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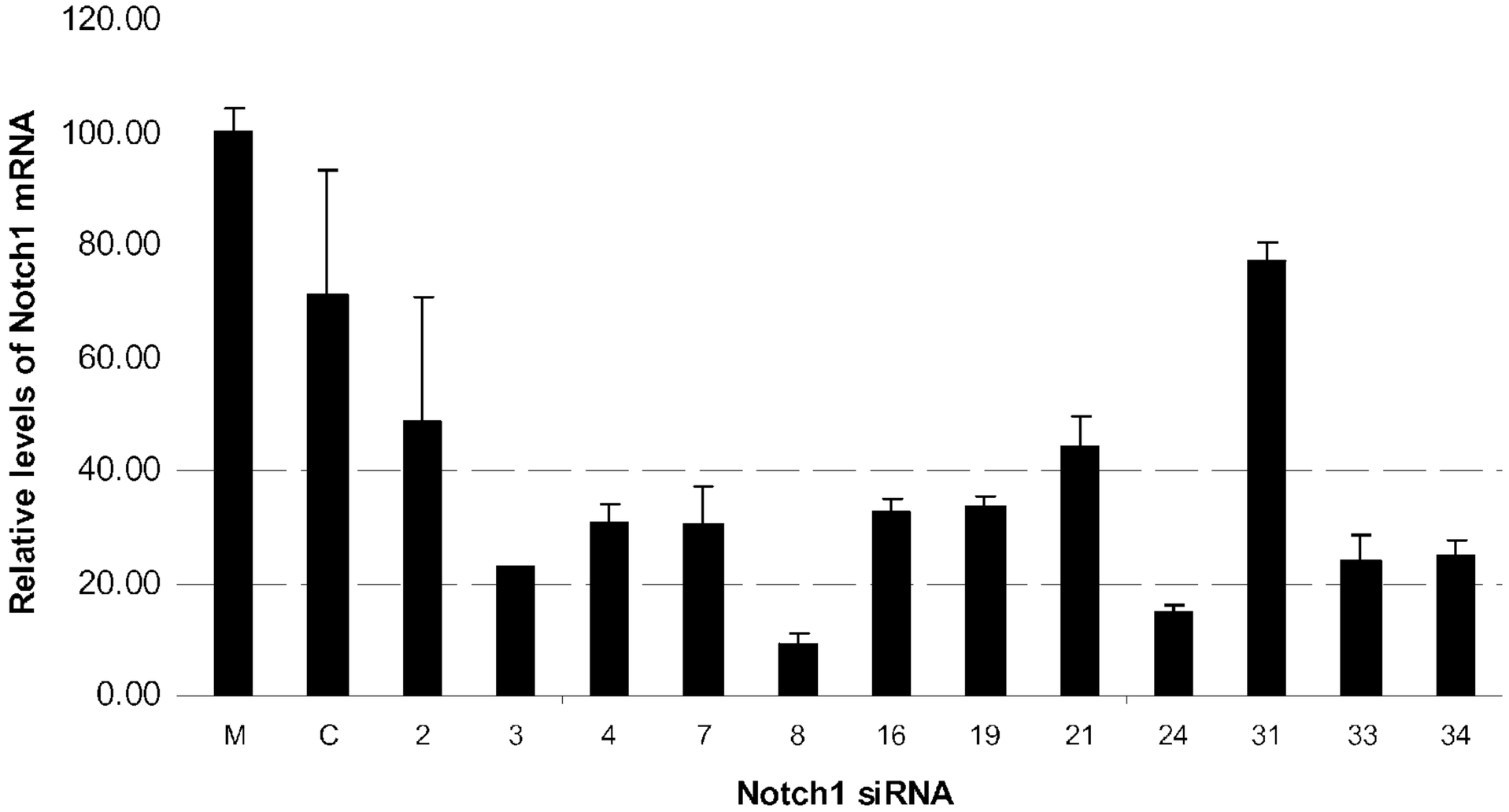
(54) Titre : COMPOSITIONS COMPRENANT DU SIARN NOTCH1 ET PROCEDES D'UTILISATION DE CELLES-CI
(54) Title: COMPOSITIONS COMPRISING NOTCH1 SIRNA AND METHODS OF USE THEREOF



Knockdown of human Notch 1 mRNA in HepG2 cells transfected with 3 nM of Notch1 siRNA at 72 hours post-transfection.

FIG. 2

(57) Abrégé/Abstract:
The present invention provides siRNA nucleic acid molecules that inhibit Notch1 expression. Methods of using the nucleic acid molecules are also provided.

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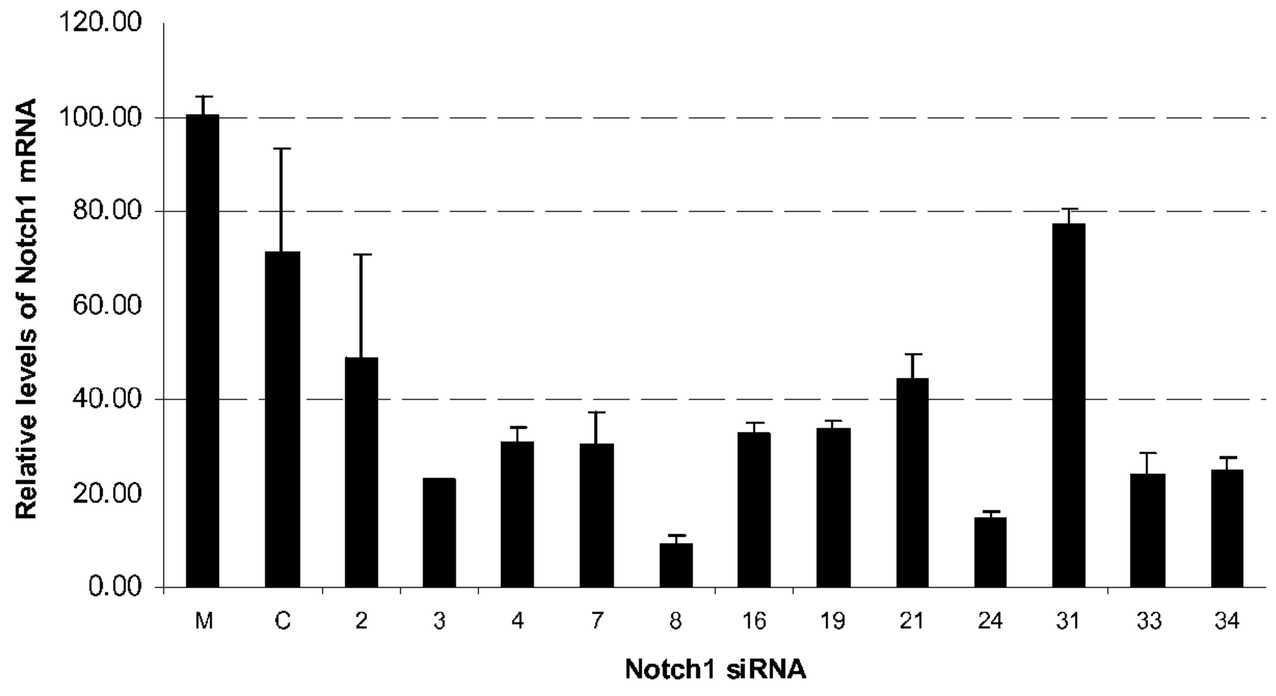

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WO 2009/114726 A1

COMPOSITIONS COMPRISING NOTCH1 SIRNA AND METHODS OF USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. provisional patent application no. 61/035,995 filed march 12, 2008 which provisional application is incorporated herein by reference in its entirety.

STATEMENT REGARDING SEQUENCE LISTING

The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is 480251_411PC_SEQUENCE_LISTING.txt. The text file is 100 KB, was created on March 12, 2009, and is being submitted electronically via EFS-Web, concurrent with the filing of the specification.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to siRNA molecules for modulating the expression of Notch1.

Description of the Related Art

The Notch signaling network is an evolutionarily conserved intercellular signaling pathway which regulates interactions between physically adjacent cells. In Drosophilia, notch interaction with its cell-bound ligands (delta, serrate) establishes an intercellular signaling pathway that plays a key role in development. Homologues of the notch ligands have also been identified

in human, but precise interactions between these ligands and the human notch homologues remain to be determined.

Notch receptors 1-4 are transmembrane receptor proteins that signal through a pathway that relies on regulated proteolysis. Following ligand-binding, the receptor is sequentially: i) cleaved extracellularly by metalloproteases of the Adam family (Brou, et al., Mol. Cell 5:207-216 (2000); Mumm, et al. Mol Cell 5:197-206 (2000)); ii) mono-ubiquitinated on a lysine residue lying just internal to the transmembrane domain (Gupta-Rossi, et al., J. Cell Biol. 166:73-83 (2004)); iii) endocytosed (Gupta-Rossi, et al., J. Cell Biol. 166:73-83 (2004)), and iv) proteolytically cleaved by a gamma-secretase enzyme (De Strooper, et al., Nature 398:518-522 (1999)). This final step in the activation process permits the intracellular portion of Notch receptors to translocate to the cell nucleus where it interacts with transcription factors to alter gene activity. Notch receptor signaling appears to play an important role in the differentiation and proliferation of cells and in controlling apoptosis, three processes that are important with respect to neoplastic transformation (see U.S. Pat. No. 6,703,221).

The Notch1 gene was discovered through its involvement in a (7;9) chromosomal translocation found in fewer than 1% of T-cell acute lymphoblastic leukemias (T-ALLs) (Ellisen, et al., Cell 66:649-661 (1991)). Notch1 is highly expressed in thymocytes (Ellisen, et al., Cell 66:649-661(1991)), where it induces common lymphoid progenitors to adopt a T cell fate (Radtke, et al., Immunity 10:547-558). Subsequently, it promotes the assembly of pre-T cell receptor complexes which play a critical role in driving a proliferative burst that accompanies maturation of CD4-/CD8-thymocytes to the CD4+/CD8+ developmental stage (Wolfer, et al., Immunity 16:869-879 (2002)).

In its resting state, mature Notch1 is a heterodimeric receptor comprised of a ligand-binding extracellular subunit (NEC) and a non-covalently associated transmembrane subunit (NTM) (Rand, et al., Mol. Cell. Biol. 20:1825-1835; Logeat, et al., Proc. Natl. Acad. Sc.i USA 95:8108-8112 (1998)).

NEC consists of a ligand-binding domain comprised of epidermal growth factor-like repeats, three iterated Lin12/NOTCH repeats, and a conserved 103 amino acid sequence (hereafter termed HD, for heterodimerization domain) that is sufficient for association with the extracellular portion of NTM.

Physiologic activation of Notch receptors occurs when a ligand of the Delta-Serrate-Lag2 (DSL) family binds to the NEC subunit and initiates a cascade of successive proteolytic cleavages in the NTM subunit. The final cleavage, which is catalyzed by γ-secretase, a multiprotein complex containing presenilin-1 or -2, nicastrin, APH-1, and PEN-2 (Francis, et al., Dev. Cell 3:85-97 (2002); Kimberly, et al., Proc. Natl. Acad. Sci. USA 100:6382-6387 (2003)) releases the intracellular part of NTM (called intracellular Notch, or ICN) from the membrane, permitting it to translocate to the cell nucleus. There, it associates with the DNA-binding factor CSL and co-activators of the Mastermind family to form a short-lived transcriptional activation complex (Wallberg, et al., Mol. Cell Biol. 22:7812-7819 (2002); Fryer, et al., Genes Dev. 16:1397-1411 (2002); Nam, et al., J. Biol. Chem. 278:21232-21239 (2003)) Degradation and turnover of the complex is apparently regulated by F-box factors of the SEL-10 family (Oberg, et al., J. Biol. Chem. 276:35847-35853 (2001)).

