit also may contain a transfection reagent or other suitable delivery vehicle. For viral shRNA vectors, the kit may contain the viral vector and/or the necessary components for viral vector production (e.g., a packaging cell line as well as a vector comprising the viral vector template and additional helper vectors for packaging). The kit may also contain positive and negative control siRNAs or shRNA expression vectors (e.g., a non-targeting control siRNA or an siRNA that targets an unrelated mRNA). The kit also may contain reagents for assessing knockdown of the intended target gene (e.g., primers and probes for quantitative PCR to detect the target mRNA and/or antibodies against the corresponding protein for western blots). Alternatively, the kit may comprise an siRNA sequence or an shRNA sequence and the instructions and materials necessary to generate the siRNA by in vitro transcription or to construct a shRNA expression vector.

[0113] A pharmaceutical combination in kit form is further provided that includes, in packaged combination, a carrier means adapted to receive a container means in close confinement therewith and a first container means including an interfering RNA composition and an acceptable carrier. Such kits can further include, if desired, one or more of various conventional pharmaceutical kit components, such as, for example, containers with one or more pharmaceutically acceptable carriers, additional containers, etc., as will be readily apparent to those skilled in the art. Printed instructions, either as inserts or as labels, indicating quantities of the components to be administered, guidelines for administration, and/or guidelines for mixing the components, can also be included in the kit.

[0114] The ability of TACE- or TNFR1-interfering RNA to knock-down the levels of endogenous TACE or TNFR1 expression in, for example, human corneal epithelial cells is evaluated in vitro as follows. Transformed human corneal epithelial cells, for example, the CEPI-T7 cell line (Offord et al. (1999) Invest Ophthalmol Vis Sci. 40:1091-1101), are plated 24 h prior to transfection in KGM keratinoocyte medium (Cambrex, East Rutherford, N.J.). Transfection is performed using DharmaFECT™ 1 (Dharmacon, Lafayette, Colo.) according to the manufacturer’s instructions at TACE- or TNFR1-interfering RNA concentrations ranging from 0.1 nM-100 nM. Non-targeting control interfering RNA and lamin A/C interfering RNA (Dharmacon) are used as controls. Target mRNA levels are assessed by qPCR 24 h post-transfection using, for example, TaqMAN® forward and reverse primers and a probe set that encompasses the target site (Applied Biosystems, Foster City, Calif.). Target protein levels may be assessed approximately 72 h post-transfection (actual time dependent on protein turnover rate) by western blot, for example. Standard techniques for RNA and/or protein isolation from cultured cells are well-known to those skilled in the art. To reduce the chance of non-specific, off-target effects, the lowest possible concentration of TACE- or TNFR1 interfering RNA is used that produces the desired level of knock-down in target gene expression.

[0115] Those of skill in the art, in light of the present disclosure, will appreciate that obvious modifications of the embodiments disclosed herein may be made without departing from the spirit and scope of the invention. All of the embodiments disclosed herein can be made and executed without undue experimentation in light of the present disclosure. The full scope of the invention is set out in the disclosure and equivalent embodiments thereof. The specification should not be construed to unduly narrow the full scope of protection to which the present invention is entitled.

[0116] While a particular embodiment of the invention has been shown and described, numerous variations and alternate embodiments will occur to those skilled in the art. Accordingly, it is intended that the invention be limited only in terms of the appended claims.

[0117] The invention may be embodied in other specific forms without departing from its spirit or essential characteristics. The described embodiments are to be considered in all respects only as illustrative and not restrictive. The scope of the invention is, therefore, indicated by the appended claims rather than by the foregoing description. All changes to the claims that come within the meaning and range of equivalency of the claims are to be embraced within their scope. Further, all published documents, patents, and applications mentioned herein are hereby incorporated by reference, as if presented in their entirety.

EXAMPLE 1

Interfering RNA for Specifically Silencing TNFR1 in GTM-3 Cells

[0118] The present study examines the ability of TNFR1 interfering RNA to knock down the levels of endogenous TNFR1 protein expression in cultured GTM-3 cells.