The (7;9) translocation creates a Notch-T cell receptor-β fusion gene that encodes N-terminally-deleted, constitutively active Notch1 polypeptides similar to the ICN (Ellisen, et al., Cell 66:649-661 (1991); Aster, et al., Cold Spring Harb. Symp. Quant. Biol. 59:125-136 (1994); Das, et al., J. Biol. Chem., epublished May 3, 2004)) and these truncated and constitutively active forms of Notch1 induce T-ALL in mouse models (Aster, et al., Mol. Cell Biol. 20:7505-7515 (2000)). Notch1 is also the site of frequent retroviral insertions that cooperate with the E2A-PBX1 and cMYC transgenes in multistep pathways leading to the development of murine T-ALL (Hoemann, et al., Mol. Cell. Biol. 20:3831-3842 (2000); Feldman, et al., Blood 96:1906-1913(2000)). Further, Notch inhibitors cause a G_0/G_1 cell cycle arrest in cell lines derived from human

and murine Notch1-associated T-ALLs, indicating that Notch signaling is required for the sustained growth of these leukemias (Weng, et al., Mol. Cell Biol. 23:655-664 (2003)).

The (7;9) translocation has only been associated with a very small percentage of patients having T-ALL. The identification of additional mutations associated with Notch1 would have important implications for the pathogenesis of this type of cancer. Although mutations in Notch-2, Notch -3, and Notch -4 have not been identified in human cancer, it is known that abnormal increases in function of these Notch receptors in other mammals can cause T-ALL (Notch-2 and -3, Bellavia, et al., Embo J. 19:3337-3348 (2000); Rohn, J. Virol. 70:8071-8080 (1996); Weng, et al., Mol. Cell. Biol. 23:655-664)) and breast cancer (NOTCH-4, Callahan and Rafat, J. Mammary Gland Biol Neoplasia 6:23-36 (2001)).

Notch1 signaling has been shown to be associated with diseases including aortic valve disease and a variety of cancers. Therefore, identification of compound(s) that inhibit the Notch1 pathway could be a potential strategy to treat diseases associated with expression of Notch1. The present invention provides this and other advantages.

RNAi technology is emerging as an effective means for reducing the expression of specific gene products and may therefore prove to be uniquely useful in a number of therapeutic, diagnostic, and research applications for the modulation of expression of Notch1. The present invention provides compositions and methods for modulating expression of these proteins using RNAi technology.

The following is a discussion of relevant art pertaining to RNAi. The discussion is provided only for understanding of the invention that follows. The summary is not an admission that any of the work described below is prior art to the claimed invention.

RNA interference refers to the process of sequence-specific post-transcriptional gene silencing in animals mediated by short interfering RNAs

(siRNAs) (Zamore *et al.*, 2000, Cell, 101, 25-33; Fire *et al.*, 1998, Nature, 391, 806; Hamilton *et al.*, 1999, Science, 286, 950-951; Lin *et al.*, 1999, Nature, 402, 128-129; Sharp, 1999, Genes & Dev., 13, 139-141; and Strauss, 1999, Science, 286, 886). The corresponding process in plants (Heifetz *et al.*, International PCT Publication No. WO 99/61631) is commonly referred to as post-transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes and is commonly shared by diverse flora and phyla (Fire *et al.*, 1999, Trends Genet., 15, 358). Such protection from foreign gene expression may have evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or from the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA. The presence of dsRNA in cells triggers the RNAi response through a mechanism that has yet to be fully characterized. This mechanism appears to be different from other known mechanisms involving double stranded RNA-specific ribonucleases, such as the interferon response that results from dsRNA-mediated activation of protein kinase PKR and 2´,5´-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L (see for example U.S. Pat. Nos. 6,107,094; 5,898,031; Clemens *et al.*, 1997, J. Interferon & Cytokine Res., 17, 503-524; Adah *et al.*, 2001, Curr. Med. Chem., 8, 1189).

The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as dicer (Bass, 2000, Cell, 101, 235; Zamore *et al.*, 2000, Cell, 101, 25-33; Hammond *et al.*, 2000, Nature, 404, 293). Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs) (Zamore *et al.*, 2000, Cell, 101, 25-33; Bass, 2000, Cell, 101, 235; Berstein *et al.*, 2001, Nature, 409, 363). Short interfering RNAs derived from dicer activity are typically about 21 to about 23

nucleotides in length and comprise about 19 base pair duplexes (Zamore *et al.*, 2000, Cell, 101, 25-33; Elbashir *et al.*, 2001, Genes Dev., 15, 188). Dicer has also been implicated in the excision of 21- and 22-nucleotide small temporal RNAs (stRNAs) from precursor RNA of conserved structure that are implicated in translational control (Hutvagner *et al.*, 2001, Science, 293, 834). The RNAi response also features an endonuclease complex, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded RNA having sequence complementary to the antisense strand of the siRNA duplex. Cleavage of the target RNA takes place in the middle of the region complementary to the antisense strand of the siRNA duplex (Elbashir *et al.*, 2001, Genes Dev., 15, 188).

RNAi has been studied in a variety of systems. Fire *et al.*, 1998, Nature, 391, 806, were the first to observe RNAi in C. elegans. Bahramian and Zarbl, 1999, Molecular and Cellular Biology, 19, 274-283 and Wianny and Goetz, 1999, Nature Cell Biol., 2, 70, describe RNAi mediated by dsRNA in mammalian systems. Hammond *et al.*, 2000, Nature, 404, 293, describe RNAi in Drosophila cells transfected with dsRNA. Elbashir *et al.*, 2001, Nature, 411, 494 and Tuschl *et al.*, International PCT Publication No. WO 01/75164, describe RNAi induced by introduction of duplexes of synthetic 21-nucleotide RNAs in cultured mammalian cells including human embryonic kidney and HeLa cells.

The use of longer dsRNA has been described. For example, Beach *et al.*, International PCT Publication No. WO 01/68836, describes specific methods for attenuating gene expression using endogenously-derived dsRNA. Tuschl *et al.*, International PCT Publication No. WO 01/75164, describe a Drosophila in vitro RNAi system and the use of specific siRNA molecules for certain functional genomic and certain therapeutic applications; although Tuschl, 2001, Chem. Biochem., 2, 239-245, doubts that RNAi can be used to cure genetic diseases or viral infection due to the danger of activating interferon response. Li *et al.*, International PCT Publication No. WO 00/44914,

describe the use of specific long (141 bp-488 bp) enzymatically synthesized or vector expressed dsRNAs for attenuating the expression of certain target genes. Zernicka-Goetz *et al.*, International PCT Publication No. WO 01/36646, describe certain methods for inhibiting the expression of particular genes in mammalian cells using certain long (550 bp-714 bp), enzymatically synthesized or vector expressed dsRNA molecules. Fire *et al.*, International PCT Publication No. WO 99/32619, describe particular methods for introducing certain long dsRNA molecules into cells for use in inhibiting gene expression in nematodes. Plaetinck *et al.*, International PCT Publication No. WO 00/01846, describe certain methods for identifying specific genes responsible for conferring a particular phenotype in a cell using specific long dsRNA molecules. Mello *et al.*, International PCT Publication No. WO 01/29058, describe the identification of specific genes involved in dsRNA-mediated RNAi. Pachuck *et al.*, International PCT Publication No. WO 00/63364, describe certain long (at least 200 nucleotide) dsRNA constructs. Deschamps Depaillette *et al.*, International PCT Publication No. WO 99/07409, describe specific compositions consisting of particular dsRNA molecules combined with certain anti-viral agents. Waterhouse *et al.*, International PCT Publication No. 99/53050 and 1998, PNAS, 95, 13959-13964, describe certain methods for decreasing the phenotypic expression of a nucleic acid in plant cells using certain dsRNAs. Driscoll *et al.*, International PCT Publication No. WO 01/49844, describe specific DNA expression constructs for use in facilitating gene silencing in targeted organisms.

Others have reported on various RNAi and gene-silencing systems. For example, Parrish *et al.*, 2000, Molecular Cell, 6, 1077-1087, describe specific chemically-modified dsRNA constructs targeting the unc-22 gene of C. elegans. Grossniklaus, International PCT Publication No. WO 01/38551, describes certain methods for regulating polycomb gene expression in plants using certain dsRNAs. Churikov *et al.*, International PCT Publication No. WO 01/42443, describe certain methods for modifying genetic

characteristics of an organism using certain dsRNAs. Cogoni *et al.*, International PCT Publication No. WO 01/53475, describe certain methods for isolating a Neurospora silencing gene and uses thereof. Reed *et al.*, International PCT Publication No. WO 01/68836, describe certain methods for gene silencing in plants. Honer *et al.*, International PCT Publication No. WO 01/70944, describe certain methods of drug screening using transgenic nematodes as Parkinson's Disease models using certain dsRNAs. Deak *et al.*, International PCT Publication No. WO 01/72774, describe certain Drosophila-derived gene products that may be related to RNAi in Drosophila. Arndt *et al.*, International PCT Publication No. WO 01/92513 describe certain methods for mediating gene suppression by using factors that enhance RNAi. Tuschl *et al.*, International PCT Publication No. WO 02/44321, describe certain synthetic siRNA constructs. Pachuk *et al.*, International PCT Publication No. WO 00/63364, and Satishchandran *et al.*, International PCT Publication No. WO 01/04313, describe certain methods and compositions for inhibiting the function of certain polynucleotide sequences using certain long (over 250 bp), vector expressed dsRNAs. Echeverri *et al.*, International PCT Publication No. WO 02/38805, describe certain C. elegans genes identified via RNAi. Kreutzer *et al.*, International PCT Publications Nos. WO 02/055692, WO 02/055693, and EP 1144623 B1 describes certain methods for inhibiting gene expression using dsRNA. Graham *et al.*, International PCT Publications Nos. WO 99/49029 and WO 01/70949, and AU 4037501 describe certain vector expressed siRNA molecules. Fire *et al.*, U.S. Pat. No. 6,506,559, describe certain methods for inhibiting gene expression in vitro using certain long dsRNA (299 bp-1033 bp) constructs that mediate RNAi. Martinez *et al.*, 2002, Cell, 110, 563-574, describe certain single stranded siRNA constructs, including certain 5´-phosphorylated single stranded siRNAs that mediate RNA interference in Hela cells. Harborth *et al.*, 2003, Antisense & Nucleic Acid Drug Development, 13, 83-105, describe certain chemically and structurally modified siRNA molecules. Chiu and Rana, 2003, RNA, 9, 1034-1048, describe certain chemically and

structurally modified siRNA molecules. Woolf *et al.*, International PCT Publication Nos. WO 03/064626 and WO 03/064625 describe certain chemically modified dsRNA constructs. Hornung *et al.*, 2005, Nature Medicine, 11, 263-270, describe the sequence-specific potent induction of IFN-alpha by short interfering RNA in plasmacytoid dendritic cells through TLR7. Judge *et al.*, 2005, Nature Biotechnology, Published online: 20 Mar. 2005, describe the sequence-dependent stimulation of the mammalian innate immune response by synthetic siRNA. Yuki *et al.*, International PCT Publication Nos. WO 05/049821 and WO 04/048566, describe certain methods for designing short interfering RNA sequences and certain short interfering RNA sequences with optimized activity. Saigo *et al.*, US Patent Application Publication No. US20040539332, describe certain methods of designing oligo- or polynucleotide sequences, including short interfering RNA sequences, for achieving RNA interference. Tei *et al.*, International PCT Publication No. WO 03/044188, describe certain methods for inhibiting expression of a target gene, which comprises transfecting a cell, tissue, or individual organism with a double-stranded polynucleotide comprising DNA and RNA having a substantially identical nucleotide sequence with at least a partial nucleotide sequence of the target gene.

BRIEF SUMMARY OF THE INVENTION

One aspect of the invention provides an isolated small interfering RNA (siRNA) polynucleotide, comprising at least one nucleotide sequence selected from the group consisting of SEQ ID NOs: 25, 26, 5, 6, 83, 84 and 135-138 and the complementary polynucleotide thereto.

Another aspect of the present invention provides an isolated small interfering RNA (siRNA) polynucleotide, comprising at least one nucleotide sequence selected from the group consisting of SEQ ID NOs:1-160. In one embodiment, the siRNA polynucleotide of the present invention comprises at least one nucleotide sequence selected from the group consisting of SEQ ID NOs:1-160 and the complementary polynucleotide thereto. In a further

embodiment, the small interfering RNA polynucleotide inhibits expression of a Notch1 polypeptide, wherein the Notch1 polypeptide comprises either of the amino acid sequences as set forth in SEQ ID NOs:163 and 164, or that is encoded by either one of the polynucleotides as set forth in SEQ ID NO:161 and 162. In another embodiment, the nucleotide sequence of the siRNA polynucleotide differs by one, two, three or four nucleotides at any positions of the siRNA polynucleotides as described herein, such as those provided in SEQ ID NOS: 1-160, or the complement thereof. In yet another embodiment, the nucleotide sequence of the siRNA polynucleotide differs by at least one mismatched base pair between a 5' end of an antisense strand and a 3' end of a sense strand of a sequence selected from the group consisting of the sequences set forth in SEQ ID NOS:1-160. In this regard, the mismatched base pair may include, but are not limited to G:A, C:A, C:U, G:G, A:A, C:C, U:U, C:T, and U:T mismatches. In a further embodiment, the mismatched base pair comprises a wobble base pair between the 5' end of the antisense strand and the 3' end of the sense strand. In another embodiment, the siRNA polynucleotide comprises at least one synthetic nucleotide analogue of a naturally occurring nucleotide. In certain embodiments, wherein the siRNA polynucleotide is linked to a detectable label, such as a reporter molecule or a magnetic or paramagnetic particle. Reporter molecules are well known to the skilled artisan. Illustrative reporter molecules include, but are in no way limited to, a dye, a radionuclide, a luminescent group, a fluorescent group, and biotin.

Another aspect of the invention provides an isolated siRNA molecule that inhibits expression of a Notch1 gene, wherein the siRNA molecule comprises a nucleic acid that targets either one of the sequences provided in SEQ ID NOs:161 and 162, or a variant thereof, where such variants of Notch1 may demonstrate altered (increased or decreased) Notch1 signaling activity. Any of a variety of assays known to the skilled artisan may be used to assess Notch1 activation or related cellular and signaling functions. Such assays include commercially available reporter assays (see *e.g.*, reporter assay

kits from SuperArray Bioscience Corp., Frederick, MD; or cell signaling assays from Promega Corp., Madison, WI) or those described, for example, in *Current Protocols in Cell Biology*, published by John Wiley & Sons, Inc., Boston, MA.

In certain embodiments, the siRNA comprises any one of the single stranded RNA sequences provided in SEQ ID NOs:1-160, or a double-stranded RNA thereof. In one embodiment of the invention, the siRNA molecule down regulates expression of a Notch1 gene via RNA interference (RNAi).

Another aspect of the invention provides compositions comprising any one or more of the siRNA polynucleotides described herein and a physiologically acceptable carrier. For example, the nucleic acid compositions prepared for delivery as described in US Patent Nos. 6,692,911, 7,163,695 and 7,070,807. In this regard, in one embodiment, the present invention provides a nucleic acid of the present invention in a composition comprising copolymers of lysine and histidine (HK) as described in US Patents 7,163,695, 7,070,807, and 6,692,911 either alone or in combination with PEG (*e.g.*, branched or unbranched PEG or a mixture of both) or in combination with PEG and a targeting moiety. Any combination of the above can also be combined with crosslinking to provide additional stability.

Another aspect of the invention provides a method for treating or preventing cardiovascular disorders such as aortic valve disease and atherosclerosis, certain inflammatory disorders and cancers including but not limited to T-ALL, breast, pancreatic cancer, colorectal cancer, gastric cancer, melanoma, leukemia, lymphoma, glioma, and lung cancer, in a subject having or suspected of being at risk for having one or more of these diseases, comprising administering to the subject a composition of the invention, such as a composition comprising the siRNa molecules of the invention, thereby treating or preventing the disease.

A further aspect of the invention provides a method for inhibiting the synthesis or expression of Notch1 comprising contacting a cell expressing

Notch1 with any one or more siRNA molecules wherein the one or more siRNA molecules comprises a sequence selected from the sequences provided in SEQ ID NOs:1-160, or a double-stranded RNA thereof. In one embodiment, a nucleic acid sequence encoding Notch1 comprises the sequence set forth in SEQ ID NO:161 or 162.

Yet a further aspect of the invention provides a method for reducing the severity of cardiovascular disorders such as aortic valve disease and atherosclerosis, certain inflammatory disorders and cancers including but not limited to T-ALL, breast, pancreatic cancer, colorectal cancer, gastric cancer, melanoma, leukemia, lymphoma, glioma, and lung cancer in a subject, comprising administering to the subject a composition comprising the siRNA as described herein, thereby reducing the severity of such diseases.

Another aspect of the invention provides a recombinant nucleic acid construct comprising a nucleic acid that is capable of directing transcription of a small interfering RNA (siRNA), the nucleic acid comprising: (a) a first promoter; (b) a second promoter; and (c) at least one DNA polynucleotide segment comprising at least one polynucleotide that is selected from the group consisting of (i) a polynucleotide comprising the nucleotide sequence set forth in any one of SEQ ID NOs:1-160, and (ii) a polynucleotide of at least 18 nucleotides that is complementary to the polynucleotide of (i), wherein the DNA polynucleotide segment is operably linked to at least one of the first and second promoters, and wherein the promoters are oriented to direct transcription of the DNA polynucleotide segment and of the complement thereto. In one embodiment, the recombinant nucleic acid construct comprises at least one enhancer that is selected from a first enhancer operably linked to the first promoter and a second enhancer operably linked to the second promoter. In another embodiment, the recombinant nucleic acid construct comprises at least one transcriptional terminator that is selected from (i) a first transcriptional terminator that is positioned in the construct to terminate transcription directed by the first promoter and (ii) a second transcriptional terminator that is

positioned in the construct to terminate transcription directed by the second promoter.

Another aspect of the invention provides isolated host cells transformed or transfected with a recombinant nucleic acid construct as described herein.

One aspect of the present invention provides a nucleic acid molecule that down regulates expression of Notch1, wherein the nucleic acid molecule comprises a nucleic acid that targets Notch1 mRNA, whose representative sequences are provided in SEQ ID NOs:161 and 162. Corresponding amino acid sequences are set forth in SEQ ID NOs:163 and 164. In one embodiment, the nucleic acid is an siRNA molecule. In a further embodiment, the siRNA comprises any one of the single stranded RNA sequences provided in SEQ ID NOs:1-160, or a double-stranded RNA thereof. In another embodiment, the nucleic acid molecule down regulates expression of Notch1 gene via RNA interference (RNAi).

A further aspect of the invention provides a composition comprising any one or more of the siRNA molecules of the invention as set forth in SEQ ID NOs:1-160. In this regard, the composition may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more siRNA molecules of the invention. In this regard, the siRNA molecules may be selected from the siRNA molecules provided in SEQ ID NOs:1-160, or a double-stranded RNA thereof. Thus, the siRNA molecules may target Notch1 and may be a mixture of siRNA molecules that target different regions of this gene. In certain embodiments, the compositions may comprise a targeting moiety or ligand, such as a targeting moeity that will target the siRNA composition to a desired cell.

These and other aspects of the present invention will become apparent upon reference to the following detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a bar graph showing knockdown of human Notch1 mRNA levels in HepG2 cells (Human hepatocellular liver carcinoma **cell** line) transfected with 10 nM of Notch1-siRNA at 72 hours post-transfection. siRNA transfection was conducted using Lipofectamine®RNAiMAX as described in Example 2. 1-40: human Notch1 25-mer siRNA #1-40; M: Mock transfection; Ctrl: negative control siRNA transfection. Data are presented as Mean +/- STD.

Figure 2 is a bar graph showing knockdown of human Notch1 mRNA levels in HepG2 cells transfected with 3 nM of 12 selected Notch1 siRNA at 72 hours post-transfection. siRNA transfection was conducted using Lipofectamine®RNAiMAX as described in Example 2. 2-34: human Notch1 25-mer siRNA #2-34; M: Mock transfection; C: negative control siRNA transfection. Data are presented as Mean +/- STD.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to nucleic acid molecules for modulating the expression of Notch1. In certain embodiments the nucleic acid is ribonucleic acid (RNA). In certain embodiments, the RNA molecules are single or double stranded. In this regard, the nucleic acid based molecules of the present invention, such as siRNA, inhibit or down-regulate expression of Notch1.

The present invention relates to compounds, compositions, and methods for the study, diagnosis, and treatment of traits, diseases and conditions that respond to the modulation of Notch1 gene expression and/or activity. The present invention is also directed to compounds, compositions, and methods relating to traits, diseases and conditions that respond to the modulation of expression and/or activity of genes involved in Notch1 gene expression pathways or other cellular processes that mediate the maintenance or development of such traits, diseases and conditions. Specifically, the invention relates to double stranded nucleic acid molecules including small

nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules capable of mediating RNA interference (RNAi) against Notch1 gene expression, including cocktails of such small nucleic acid molecules and nanoparticle formulations of such small nucleic acid molecules. The present invention also relates to small nucleic acid molecules, such as siNA, siRNA, and others that can inhibit the function of endogenous RNA molecules, such as endogenous micro-RNA (miRNA) (*e.g*, miRNA inhibitors) or endogenous short interfering RNA (siRNA), (*e.g.*, siRNA inhibitors) or that can inhibit the function of RISC (*e.g.*, RISC inhibitors), to modulate Notch1 gene expression by interfering with the regulatory function of such endogenous RNAs or proteins associated with such endogenous RNAs (*e.g.*, RISC), including cocktails of such small nucleic acid molecules and nanoparticle formulations of such small nucleic acid molecules. Such small nucleic acid molecules are useful, for example, in providing compositions to prevent, inhibit, or reduce cardiovascular disorders such as aortic valve disease and atherosclerosis, certain inflammatory disorders and cancers including but not limited to T-ALL, breast, pancreatic cancer, colorectal cancer, gastric cancer, melanoma, leukemia, lymphoma, glioma, and lung cancer and/or other disease states, conditions, or traits associated with Notch1 gene expression or activity in a subject or organism.

By “inhibit” or “down-regulate” it is meant that the expression of the gene, or level of mRNA encoding a Notch1 protein, levels of Notch1 protein, or activity of Notch1, is reduced below that observed in the absence of the nucleic acid molecules of the invention. In one embodiment, inhibition or down-regulation with the nucleic acid molecules of the invention is below that level observed in the presence of an inactive control or attenuated molecule that is able to bind to the same target mRNA, but is unable to cleave or otherwise silence that mRNA. In another embodiment, inhibition or down-regulation with the nucleic acid molecules of the invention is preferably below that level

observed in the presence of, for example, a nucleic acid with scrambled sequence or with mismatches. In another embodiment, inhibition or down-regulation of Notch1 with the nucleic acid molecule of the instant invention is greater in the presence of the nucleic acid molecule than in its absence.

By “modulate” is meant that the expression of the gene, or level of RNAs or equivalent RNAs encoding one or more protein subunits, or activity of one or more protein subunit(s) is up-regulated or down-regulated, such that the expression, level, or activity is greater than or less than that observed in the absence of the nucleic acid molecules of the invention.