[0119] Transfection of GTM-3 cells (Pang, I. H. et al., 1994. *Curr. Eye Res. 13:51-63*) was accomplished using standard in vitro concentrations (0.1-10 nM) of TNFR1 siRNAs, siCONTROL RISC-free siRNA #1, or siCONTROL Non-targeting siRNA #2 (NTC2) and DharmaFECT™ #1 transfection reagent (Dharmacon, Lafayette, Colo.). All siRNAs were dissolved in 1x siRNA buffer, an aqueous solution of 20 mM KCl, 6 mM HEPES (pH 7.5), 0.2 mM MgCl₂. Control samples included a buffer control in which the volume of siRNA was replaced with an equal volume of 1x siRNA buffer (-siRNA). Western blots using an anti-TNFRI antibody (Santa Cruz Biotechnology, Santa Cruz, Calif.) were performed to assess TNFRI protein expression. The TNFR1 siRNAs are double-stranded interfering RNAs having specificity for the following targets: siTNFR1 #1 targets the sequence CAAAGGAACCUAC-UUGUAC (SEQ ID NO: 202); siTNFR1 #2 targets the sequence GAGCUUGAACUGAUCA (SEQ ID NO: 203); siTNFR1 #3 targets the sequence CACAGGCUAC-UUGUAC (SEQ ID NO: 204); siTNFR1 #4 targets the sequence UCAAGCUACGCCAUG (SEQ ID NO: 205). As shown by the data in FIG. 1, siTNFR1 #1, siTNFR1 #2, and siTNFR1 #3 siRNAs reduced TNFR1 protein expression significantly at the 10 nM and 1 nM concentrations relative to the control siRNAs, but exhibited reduced efficacy at 0.1 nM. The siTNFR1 #2 and siTNFR1 #3 siRNAs were particularly effective. The siTNFR1 #4 siRNA also showed a concentration dependent reduction in TNFRI protein expression as expected.
SEQUENCE LISTING

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420
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**LOCATION:** (20)...(21)  
**OTHER INFORMATION:** any, A, T/U, C, G

**SEQUENCE:**
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**FEATURE:**  
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SEQ ID NO 12
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SEQ ID NO 13
LENGTH: 27
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ORGANISM: Artificial
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**TYPE:** DNA

**ORGANISM:** Artificial

**FEATURE:** OTHER INFORMATION: Target Sequence

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**LENGTH:** 19

**TYPE:** DNA

**ORGANISM:** Artificial

**FEATURE:** OTHER INFORMATION: Target Sequence

**SEQUENCE:** 19
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**LENGTH:** 19

**TYPE:** DNA

**ORGANISM:** Artificial

**FEATURE:** OTHER INFORMATION: Target Sequence

**SEQUENCE:** 19
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**LENGTH:** 19

**TYPE:** DNA

**ORGANISM:** Artificial

**FEATURE:** OTHER INFORMATION: Target Sequence

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**LENGTH:** 19

**TYPE:** DNA

**ORGANISM:** Artificial

**FEATURE:** OTHER INFORMATION: Target Sequence

**SEQUENCE:** 19
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**SEQ ID NO:** 77

**LENGTH:** 19

**TYPE:** DNA

**ORGANISM:** Artificial

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TYPE: DNA
ORGANISM: Artificial
FEATURE:
OTHER INFORMATION: Target Sequence

SEQUENCE: 19
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SEQ ID NO 79
LENGTH: 19
TYPE: DNA
ORGANISM: Artificial
FEATURE:
OTHER INFORMATION: Target Sequence

SEQUENCE: 19
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SEQ ID NO 80
LENGTH: 19
TYPE: DNA
ORGANISM: Artificial
FEATURE:
OTHER INFORMATION: Target Sequence

SEQUENCE: 19
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SEQ ID NO 81
LENGTH: 19
TYPE: DNA
ORGANISM: Artificial
FEATURE:
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SEQUENCE: 19
ttgaagcagc tactactaa

SEQ ID NO 82
LENGTH: 19
TYPE: DNA
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FEATURE:
OTHER INFORMATION: Target Sequence

SEQUENCE: 19
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SEQ ID NO 83
LENGTH: 19
TYPE: DNA
ORGANISM: Artificial
FEATURE:
OTHER INFORMATION: Target Sequence

SEQUENCE: 19
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SEQ ID NO 84
LENGTH: 19
TYPE: DNA
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FEATURE:
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SEQUENCE: 19
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SEQ ID NO 85
LENGTH: 19
TYPE: DNA
ORGANISM: Artificial
FEATURE:
OTHER INFORMATION: Target Sequence