By “double stranded RNA” or “dsRNA” is meant a double stranded RNA that matches a predetermined gene sequence that is capable of activating cellular enzymes that degrade the corresponding messenger RNA transcripts of the gene. These dsRNAs are referred to as small interfering RNA (siRNA) and can be used to inhibit gene expression (see for example Elbashir *et al.*, 2001, Nature, 411, 494-498; and Bass, 2001, Nature, 411, 428-429). The term “double stranded RNA” or “dsRNA” as used herein also refers to a double stranded RNA molecule capable of mediating RNA interference “RNAi”, including small interfering RNA “siRNA” (see for example Bass, 2001, Nature, 411, 428-429; Elbashir *et al.*, 2001, Nature, 411, 494-498; and Kreutzer *et al.*, International PCT Publication No. WO 00/44895; Zernicka-Goetz *et al.*, International PCT Publication No. WO 01/36646; Fire, International PCT Publication No. WO 99/32619; Plaetinck *et al.*, International PCT Publication No. WO 00/01846; Mello and Fire, International PCT Publication No. WO 01/29058; Deschamps-Depaillette, International PCT Publication No. WO 99/07409; and Li *et al.*, International PCT Publication No. WO 00/44914).

By “gene” it is meant a nucleic acid that encodes an RNA, for example, nucleic acid sequences including but not limited to structural genes encoding a polypeptide.

By “a nucleic acid that target” is meant a nucleic acid as described herein that matches, is complementary to or otherwise specifically binds or

specifically hybridizes to and thereby can modulate the expression of the gene that comprises the target sequence, or level of mRNAs or equivalent RNAs encoding one or more protein subunits, or activity of one or more protein subunit(s) encoded by the gene.

"Complementarity" refers to the ability of a nucleic acid to form hydrogen bond(s) with another RNA sequence by either traditional Watson-Crick or other non-traditional types. In reference to the nucleic molecules of the present invention, the binding free energy for a nucleic acid molecule with its target or complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, *e.g.*, enzymatic nucleic acid cleavage, antisense or triple helix inhibition. Determination of binding free energies for nucleic acid molecules is well known in the art (see, *e.g.*, Turner *et al.*, 1987, CSH Symp. Quant. Biol. LII, pp. 123-133; Frier *et al.*, 1986, Proc. Nat. Acad. Sci. USA 83, 9373-9377; Turner *et al.*, 1987, J. Am. Chem. Soc. 109, 3783-3785). A percent complementarity indicates the percentage of contiguous residues in a nucleic acid molecule which can form hydrogen bonds (*e.g.*, Watson-Crick base pairing) with a second nucleic acid sequence (*e.g.*, 5, 6, 7, 8, 9, 10 out of 10 being 50%, 60%, 70%, 80%, 90%, and 100% complementary). "Perfectly complementary" means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence.

By "RNA" is meant a molecule comprising at least one ribonucleotide residue. By "ribonucleotide" or "2´-OH" is meant a nucleotide with a hydroxyl group at the 2´ position of a β-D-ribo-furanose moiety.

By "RNA interference" or "RNAi" is meant a biological process of inhibiting or down regulating gene expression in a cell as is generally known in the art and which is mediated by short interfering nucleic acid molecules, see for example Zamore and Haley, 2005, Science, 309, 1519-1524; Vaughn and Martienssen, 2005, Science, 309, 1525-1526; Zamore *et al.*, 2000, Cell, 101, 25-33; Bass, 2001, Nature, 411, 428-429; Elbashir *et al.*, 2001, Nature, 411,

494-498; and Kreutzer *et al.*, International PCT Publication No. WO 00/44895; Zemicka-Goetz *et al.*, International PCT Publication No. WO 01/36646; Fire, International PCT Publication No. WO 99/32619; Plaetinck *et al.*, International PCT Publication No. WO 00/01846; Mello and Fire, International PCT Publication No. WO 01/29058; Deschamps-Depaillette, International PCT Publication No. WO 99/07409; and Li *et al.*, International PCT Publication No. WO 00/44914; Allshire, 2002, Science, 297, 1818-1819; Volpe *et al.*, 2002, Science, 297, 1833-1837; Jenuwein, 2002, Science, 297, 2215-2218; and Hall *et al.*, 2002, Science, 297, 2232-2237; Hutvagner and Zamore, 2002, Science, 297, 2056-60; McManus *et al.*, 2002, RNA, 8, 842-850; Reinhart *et al.*, 2002, Gene & Dev., 16, 1616-1626; and Reinhart & Bartel, 2002, Science, 297, 1831). In addition, as used herein, the term RNAi is meant to be equivalent to other terms used to describe sequence specific RNA interference, such as post transcriptional gene silencing, translational inhibition, transcriptional inhibition, or epigenetics. For example, siRNA molecules of the invention can be used to epigenetically silence genes at both the post-transcriptional level or the pre-transcriptional level. In a non-limiting example, epigenetic modulation of gene expression by siRNA molecules of the invention can result from siRNA mediated modification of chromatin structure or methylation patterns to alter gene expression (see, for example, Verdel *et al.*, 2004, Science, 303, 672-676; Pal-Bhadra *et al.*, 2004, Science, 303, 669-672; Allshire, 2002, Science, 297, 1818-1819; Volpe *et al.*, 2002, Science, 297, 1833-1837; Jenuwein, 2002, Science, 297, 2215-2218; and Hall *et al.*, 2002, Science, 297, 2232-2237). In another non-limiting example, modulation of gene expression by siRNA molecules of the invention can result from siRNA mediated cleavage of RNA (either coding or non-coding RNA) via RISC, or alternately, translational inhibition as is known in the art. In another embodiment, modulation of gene expression by siRNA molecules of the invention can result from transcriptional inhibition (see for example Janowski *et al.*, 2005, Nature Chemical Biology, 1, 216-222).

Two types of about 21 nucleotide RNAs trigger post-transcriptional gene silencing in animals: small interfering RNAs (siRNAs) and microRNAs (miRNAs). Both siRNAs and miRNAs are produced by the cleavage of double-stranded RNA (dsRNA) precursors by Dicer, a nuclease of the RNase III family of dsRNA-specific endonucleases (Bernstein *et al*., (2001). Nature 409, 363-366; Billy, E., *et al*. (2001). Proc Natl Acad Sci USA 98, 14428-14433; Grishok *et al*., 2001, Cell 106, 23-34; Hutvgner *et al*., 2001, Science 293, 834-838; Ketting *et al*., 2001, Genes Dev 15, 2654-2659; Knight and Bass, 2001, Science 293, 2269-2271; Paddison *et al*., 2002, Genes Dev 16, 948-958; Park *et al*., 2002, Curr Biol 12, 1484-1495; Provost *et al*., 2002, EMBO J. 21, 5864-5874; Reinhart *et al*., 2002, Science. 297: 1831; Zhang *et al*., 2002, EMBO J. 21, 5875-5885; Doi *et al*., 2003, Curr Biol 13, 41-46; Myers *et al*., 2003, Nature Biotechnology Mar;21(3):324-8). siRNAs result when transposons, viruses or endogenous genes express long dsRNA or when dsRNA is introduced experimentally into plant or animal cells to trigger gene silencing, also called RNA interference (RNAi) (Fire *et al*., 1998; Hamilton and Baulcombe, 1999; Zamore *et al*., 2000; Elbashir *et al*., 2001a; Hammond *et al*., 2001; Sijen *et al*., 2001; Catalanotto *et al*., 2002). In contrast, miRNAs are the products of endogenous, non-coding genes whose precursor RNA transcripts can form small stem-loops from which mature miRNAs are cleaved by Dicer (Lagos-Quintana *et al*., 2001; Lau *et al*., 2001; Lee and Ambros, 2001; Lagos-Quintana *et al*., 2002; Mourelatos *et al*., 2002; Reinhart *et al*., 2002; Ambros *et al*., 2003; Brennecke *et al*., 2003; Lagos-Quintana *et al*., 2003; Lim *et al*., 2003a; Lim *et al*., 2003b). miRNAs are encoded by genes distinct from the mRNAs whose expression they control.

siRNAs were first identified as the specificity determinants of the RNA interference (RNAi) pathway (Hamilton and Baulcombe, 1999; Hammond *et al*., 2000), where they act as guides to direct endonucleolytic cleavage of their target RNAs (Zamore *et al*., 2000; Elbashir *et al*., 2001a). Prototypical siRNA duplexes are 21 nt, double-stranded RNAs that contain 19 base pairs, with two-

nucleotide, 3' overhanging ends (Elbashir *et al*., 2001a; Nyknen *et al*., 2001; Tang *et al*., 2003). Active siRNAs contain 5' phosphates and 3' hydroxyls (Zamore *et al*., 2000; Boutla *et al*., 2001; Nyknen *et al*., 2001; Chiu and Rana, 2002). Similarly, miRNAs contain 5' phosphate and 3' hydroxyl groups, reflecting their production by Dicer (Hutvgner *et al*., 2001; Mallory *et al*., 2002)

Thus, the present invention is directed in part to the discovery of short RNA polynucleotide sequences that are capable of specifically modulating expression of a target Notch1 polypeptide, such as encoded by the sequences provided in SEQ ID NOs:161 and 162, or a variant thereof. Illustrative siRNA polynucleotide seqeunces that specifically modulate the expression of Notch1 are provided in SEQ ID NOs:1-160. Without wishing to be bound by theory, the RNA polynucleotides of the present invention specifically reduce expression of a desired target polypeptide through recruitment of small interfering RNA (siRNA) mechanisms. In particular, and as described in greater detail herein, according to the present invention there are provided compositions and methods that relate to the identification of certain specific RNAi oligonucleotide sequences of 19, 20, 21, 22, 23, 24, 25, 26 or 27 nucleotides that can be derived from corresponding polynucleotide sequences encoding the desired Notch1 target polypeptide.

In certain embodiments of the invention, the siRNA polynucleotides interfere with expression of a Notch1 target polypeptide or a variant thereof, and comprises a RNA oligonucleotide or RNA polynucleotide uniquely corresponding in its nucleotide base sequence to the sequence of a portion of a target polynucleotide encoding the target polypeptide, for instance, a target mRNA sequence or an exonic sequence encoding such mRNA. The invention relates in certain embodiments to siRNA polynucleotides that interfere with expression (sometimes referred to as silencing) of specific polypeptides in mammals, which in certain embodiments are humans and in certain other embodiments are non-human mammals. Hence, according to non-limiting theory, the siRNA polynucleotides of the present invention direct sequence-

specific degradation of mRNA encoding a desired target polypeptide, such as Notch1.

In certain embodiments, the term “siRNA” means either: (i) a double stranded RNA oligonucleotide, or polynucleotide, that is 18 base pairs, 19 base pairs, 20 base pairs, 21 base pairs, 22 base pairs, 23 base pairs, 24 base pairs, 25 base pairs, 26 base pairs, 27 base pairs, 28 base pairs, 29 base pairs or 30 base pairs in length and that is capable of interfering with expression and activity of a Notch1 polypeptide, or a variant of the Notch1 polypeptide, wherein a single strand of the siRNA comprises a portion of a RNA polynucleotide sequence that encodes the Notch1 polypeptide, its variant, or a complementary sequence thereto; (ii) a single stranded oligonucleotide, or polynucleotide of 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, 29 nucleotides or 30 nucleotides in length and that is either capable of interfering with expression and/or activity of a target Notch1 polypeptide, or a variant of the Notch1 polypeptide, or that anneals to a complementary sequence to result in a dsRNA that is capable of interfering with target polypeptide expression, wherein such single stranded oligonucleotide comprises a portion of a RNA polynucleotide sequence that encodes the Notch1 polypeptide, its variant, or a complementary sequence thereto; or (iii) an oligonucleotide, or polynucleotide, of either (i) or (ii) above wherein such oligonucleotide, or polynucleotide, has one, two, three or four nucleic acid alterations or substitutions therein. Certain RNAi oligonucleotide sequences described below are complementary to the 3’ non-coding region of target mRNA that encodes the Notch1 polypeptide.