SEQUENCE: 19
acaagccaca gagcctaga

SEQ ID NO 86
LENGTH: 19
TYPE: DNA
ORGANISM: Artificial
FEATURE:
OTHER INFORMATION: Target Sequence

SEQUENCE: 19
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SEQ ID NO 87
LENGTH: 19
TYPE: DNA
ORGANISM: Artificial
FEATURE:
OTHER INFORMATION: Target Sequence

SEQUENCE: 19
tgtacgccgt ggtggagaa

SEQ ID NO 88
LENGTH: 19
TYPE: DNA
ORGANISM: Artificial
FEATURE:
OTHER INFORMATION: Target Sequence

SEQUENCE: 19
acaagccaca gagcctaga

SEQ ID NO 89
LENGTH: 19
TYPE: DNA
ORGANISM: Artificial
FEATURE:
OTHER INFORMATION: Target Sequence

SEQUENCE: 19
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SEQ ID NO 90
LENGTH: 19
TYPE: DNA
ORGANISM: Artificial
FEATURE:
OTHER INFORMATION: Target Sequence

SEQUENCE: 19
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SEQ ID NO 91
LENGTH: 19
TYPE: DNA
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FEATURE:
OTHER INFORMATION: Target Sequence

SEQUENCE: 19
tgtacgccgt ggtggagaa

SEQ ID NO 92
LENGTH: 19
TYPE: DNA
ORGANISM: Artificial
FEATURE:
OTHER INFORMATION: Target Sequence

SEQUENCE: 19
tgtacgccgt ggtggagaa

SEQ ID NO 93
LENGTH: 19
TYPE: DNA
ORGANISM: Artificial
FEATURE:
OTHER INFORMATION: Target Sequence

SEQUENCE: 19
tgtacgccgt ggtggagaa

SEQ ID NO 94
LENGTH: 19
TYPE: DNA
ORGANISM: Artificial
FEATURE:
OTHER INFORMATION: Target Sequence

SEQUENCE: 19
tgtacgccgt ggtggagaa

SEQ ID NO 95
LENGTH: 19
TYPE: DNA
ORGANISM: Artificial
FEATURE:
OTHER INFORMATION: Target Sequence

SEQUENCE: 19
tgtacgccgt ggtggagaa

SEQ ID NO 96
LENGTH: 19
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FEATURE:
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TYPE: DNA
ORGANISM: Artificial
FEATURE:
OTHER INFORMATION: Target Sequence

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TYPE: DNA
ORGANISM: Artificial
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OTHER INFORMATION: Target Sequence

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TYPE: DNA
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FEATURE:
OTHER INFORMATION: Target Sequence

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TYPE: DNA
ORGANISM: Artificial
FEATURE:
OTHER INFORMATION: Target Sequence

SEQ ID NO 152
LENGTH: 19
TYPE: DNA
ORGANISM: Artificial
FEATURE:
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SEQ ID NO 153
LENGTH: 19
TYPE: DNA
ORGANISM: Artificial
FEATURE:
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aattattggt ggttagcaga 19

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atcatgcctt ctacagata 19

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atcatgggc agaggggaa 19

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ggsgagaggg ggaggagagt 19

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ggsgagaggt acaacactca 19

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gaagagagta caactacaa 19

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gaggtacacca ctacaa att 19

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GCTAATGAC AGATGGAT 19

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CGGAACACTT CATGGGATA 19

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GGATAATGCA GGTAAATTAA 19

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AGGCTATGGA ATACAGATA 19

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GAAATACAGAT AGAGCAGAT 19

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GGTAATACTG GGTAAAAG 19

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tatggaact ctggatta

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tgacgagcac aaagaat

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gacaagaaatatggtaa

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gttacact ctgatttg
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<223> OTHER INFORMATION: Target Sequence

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actcatgaat tgggacata

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gtgccgatca cgagaacaa

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cataagacc attgaaagt

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<400> SEQUENCE: 188
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gcaataagtc tgtggaac

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<400> SEQUENCE: 191
gaagggtgat gaggagaa

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ggtgagaagga gaggagtgat

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<400> SEQUENCE: 194
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gatggcata ctaagatga

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<400> SEQUENCE: 196
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<400> SEQUENCE: 197
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<210> SEQ ID NO 198
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<400> SEQUENCE: 190

ggataaacag tagatctct

<210> SEQ ID NO 199
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<212> TYPE: DNA
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<400> SEQUENCE: 199
cotttaaact gcagegtca