A siRNA polynucleotide is a RNA nucleic acid molecule that mediates the effect of RNA interference, a post-transcriptional gene silencing mechanism. In certain embodiments, a siRNA polynucleotide comprises a double-stranded RNA (dsRNA) but is not intended to be so limited and may comprise a single-stranded RNA (*see, e.g.,* Martinez *et al*. *Cell* 110:563-74 (2002)). A siRNA polynucleotide may comprise other naturally occurring,

recombinant, or synthetic single-stranded or double-stranded polymers of nucleotides (ribonucleotides or deoxyribonucleotides or a combination of both) and/or nucleotide analogues as provided herein (*e.g.,* an oligonucleotide or polynucleotide or the like, typically in 5' to 3' phosphodiester linkage). Accordingly it will be appreciated that certain exemplary sequences disclosed herein as DNA sequences capable of directing the transcription of the subject invention siRNA polynucleotides are also intended to describe the corresponding RNA sequences and their complements, given the well established principles of complementary nucleotide base-pairing. A siRNA may be transcribed using as a template a DNA (genomic, cDNA, or synthetic) that contains a RNA polymerase promoter, for example, a U6 promoter or the H1 RNA polymerase III promoter, or the siRNA may be a synthetically derived RNA molecule. In certain embodiments the subject invention siRNA polynucleotide may have blunt ends, that is, each nucleotide in one strand of the duplex is perfectly complementary (*e.g.*, by Watson-Crick base-pairing) with a nucleotide of the opposite strand. In certain other embodiments, at least one strand of the subject invention siRNA polynucleotide has at least one, and in certain embodiments, two nucleotides that "overhang" (*i.e.*, that do not base pair with a complementary base in the opposing strand) at the 3′ end of either strand, or in certain embodiments, both strands, of the siRNA polynucleotide. In one embodiment of the invention, each strand of the siRNA polynucleotide duplex has a two-nucleotide overhang at the 3′ end. The two-nucleotide overhang may be a thymidine dinucleotide (TT) but may also comprise other bases, for example, a TC dinucleotide or a TG dinucleotide, or any other dinucleotide. For a discussion of 3' ends of siRNA polynucleotides see, *e.g.,* WO 01/75164.

Certain illustrative siRNA polynucleotides comprise double-stranded oligomeric nucleotides of about 18-30 nucleotide base pairs. In certain embodiments, the siRNA molecules of the invention comprise about 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27 base pairs, and in other particular embodiments about 19, 20, 21, 22 or 23 base pairs, or about 27 base pairs,

whereby the use of “about” indicates, as described above, that in certain embodiments and under certain conditions the processive cleavage steps that may give rise to functional siRNA polynucleotides that are capable of interfering with expression of a selected polypeptide may not be absolutely efficient. Hence, siRNA polynucleotides, for instance, of “about” 18, 19, 20, 21, 22, 23, 24, or 25 base pairs may include one or more siRNA polynucleotide molecules that may differ (*e.g.,* by nucleotide insertion or deletion) in length by one, two, three or four base pairs, by way of non-limiting theory as a consequence of variability in processing, in biosynthesis, or in artificial synthesis. The contemplated siRNA polynucleotides of the present invention may also comprise a polynucleotide sequence that exhibits variability by differing (*e.g.,* by nucleotide substitution, including transition or transversion) at one, two, three or four nucleotides from a particular sequence, the differences occurring at any of positions 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 of a particular siRNA polynucleotide sequence, or at positions 20, 21, 22, 23, 24, 25, 26, or 27 of siRNA polynucleotides depending on the length of the molecule, whether situated in a sense or in an antisense strand of the double-stranded polynucleotide. The nucleotide substitution may be found only in one strand, by way of example in the antisense strand, of a double-stranded polynucleotide, and the complementary nucleotide with which the substitute nucleotide would typically form hydrogen bond base pairing may not necessarily be correspondingly substituted in the sense strand. In certain embodiments, the siRNA polynucleotides are homogeneous with respect to a specific nucleotide sequence. As described herein, the siRNA polynucleotides interfere with expression of a Notch1 polypeptide. These polynucleotides may also find uses as probes or primers.

In certain embodiments, the efficacy and specificity of gene/protein silencing by the siRNA nucleic acids of the present invention may be enhanced using the methods described in US Patent Application Publications 2005/0186586, 2005/0181382, 2005/0037988, and 2006/0134787.

In this regard, the RNA silencing may be enhanced by lessening the base pair strength between the 5' end of the first strand and the 3' end of a second strand of the duplex as compared to the base pair strength between the 3' end of the first strand and the 5' end of the second strand. In certain embodiments the RNA duplex may comprise at least one blunt end and may comprise two blunt ends. In other embodiments, the duplex comprises at least one overhang and may comprise two overhangs.

In one embodiment of the invention, the ability of the siRNA molecule to silence a target gene is enhanced by enhancing the ability of a first strand of a RNAi agent to act as a guide strand in mediating RNAi. This is achieved by lessening the base pair strength between the 5' end of the first strand and the 3' end of a second strand of the duplex as compared to the base pair strength between the 3' end of the first strand and the 5' end of the second strand.

In a further aspect of the invention, the efficacy of a siRNA duplex is enhanced by lessening the base pair strength between the antisense strand 5' end (AS 5') and the sense strand 3' end (S 3') as compared to the base pair strength between the antisense strand 3' end (AS 3') and the sense strand 5' end (S '5), such that efficacy is enhanced.

In certain embodiments, modifications can be made to the siRNA molecules of the invention in order to promote entry of a desired strand of an siRNA duplex into a RISC complex. This is achieved by enhancing the asymmetry of the siRNA duplex, such that entry of the desired strand is promoted. In this regard, the asymmetry is enhanced by lessening the base pair strength between the 5' end of the desired strand and the 3' end of a complementary strand of the duplex as compared to the base pair strength between the 3' end of the desired strand and the 5' end of the complementary strand. In certain embodiments, the base-pair strength is less due to fewer G:C base pairs between the 5' end of the first or antisense strand and the 3' end of the second or sense strand than between the 3' end of the first or antisense

strand and the 5' end of the second or sense strand. In other embodiments, the base pair strength is less due to at least one mismatched base pair between the 5' end of the first or antisense strand and the 3' end of the second or sense strand. In certain embodiments, the mismatched base pairs include but are not limited to G:A, C:A, C:U, G:G, A:A, C:C, U:U, C:T, and U:T. In one embodiment, the base pair strength is less due to at least one wobble base pair between the 5' end of the first or antisense strand and the 3' end of the second or sense strand. In this regard, the wobble base pair may be G:U. or G:T.

In certain embodiments, the base pair strength is less due to: (a) at least one mismatched base pair between the 5' end of the first or antisense strand and the 3' end of the second or sense strand; and (b) at least one wobble base pair between the 5' end of the first or antisense strand and the 3' end of the second or sense strand. Thus, the mismatched base pair may be selected from the group consisting of G:A, C:A, C:U, G:G, A:A, C:C and U:U. In another embodiment, the mismatched base pair is selected from the group consisting of G:A, C:A, C:T, G:G, A:A, C:C and U:T. In certain cases, the wobble base pair is G:U or G:T.

In certain embodiments, the base pair strength is less due to at least one base pair comprising a rare nucleotide such as inosine, 1-methyl inosine, pseudouridine, 5,6-dihydrouridine, ribothymidine, 2N-methylguanosine and 2,2N,N-dimethylguanosine; or a modified nucleotide, such as 2-amino-G, 2-amino-A, 2,6-diamino-G, and 2,6-diamino-A.

As used herein, the term "antisense strand" of an siRNA or RNAi agent refers to a strand that is substantially complementary to a section of about 10-50 nucleotides, *e.g.*, about 15-30, 16-25, 18-23 or 19-22 nucleotides of the mRNA of the gene targeted for silencing. The antisense strand or first strand has sequence sufficiently complementary to the desired target mRNA sequence to direct target-specific RNA interference (RNAi), *e.g.*, complementarity sufficient to trigger the destruction of the desired target mRNA by the RNAi machinery or process. The term "sense strand" or "second strand"

of an siRNA or RNAi agent refers to a strand that is complementary to the antisense strand or first strand. Antisense and sense strands can also be referred to as first or second strands, the first or second strand having complementarity to the target sequence and the respective second or first strand having complementarity to said first or second strand.

As used herein, the term "guide strand" refers to a strand of an RNAi agent, *e.g.*, an antisense strand of an siRNA duplex, that enters into the RISC complex and directs cleavage of the target mRNA.

Thus, complete complementarity of the siRNA molecules of the invention with their target gene is not necessary in order for effective silencing to occur. In particular, three or four mismatches between a guide strand of an siRNA duplex and its target RNA, properly placed so as to still permit mRNA cleavage, facilitates the release of cleaved target RNA from the RISC complex, thereby increasing the rate of enzyme turnover. In particular, the efficiency of cleavage is greater when a G:U base pair, referred to also as a G:U wobble, is present near the 5' or 3' end of the complex formed between the miRNA and the target.

Thus, at least one terminal nucleotide of the RNA molecules described herein can be substituted with a nucleotide that does not form a Watson-Crick base pair with the corresponding nucleotide in a target mRNA.

Polynucleotides that are siRNA polynucleotides of the present invention may in certain embodiments be derived from a single-stranded polynucleotide that comprises a single-stranded oligonucleotide fragment (*e.g.*, of about 18-30 nucleotides, which should be understood to include any whole integer of nucleotides including and between 18 and 30) and its reverse complement, typically separated by a spacer sequence. According to certain such embodiments, cleavage of the spacer provides the single-stranded oligonucleotide fragment and its reverse complement, such that they may anneal to form (optionally with additional processing steps that may result in addition or removal of one, two, three or more nucleotides from the 3' end

and/or the 5' end of either or both strands) the double-stranded siRNA polynucleotide of the present invention. In certain embodiments the spacer is of a length that permits the fragment and its reverse complement to anneal and form a double-stranded structure (*e.g.*, like a hairpin polynucleotide) prior to cleavage of the spacer (and, optionally, subsequent processing steps that may result in addition or removal of one, two, three, four, or more nucleotides from the 3' end and/or the 5' end of either or both strands). A spacer sequence may therefore be any polynucleotide sequence as provided herein that is situated between two complementary polynucleotide sequence regions which, when annealed into a double-stranded nucleic acid, comprise a siRNA polynucleotide. In some embodiments, a spacer sequence comprises at least 4 nucleotides, although in certain embodiments the spacer may comprise 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,17, 18, 19, 20, 21-25, 26-30, 31-40, 41-50, 51-70, 71-90, 91-110, 111-150, 151-200 or more nucleotides. Examples of siRNA polynucleotides derived from a single nucleotide strand comprising two complementary nucleotide sequences separated by a spacer have been described (*e.g.*, Brummelkamp *et al*., 2002 *Science* 296:550; Paddison *et al*., 2002 *Genes Develop*. 16:948; Paul *et al*. *Nat. Biotechnol.* 20:505-508 (2002); Grabarek *et al*., *BioTechniques* 34:734-44 (2003)).

Polynucleotide variants may contain one or more substitutions, additions, deletions, and/or insertions such that the activity of the siRNA polynucleotide is not substantially diminished, as described above. The effect on the activity of the siRNA polynucleotide may generally be assessed as described herein or using conventional methods. In certain embodiments, variants exhibit at least about 75%, 78%, 80%, 85%, 87%, 88% or 89% identity and in particular embodiments, at least about 90%, 92%, 95%, 96%, 97%, 98%, or 99% identity to a portion of a polynucleotide sequence that encodes a native Notch1. The percent identity may be readily determined by comparing sequences of the polynucleotides to the corresponding portion of a full-length Notch1 polynucleotide such as those known to the art and cited herein, using

any method including using computer algorithms well known to those having ordinary skill in the art, such as Align or the BLAST algorithm (Altschul, *J. Mol. Biol. 219*:555-565, 1991; Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA 89*:10915-10919, 1992), which is available at the NCBI website (*see* [online] Internet:<URL: ncbi dot nlm dot nih dot gov/cgi-bin/BLAST). Default parameters may be used.