<210> SEQ ID NO 200
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Target Sequence

<400> SEQUENCE: 200
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<210> SEQ ID NO 201
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<212> TYPE: DNA
<213> ORGANISM: Artificial
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<400> SEQUENCE: 201
gcaagaacaagagtgctca

<210> SEQ ID NO 202
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<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
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<400> SEQUENCE: 202
caaaggaacc uacuuguac

<210> SEQ ID NO 203
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial
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<400> SEQUENCE: 203
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<210> SEQ ID NO 204
<211> LENGTH: 19
<212> TYPE: RNA
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<220> FEATURE:
What is claimed is:

1. An interfering RNA for inhibiting the expression of a tumor necrosis factor α converting enzyme (TACE) gene, wherein the interfering RNA comprises a sense strand and an antisense strand each 19-49 nucleotides in length, wherein the antisense strand comprises a nucleotide sequence that is complementary to any of SEQ ID NO:3, SEQ ID NO:14-SEQ ID NO:58, or SEQ ID NO:155-SEQ ID NO:201 except that the T’s can be T’s or U’s, and the antisense strand comprises a nucleotide sequence that is complementary to any of SEQ ID NO:59-SEQ ID NO:154, SEQ ID NO:202-SEQ ID NO:204; and a pharmaceutically acceptable carrier.

2. The compositions of claim 1, wherein each strand of the interfering RNA molecule is 19 to 27 nucleotides in length.

3. The interfering RNA of claim 1, wherein the interfering RNA comprises one or more chemically modified nucleotides, one or more deoxyribonucleotides, and/or one or more non-phosphodiester linkages.

4. The interfering RNA of claim 3, wherein one or more of the chemically modified nucleotides have a sugar modification selected from the group consisting of: a 2’ amino group, a 2’ O-methyl group, and a 2’ methoxethyl group.

5. The interfering RNA of claim 3, wherein the interfering RNA comprises one or more chemically modified nucleotides and one or more non-phosphodiester linkages.

6. The interfering RNA of claim 3, wherein the non-nucleotide material is bound to the 5’ end and/or 3’ end of the sense strand and/or the antisense strand.

7. The interfering RNA of claim 3, wherein the non-nucleotide material is bound internally to the sense strand and/or the antisense strand.

8. The interfering RNA of claim 6, wherein the non-nucleotide material improves cellular uptake, enhances cellular targeting, assists in tracing, improves stability, and/or reduces activation of the interferon pathway.

9. The interfering RNA of claim 3, wherein the sense strand and/or the antisense strand contains a 3’ overhang.

10. The interfering RNA of claim 3, wherein the sense strand and/or the antisense strand contains a 5’ overhang.

11. The interfering RNA of claim 3, wherein the interfering RNA molecule at least one blunt end.

12. A composition for inhibiting the expression of a tumor necrosis factor α converting enzyme (TACE) gene comprising:

13. The composition of claim 12, wherein the interfering RNA comprises one or more chemically modified nucleotides, one or more deoxyribonucleotides, and/or one or more non-phosphodiester linkages.

14. The composition of claim 13, wherein one or more of the chemically modified nucleotides have a sugar modification selected from the group consisting of: a 2’ amino group, a 2’ O-methyl group, and a 2’ methoxethyl group.

15. The composition of claim 13, wherein the interfering RNA comprises one or more chemically modified nucleotides and one or more non-phosphodiester linkages.

16. The composition of claim 13, wherein non-nucleotide material is bound to the 5’ end and/or 3’ end of the sense strand and/or the antisense strand.

17. The composition of claim 13, wherein the non-nucleotide material is bound internally to the sense strand and/or the antisense strand.

18. The composition of claim 16, wherein the non-nucleotide material improves cellular uptake, enhances cellular targeting, assists in tracing, improves stability, and/or reduces activation of the interferon pathway.

19. The composition of claim 14, wherein the sense strand and/or the antisense strand contains a 3’ overhang and/or a 5’ overhang.

20. An interfering RNA for inhibiting the expression of a tumor necrosis factor α receptor-1 (TNFR1) wherein the interfering RNA comprises a sense strand and an antisense strand, wherein said antisense strand is complementary to any of any of SEQ ID NO:59-SEQ ID NO:154, or SEQ ID NO:202-SEQ ID NO:204.