Certain siRNA polynucleotide variants are substantially homologous to a portion of a native Notch1 gene. Single-stranded nucleic acids derived (*e.g.,* by thermal denaturation) from such polynucleotide variants are capable of hybridizing under moderately stringent conditions or stringent conditions to a naturally occurring DNA or RNA sequence encoding a native Notch1 polypeptide (or a complementary sequence). A polynucleotide that detectably hybridizes under moderately stringent conditions or stringent conditions may have a nucleotide sequence that includes at least 10 consecutive nucleotides, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 consecutive nucleotides complementary to a particular polynucleotide. In certain embodiments, such a sequence (or its complement) will be unique to a Notch1 polypeptide for which interference with expression is desired, and in certain other embodiments the sequence (or its complement) may be shared by Notch1 and one or more related polypeptides for which interference with polypeptide expression is desired.

Suitable moderately stringent conditions and stringent conditions are known to the skilled artisan. Moderately stringent conditions include, for example, pre-washing in a solution of 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50 °C-70°C, 5X SSC for 1-16 hours (*e.g.,* overnight); followed by washing once or twice at 22-65 °C for 20-40 minutes with one or more each of 2X, 0.5X and 0.2X SSC containing 0.05-0.1% SDS. For additional stringency, conditions may include a wash in 0.1X SSC and 0.1% SDS at 50-60 °C for 15-40 minutes. As known to those having ordinary skill in the art, variations in stringency of hybridization conditions may be achieved by

altering the time, temperature, and/or concentration of the solutions used for pre-hybridization, hybridization, and wash steps. Suitable conditions may also depend in part on the particular nucleotide sequences of the probe used, and of the blotted, proband nucleic acid sample. Accordingly, it will be appreciated that suitably stringent conditions can be readily selected without undue experimentation when a desired selectivity of the probe is identified, based on its ability to hybridize to one or more certain proband sequences while not hybridizing to certain other proband sequences.

Sequence specific siRNA polynucleotides of the present invention may be designed using one or more of several criteria. For example, to design a siRNA polynucleotide that has 19 consecutive nucleotides identical to a sequence encoding a polypeptide of interest (*e.g.*, Notch1 and other polypeptides described herein), the open reading frame of the polynucleotide sequence may be scanned for 21-base sequences that have one or more of the following characteristics: (1) an A+T/G+C ratio of approximately 1:1 but no greater than 2:1 or 1:2; (2) an AA dinucleotide or a CA dinucleotide at the 5' end; (3) an internal hairpin loop melting temperature less than 55 °C; (4) a homodimer melting temperature of less than 37 °C (melting temperature calculations as described in (3) and (4) can be determined using computer software known to those skilled in the art); (5) a sequence of at least 16 consecutive nucleotides not identified as being present in any other known polynucleotide sequence (such an evaluation can be readily determined using computer programs available to a skilled artisan such as BLAST to search publicly available databases). Alternatively, an siRNA polynculeotide sequence may be designed and chosen using a computer software available commercially from various vendors (e.g., OligoEngine™ (Seattle, WA); Dharmacon, Inc. (Lafayette, CO); Ambion Inc. (Austin, TX); and QIAGEN, Inc. (Valencia, CA)). (*See also* Elbashir *et al.*, *Genes & Development* 15:188-200 (2000); Elbashir *et al.*, *Nature* 411:494-98 (2001)) The siRNA polynucleotides may then be tested for their ability to interfere with the expression of the target

polypeptide according to methods known in the art and described herein. The determination of the effectiveness of an siRNA polynucleotide includes not only consideration of its ability to interfere with polypeptide expression but also includes consideration of whether the siRNA polynucleotide manifests undesirably toxic effects, for example, apoptosis of a cell for which cell death is not a desired effect of RNA interference (*e.g.,* interference of Notch1 expression in a cell).

In certain embodiments, the nucleic acid inhibitors comprise sequences which are complementary to any known Notch1 sequence, including variants thereof that have altered expression and/or activity, particularly variants associated with disease. Variants of Notch1 include sequences having 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher sequence identity to the wild type Notch1 sequences, such as those set forth in SEQ ID NOs:161 and 162, where such variants of Notch1 may demonstrate altered (increased or decreased) Notch1 signaling activity. Any of a variety of assays known to the skilled artisan may be used to assess Notch1 activation or related cellular and signaling functions. Such assays include commercially available reporter assays (see *e.g.*, reporter assay kits from SuperArray Bioscience Corp., Frederick, MD; or cell signaling assays from Promega Corp., Madison, WI) or those described, for example, in *Current Protocols in Cell Biology*, published by John Wiley & Sons, Inc., Boston, MA.

As would be understood by the skilled artisan, Notch1 sequences are available in any of a variety of public sequence databases including GENBANK or SWISSPROT. In one embodiment, the nucleic acid inhibitors (*e.g.*, siRNA) of the invention comprise sequences complimentary to the specific Notch1 target sequences provided in SEQ ID NOs:161 and 162, or polynucleotides encoding the amino acid sequences provided in SEQ ID NOs:163 and 164. Examples of such siRNA molecules also are shown in the Examples and provided in SEQ ID NOs:1-160.

Polynucleotides, including target polynucleotides (*e.g.*, polynucleotides capable of encoding a target polypeptide of interest), may be prepared using any of a variety of techniques, which will be useful for the preparation of specifically desired siRNA polynucleotides and for the identification and selection of desirable sequences to be used in siRNA polynucleotides. For example, a polynucleotide may be amplified from cDNA prepared from a suitable cell or tissue type. Such polynucleotides may be amplified via polymerase chain reaction (PCR). For this approach, sequence-specific primers may be designed based on the sequences provided herein and may be purchased or synthesized. An amplified portion may be used to isolate a full-length gene, or a desired portion thereof, from a suitable library using well known techniques. Within such techniques, a library (cDNA or genomic) is screened using one or more polynucleotide probes or primers suitable for amplification. In certain embodiments, a library is size-selected to include larger molecules. Random primed libraries may also be preferred for identifying 5' and upstream regions of genes. Genomic libraries are preferred for obtaining introns and extending 5' sequences. Suitable sequences for a siRNA polynucleotide contemplated by the present invention may also be selected from a library of siRNA polynucleotide sequences.

For hybridization techniques, a partial sequence may be labeled (*e.g.,* by nick-translation or end-labeling with ^{32}P) using well known techniques. A bacterial or bacteriophage library may then be screened by hybridizing filters containing denatured bacterial colonies (or lawns containing phage plaques) with the labeled probe (*see, e.g.,* Sambrook *et al*., *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 2001). Hybridizing colonies or plaques are selected and expanded, and the DNA is isolated for further analysis. Clones may be analyzed to determine the amount of additional sequence by, for example, PCR using a primer from the partial sequence and a primer from the vector. Restriction maps and partial sequences may be generated to identify one or more overlapping clones. A

full-length cDNA molecule can be generated by ligating suitable fragments, using well known techniques.

Alternatively, numerous amplification techniques are known in the art for obtaining a full-length coding sequence from a partial cDNA sequence. Within such techniques, amplification is generally performed via PCR. One such technique is known as "rapid amplification of cDNA ends" or RACE. This technique involves the use of an internal primer and an external primer, which hybridizes to a polyA region or vector sequence, to identify sequences that are 5' and 3' of a known sequence. Any of a variety of commercially available kits may be used to perform the amplification step. Primers may be designed using, for example, software well known in the art. Primers (or oligonucleotides for other uses contemplated herein, including, for example, probes and antisense oligonucleotides) are generally 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31 or 32 nucleotides in length, have a GC content of at least 40% and anneal to the target sequence at temperatures of about 54 °C to 72 °C. The amplified region may be sequenced as described above, and overlapping sequences assembled into a contiguous sequence. Certain oligonucleotides contemplated by the present invention may, for some embodiments, have lengths of 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33-35, 35-40, 41-45, 46-50, 56-60, 61-70, 71-80, 81-90 or more nucleotides.

In general, polypeptides and polynucleotides as described herein are isolated. An "isolated" polypeptide or polynucleotide is one that is removed from its original environment. For example, a naturally occurring protein is isolated if it is separated from some or all of the coexisting materials in the natural system. In certain embodiments, such polypeptides are at least about 90% pure, at least about 95% pure and in certain embodiments, at least about 99% pure. A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of the natural environment.

A number of specific siRNA polynucleotide sequences useful for interfering with Notch1 polypeptide expression are described herein in the Examples and are provided in the Sequence Listing. SiRNA polynucleotides may generally be prepared by any method known in the art, including, for example, solid phase chemical synthesis. Modifications in a polynucleotide sequence may also be introduced using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis. Further, siRNAs may be chemically modified or conjugated to improve their serum stability and/or delivery properties as described further herein. Included as an aspect of the invention are the siRNAs described herein wherein the ribose has been removed therefrom. Alternatively, siRNA polynucleotide molecules may be generated by *in vitro* or *in vivo* transcription of suitable DNA sequences (*e.g.*, polynucleotide sequences encoding a PTP, or a desired portion thereof), provided that the DNA is incorporated into a vector with a suitable RNA polymerase promoter (such as T7, U6, H1, or SP6). In addition, a siRNA polynucleotide may be administered to a patient, as may be a DNA sequence (*e.g.*, a recombinant nucleic acid construct as provided herein) that supports transcription (and optionally appropriate processing steps) such that a desired siRNA is generated *in vivo*.

As discussed above, siRNA polynucleotides exhibit desirable stability characteristics and may, but need not, be further designed to resist degradation by endogenous nucleolytic enzymes by using such linkages as phosphorothioate, methylphosphonate, sulfone, sulfate, ketyl, phosphorodithioate, phosphoramidate, phosphate esters, and other such linkages (*see*, *e.g.*, Agrwal *et al.*, *Tetrahedron Lett.* 28:3539-3542 (1987); Miller *et al.*, *J. Am. Chem. Soc.* 93:6657-6665 (1971); Stec *et al.*, *Tetrahedron Lett.* *26*:2191-2194 (1985); Moody *et al.*, *Nucleic Acids Res.* 12:4769-4782 (1989); Uznanski *et al.*, *Nucleic Acids Res.* (1989); Letsinger *et al.*, *Tetrahedron* 40:137-143 (1984); Eckstein, *Annu. Rev. Biochem.* 54:367-402 (1985); Eckstein, *Trends Biol. Sci.* 14:97-100 (1989); Stein, In: *Oligodeoxynucleotides.*

Antisense Inhibitors of Gene Expression, Cohen, ed., Macmillan Press, London, pp. 97-117 (1989); Jager *et al.*, *Biochemistry* 27:7237-7246 (1988)).

Any polynucleotide of the invention may be further modified to increase stability or reduce cytokine production *in vivo*. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiester linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine, and wybutosine and the like, as well as acetyl- methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine, and uridine. See for example Molecular Therapy, Vol. 15, no. 9, 1663-1669 (Sept.2007) These polynucleotide variants may be modified such that the activity of the siRNA polynucleotide is not substantially diminished, as described above. The effect on the activity of the siRNA polynucleotide may generally be assessed as described herein or using conventional methods.

In certain embodiments, “vectors” mean any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

By “subject” is meant an organism which is a recipient of the nucleic acid molecules of the invention. “Subject” also refers to an organism to which the nucleic acid molecules of the invention can be administered. In certain embodiments, a subject is a mammal or mammalian cells. In further embodiments, a subject is a human or human cells. Subjects of the present invention include, but are not limited to mice, rats, pigs, and non-human primates.

Nucleic acids can be synthesized using protocols known in the art as described in Caruthers *et al.*, 1992, Methods in Enzymology 211, 3-19; Thompson *et al.*, International PCT Publication No. WO 99/54459; Wincott *et al.*, 1995, Nucleic Acids Res. 23, 2677-2684; Wincott *et al.*, 1997, Methods Mol. Bio., 74, 59-68; Brennan *et al.*, 1998, Biotechnol Bioeng., 61, 33-45; and Brennan, U.S. Pat. No. 6,001,311). The synthesis of nucleic acids makes use

of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5′-end, and phosphoramidites at the 3′-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2 μM scale protocol with a 2.5 min coupling step for 2′-O-methylated nucleotides and a 45 second coupling step for 2′-deoxy nucleotides. Alternatively, syntheses at the 0.2 μM scale can be performed on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, Calif.) with minimal modification to the cycle. A 33-fold excess (60 μL of 0.11 M=6.6 μM) of 2′-O-methyl phosphoramidite and a 105-fold excess of S-ethyl tetrazole (60 μL of 0.25 M=15 μM) can be used in each coupling cycle of 2′-O-methyl residues relative to polymer-bound 5′-hydroxyl. A 22-fold excess (40 μL of 0.11 M=4.4 μM) of deoxy phosphoramidite and a 70-fold excess of S-ethyl tetrazole (40 μL of 0.25 M=10 μM) can be used in each coupling cycle of deoxy residues relative to polymer-bound 5′-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by calorimetric quantitation of the trityl fractions, are typically 97.5 99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include; detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% N-methylimidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); and oxidation solution is 16.9 mM I_2, 49 mM pyridine, 9% water in THF. Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide, 0.05 M in acetonitrile) is used.

By “nucleotide” is meant a heterocyclic nitrogenous base in N-glycosidic linkage with a phosphorylated sugar. Nucleotides are recognized in the art to include natural bases (standard), and modified bases well known in the art. Such bases are generally located at the 1′ position of a nucleotide

sugar moiety. Nucleotides generally comprise a base, sugar and a phosphate group. The nucleotides can be unmodified or modified at the sugar, phosphate and/or base moiety, (also referred to interchangeably as nucleotide analogs, modified nucleotides, non-natural nucleotides, non-standard nucleotides and other (see for example, Usman and McSwiggen, *supra*; Eckstein *et al*., International PCT Publication No. WO 92/07065; Usman *et al*., International PCT Publication No. WO 93/15187; Uhlman & Peyman, *supra*). There are several examples of modified nucleic acid bases known in the art as summarized by Limbach *et al*., (1994, Nucleic Acids Res. 22, 2183-2196).

Exemplary chemically modified and other natural nucleic acid bases that can be introduced into nucleic acids include, for example, inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2,4,6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidines (*e.g.*, 5-methylcytidine), 5-alkyluridines (*e.g.*, ribothymidine), 5-halouridine (*e.g.*, 5-bromouridine) or 6-azapyrimidines or 6-alkylpyrimidines (*e.g.* 6-methyluridine), propyne, quesosine, 2-thiouridine, 4-thiouridine, wybutosine, wybutoxosine, 4-acetyltidine, 5-(carboxyhydroxymethyl)uridine, 5´-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluridine, beta-D-galactosylqueosine, 1-methyladenosine, 1-methylinosine, 2,2-dimethylguanosine, 3-methylcytidine, 2-methyladenosine, 2-methylguanosine, N6-methyladenosine, 7-methylguanosine, 5-methoxyaminomethyl-2-thiouridine, 5-methylaminomethyluridine, 5-methylcarbonyhnethyluridine, 5-methyloxyuridine, 5-methyl-2-thiouridine, 2-methylthio-N6-isopentenyladenosine, beta-D-mannosylqueosine, uridine-5-oxyacetic acid, 2-thiocytidine, threonine derivatives and others (Burgin *et al*., 1996, Biochemistry, 35, 14090; Uhlman & Peyman, *supra*). By "modified bases" in this aspect is meant nucleotide bases other than adenine, guanine, cytosine and uracil at 1´ position or their equivalents; such bases can be used at any position, for example, within the catalytic core of an enzymatic nucleic acid molecule and/or in the substrate-binding regions of the nucleic acid molecule.

By “nucleoside” is meant a heterocyclic nitrogenous base in N-glycosidic linkage with a sugar. Nucleosides are recognized in the art to include natural bases (standard), and modified bases well known in the art. Such bases are generally located at the 1´ position of a nucleoside sugar moiety. Nucleosides generally comprise a base and sugar group. The nucleosides can be unmodified or modified at the sugar, and/or base moiety, (also referred to interchangeably as nucleoside analogs, modified nucleosides, non-natural nucleosides, non-standard nucleosides and other (see for example, Usman and McSwiggen, *supra*; Eckstein *et al*., International PCT Publication No. WO 92/07065; Usman *et al.*, International PCT Publication No. WO 93/15187; Uhlman & Peyman). There are several examples of modified nucleic acid bases known in the art as summarized by Limbach *et al*. (1994, Nucleic Acids Res. 22, 2183-2196). Exemplary chemically modified and other natural nucleic acid bases that can be introduced into nucleic acids include, inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2,4,6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidines (*e.g.*, 5-methylcytidine), 5-alkyluridines (*e.g.*, ribothymidine), 5-halouridine (*e.g.*, 5-bromouridine) or 6-azapyrimidines or 6-alkylpyrimidines (*e.g.*, 6-methyluridine), propyne, quesosine, 2-thiouridine, 4-thiouridine, wybutosine, wybutoxosine, 4-acetylcytidine, 5-(carboxyhydroxymethyl)uridine, 5´-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluridine, beta-D-galactosylqueosine, 1-methyladenosine, 1-methylinosine, 2,2-dimethylguanosine, 3-methylcytidine, 2-methyladenosine, 2-methylguanosine, N6-methyladenosine, 7-methylguanosine, 5-methoxyaminomethyl-2-thiouridine, 5-methylaminomethyluridine, 5-methylcarbonylmethyluridine, 5-methyloxyuridine, 5-methyl-2-thiouridine, 2-methylthio-N6-isopentenyladenosine, beta-D-mannosylqueosine, uridine-5-oxyacetic acid, 2-thiocytidine, threonine derivatives and others (Burgin *et al.*, 1996, Biochemistry, 35, 14090-14097; Uhlman & Peyman, *supra*). By “modified bases” in this aspect is meant

nucleoside bases other than adenine, guanine, cytosine and uracil at 1´ position or their equivalents; such bases can be used at any position, for example, within the catalytic core of an enzymatic nucleic acid molecule and/or in the substrate-binding regions of the nucleic acid molecule.

Nucleotide sequences as described herein may be joined to a variety of other nucleotide sequences using established recombinant DNA techniques. For example, a polynucleotide may be cloned into any of a variety of cloning vectors, including plasmids, phagemids, lambda phage derivatives, and cosmids. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors, and sequencing vectors. In general, a suitable vector contains an origin of replication functional in at least one organism, convenient restriction endonuclease sites, and one or more selectable markers. (*See, e.g.,* WO 01/96584; WO 01/29058; U.S. Pat. No. 6,326,193; U.S. 2002/0007051). Other elements will depend upon the desired use, and will be apparent to those having ordinary skill in the art. For example, the invention contemplates the use of siRNA polynucleotide sequences in the preparation of recombinant nucleic acid constructs including vectors for interfering with the expression of a desired target polypeptide such as a Notch1 polypeptide *in vivo*; the invention also contemplates the generation of siRNA transgenic or "knock-out" animals and cells (*e.g.,* cells, cell clones, lines or lineages, or organisms in which expression of one or more desired polypeptides (*e.g.*, a target polypeptide) is fully or partially compromised). An siRNA polynucleotide that is capable of interfering with expression of a desired polypeptide (*e.g.*, a target polypeptide) as provided herein thus includes any siRNA polynucleotide that, when contacted with a subject or biological source as provided herein under conditions and for a time sufficient for target polypeptide expression to take place in the absence of the siRNA polynucleotide, results in a statistically significant decrease (alternatively referred to as "knockdown" of expression) in the level of target polypeptide expression that can be detected. In certain embodiments, the decrease is

greater than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or 98% relative to the expression level of the polypeptide detected in the absence of the siRNA, using conventional methods for determining polypeptide expression as known to the art and provided herein. In certain embodiments, the presence of the siRNA polynucleotide in a cell does not result in or cause any undesired toxic effects, for example, apoptosis or death of a cell in which apoptosis is not a desired effect of RNA interference.

The present invention also relates to vectors and to constructs that include or encode siRNA polynucleotides of the present invention, and in particular to "recombinant nucleic acid constructs" that include any nucleic acids that may be transcribed to yield target polynucleotide-specific siRNA polynucleotides (*i.e.*, siRNA specific for a polynucleotide that encodes a target polypeptide, such as a mRNA) according to the invention as provided above; to host cells which are genetically engineered with vectors and/or constructs of the invention and to the production of siRNA polynucleotides, polypeptides, and/or fusion proteins of the invention, or fragments or variants thereof, by recombinant techniques. SiRNA sequences disclosed herein as RNA polynucleotides may be engineered to produce corresponding DNA sequences using well established methodologies such as those described herein. Thus, for example, a DNA polynucleotide may be generated from any siRNA sequence described herein (including in the Sequence Listing), such that the present siRNA sequences will be recognized as also providing corresponding DNA polynucleotides (and their complements). These DNA polynucleotides are therefore encompassed within the contemplated invention, for example, to be incorporated into the subject invention recombinant nucleic acid constructs from which siRNA may be transcribed.

According to the present invention, a vector may comprise a recombinant nucleic acid construct containing one or more promoters for transcription of an RNA molecule, for example, the human U6 snRNA promoter (*see, e.g.,* Miyagishi *et al*, *Nat. Biotechnol.* 20:497-500 (2002); Lee *et al*., *Nat.*

Biotechnol. 20:500-505 (2002); Paul *et al*., *Nat. Biotechnol.* 20:505-508 (2002); Grabarek *et al*., *BioTechniques* 34:73544 (2003); *see also* Sui *et al*., *Proc. Natl. Acad. Sci. USA* 99:5515-20 (2002)). Each strand of a siRNA polynucleotide may be transcribed separately each under the direction of a separate promoter and then may hybridize within the cell to form the siRNA polynucleotide duplex. Each strand may also be transcribed from separate vectors (*see* Lee *et al*., *supra*). Alternatively, the sense and antisense sequences specific for a Notch1 sequence may be transcribed under the control of a single promoter such that the siRNA polynucleotide forms a hairpin molecule (Paul *et al*., *supra*). In such an instance, the complementary strands of the siRNA specific sequences are separated by a spacer that comprises at least four nucleotides, but may comprise at least 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, 94 18 nucleotides or more nucleotides as described herein. In addition, siRNAs transcribed under the control of a U6 promoter that form a hairpin may have a stretch of about four uridines at the 3' end that act as the transcription termination signal (Miyagishi *et al*., *supra*; Paul *et al*., *supra*). By way of illustration, if the target sequence is 19 nucleotides, the siRNA hairpin polynucleotide (beginning at the 5' end) has a 19-nucleotide sense sequence followed by a spacer (which as two uridine nucleotides adjacent to the 3' end of the 19-nucleotide sense sequence), and the spacer is linked to a 19 nucleotide antisense sequence followed by a 4-uridine terminator sequence, which results in an overhang. SiRNA polynucleotides with such overhangs effectively interfere with expression of the target polypeptide (*see id.*). A recombinant construct may also be prepared using another RNA polymerase III promoter, the H1 RNA promoter, that may be operatively linked to siRNA polynucleotide specific sequences, which may be used for transcription of hairpin structures comprising the siRNA specific sequences or separate transcription of each strand of a siRNA duplex polynucleotide (*see, e.g.,* Brummelkamp *et al*., Science 296:550-53 (2002); Paddison *et al*., *supra*). DNA vectors useful for insertion of sequences for transcription of an siRNA polynucleotide include pSUPER vector (*see, e.g.,*

Brummelkamp *et al*., supra); pAV vectors derived from pCWRSVN (*see, e.g.,* Paul *et al*., *supra*); and pIND (*see, e.g.*, Lee *et al*., *supra*), or the like.

In certain embodiments, the nucleic acid molecules of the instant invention can be expressed within cells from eukaryotic promoters (*e.g.*, Izant and Weintraub, 1985, Science, 229, 345-352; McGarry and Lindquist, 1986, Proc. Natl. Acad. Sci., USA, 83, 399-403; Scanlon *et al*., 1991, Proc. Natl. Acad. Sci. USA, 88, 10591-10595; Kashani-Sabet *et al*., 1992, Antisense Res. Dev., 2, 3-15; Dropulic *et al*., 1992, J. Virol., 66, 1432-1441; Weerasinghe *et al*., 1991, J. Virol., 65, 5531-5534; Ojwang *et al*., 1992, Proc. Natl. Acad. Sci. USA, 89, 10802-10806; Chen *et al*., 1992, Nucleic Acids Res., 20, 4581-4589; Sarver *et al*., 1990 Science, 247, 1222-1225; Thompson *et al*., 1995, Nucleic Acids Res., 23, 2259-2268; Good *et al*., 1997, Gene Therapy, 4, 45-54). Those skilled in the art will realize that any nucleic acid can be expressed in eukaryotic cells from the appropriate DNA/RNA vector. The activity of such nucleic acids can be augmented by their release from the primary transcript by an enzymatic nucleic acid (Draper *et al*., PCT WO 93/23569, and Sullivan *et al*., PCT WO 94/02595; Ohkawa *et al*., 1992, Nucleic Acids Symp. Ser., 27, 15-16; Taira *et al*., 1991, Nucleic Acids Res., 19, 5125-5130; Ventura *et al*., 1993, Nucleic Acids Res., 21, 3249-3255; Chowrira *et al*., 1994, J. Biol. Chem., 269, 25856-25864).

In another aspect of the invention, nucleic acid molecules of the present invention, such as RNA molecules, are expressed from transcription units (see for example Couture *et al*., 1996, TIG., 12, 510-515) inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. RNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, lentivirus, or alphavirus. Preferably, the recombinant vectors capable of expressing the nucleic acid molecules are delivered as described above, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of nucleic acid molecules. Such vectors can be repeatedly

administered as necessary. Once expressed, the nucleic acid molecule binds to the target mRNA and induces RNAi within cell. Delivery of nucleic acid molecule expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient or subject followed by reintroduction into the patient or subject, or by any other means that would allow for introduction into the desired target cell (for a review see Couture *et al*., 1996, TIG., 12, 510-515).

In one aspect, the invention features an expression vector comprising a nucleic acid sequence encoding at least one of the nucleic acid molecules of the instant invention is disclosed. The nucleic acid sequence encoding the nucleic acid molecule of the instant invention is operably linked in a manner which allows expression of that nucleic acid molecule.

In another aspect the invention features an expression vector comprising: a) a transcription initiation region (*e.g.*, eukaryotic pol I, II or III initiation region); b) a transcription termination region (*e.g.*, eukaryotic pol I, II or III termination region); c) a nucleic acid sequence encoding at least one of the nucleic acid catalyst of the instant invention; and wherein said sequence is operably linked to said initiation region and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule. The vector can optionally include an open reading frame (ORF) for a protein operably linked on the 5′ side or the 3′-side of the sequence encoding the nucleic acid catalyst of the invention; and/or an intron (intervening sequences).

Transcription of the nucleic acid molecule sequences may be driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters are expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type depends on the nature of the gene regulatory sequences (enhancers, silencers, *etc*.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and

Moss, 1990, Proc. Natl. Acad. Sci. USA, 87, 6743-6747; Gao and Huang 1993, Nucleic Acids Res., 21, 2867-2872; Lieber *et al*., 1993, Methods Enzymol., 217, 47-66; Zhou *et al*., 1990, Mol. Cell. Biol., 10, 4529-4537). Several investigators have demonstrated that nucleic acid molecules, such as ribozymes expressed from such promoters can function in mammalian cells (*e.g.*, Kashani-Sabet *et al*., 1992, Antisense Res. Dev., 2, 3-15; Ojwang *et al*., 1992, Proc. Natl. Acad. Sci. USA, 89, 10802-10806; Chen *et al.*, 1992, Nucleic Acids Res., 20, 4581-4589; Yu *et al*., 1993, Proc. Natl. Acad. Sci. USA, 90, 6340-6344; L'Huillier *et al*., 1992, EMBO J., 11, 4411-4418; Lisziewicz *et al*., 1993, Proc. Natl. Acad. Sci. U.S.A, 90, 8000-8004; Thompson *et al*., 1995, Nucleic Acids Res., 23, 2259-2268; Sullenger & Cech, 1993, Science, 262, 1566-1569). More specifically, transcription units such as the ones derived from genes encoding U6 small nuclear (snRNA), transfer RNA (tRNA) and adenovirus VA RNA are useful in generating high concentrations of desired RNA molecules such as ribozymes in cells (Thompson *et al*., *supra*; Couture and Stinchcomb, 1996, *supra*; Noonberg *et al*., 1994, Nucleic Acid Res., 22, 2830-2836; Noonberg *et al*., U.S. Pat. No. 5,624,803; Good *et al*., 1997, Gene Ther., 4, 45-54; Beigelman *et al*., International PCT Publication No. WO 96/18736). The above ribozyme transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors) (for a review see Couture and Stinchcomb, 1996, *supra*).

In another aspect, the invention features an expression vector comprising nucleic acid sequence encoding at least one of the nucleic acid molecules of the invention, in a manner which allows expression of that nucleic acid molecule. The expression vector comprises in one embodiment; a) a transcription initiation region; b) a transcription termination region; c) a nucleic acid sequence encoding at least one said nucleic acid molecule; and wherein said sequence is operably linked to said initiation region and said termination

region, in a manner which allows expression and/or delivery of said nucleic acid molecule.

In another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an open reading frame; d) a nucleic acid sequence encoding at least one said nucleic acid molecule, wherein said sequence is operably linked to the 3´-end of said open reading frame; and wherein said sequence is operably linked to said initiation region, said open reading frame and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule. In yet another embodiment the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; d) a nucleic acid sequence encoding at least one said nucleic acid molecule; and wherein said sequence is operably linked to said initiation region, said intron and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule.

In yet another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; d) an open reading frame; e) a nucleic acid sequence encoding at least one said nucleic acid molecule, wherein said sequence is operably linked to the 3´-end of said open reading frame; and wherein said sequence is operably linked to said initiation region, said intron, said open reading frame and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule.

In another example, the nucleic acids of the invention as described herein (*e.g.,* DNA sequences from which siRNA may be transcribed) herein may be included in any one of a variety of expression vector constructs as a recombinant nucleic acid construct for expressing a target polynucleotide-specific siRNA polynucleotide. Such vectors and constructs include chromosomal, nonchromosomal and synthetic DNA sequences, *e.g.*, derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast

plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA, such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used for preparation of a recombinant nucleic acid construct as long as it is replicable and viable in the host.

The appropriate DNA sequence(s) may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are those known and commonly employed by those skilled in the art. A number of standard techniques are described, for example, in Ausubel *et al*. (1993 *Current Protocols in Molecular Biology*, Greene Publ. Assoc. Inc. & John Wiley & Sons, Inc., Boston, MA); Sambrook *et al*. (2001 *Molecular Cloning*, Third Ed., Cold Spring Harbor Laboratory, Plainview, NY); Maniatis *et al*. (1982 Molecular Cloning, Cold Spring Harbor Laboratory, Plainview, NY); and elsewhere.

The DNA sequence in the expression vector is operatively linked to at least one appropriate expression control sequences (*e.g.,* a promoter or a regulated promoter) to direct mRNA synthesis. Representative examples of such expression control sequences include LTR or SV40 promoter, the *E. coli lac* or *trp*, the phage lambda P_L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art, and preparation of certain particularly preferred recombinant expression constructs comprising at

least one promoter or regulated promoter operably linked to a nucleic acid encoding a polypeptide (*e.g.,* PTP, MAP kinase kinase, or chemotherapeutic target polypeptide) is described herein.

The expressed recombinant siRNA polynucleotides may be useful in intact host cells; in intact organelles such as cell membranes, intracellular vesicles or other cellular organelles; or in disrupted cell preparations including but not limited to cell homogenates or lysates, microsomes, uni- and multilamellar membrane vesicles or other preparations. Alternatively, expressed recombinant siRNA polynucleotides can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

In certain preferred embodiments of the present invention, the siRNA polynucleotides are detectably labeled, and in certain embodiments the siRNA polynucleotide is capable of generating a radioactive or a fluorescent signal. The siRNA polynucleotide can be detectably labeled by covalently or non-covalently attaching a suitable reporter molecule or moiety, for example a radionuclide such as ^{32}P (*e.g.*, Pestka *et al.*, 1999 *Protein Expr. Purif*. 17:203-14), a radiohalogen such as iodine [^{125}I or ^{131}I] (*e.g.*, Wilbur, 1992 *Bioconjug. Chem*. 3:433-70), or tritium [^{3}H]; an enzyme; or any of various luminescent (*e.g.,* chemiluminescent) or fluorescent materials (*e.g.*, a fluorophore) selected according to the particular fluorescence detection technique to be employed, as known in the art and based upon the present disclosure. Fluorescent reporter moieties and methods for labeling siRNA polynucleotides and/or PTP substrates as provided herein can be found, for example in Haugland (1996 *Handbook of Fluorescent Probes and Research Chemicals- Sixth Ed.*, Molecular Probes, Eugene, OR; 1999 *Handbook of Fluorescent Probes and*

Research Chemicals- Seventh Ed., Molecular Probes, Eugene, OR, Internet: http://www.probes.com/lit/) and in references cited therein. Particularly preferred for use as such a fluorophore in the subject invention methods are fluorescein, rhodamine, Texas Red, AlexaFluor-594, AlexaFluor-488, Oregon Green, BODIPY-FL, umbelliferone, dichlorotriazinylamine fluorescein, dansyl chloride, phycoerythrin or Cy-5. Examples of suitable enzymes include, but are not limited to, horseradish peroxidase, biotin, alkaline phosphatase, β-galactosidase and acetylcholinesterase. Appropriate luminescent materials include luminol, and suitable radioactive materials include radioactive phosphorus [^{32}P]. In certain other preferred embodiments of the present invention, a detectably labeled siRNA polynucleotide comprises a magnetic particle, for example a paramagnetic or a diamagnetic particle or other magnetic particle or the like (preferably a microparticle) known to the art and suitable for the intended use. Without wishing to be limited by theory, according to certain such embodiments there is provided a method for selecting a cell that has bound, adsorbed, absorbed, internalized or otherwise become associated with a siRNA polynucleotide that comprises a magnetic particle.

Methods of Use and Administration of Nucleic Acid Molecules

Methods for the delivery of nucleic acid molecules are described in Akhtar *et al.*, 1992, Trends Cell Bio., 2, 139; and Delivery Strategies for Antisense Oligonucleotide Therapeutics, ed. Akhtar; Sullivan *et al.*, PCT WO 94/02595, further describes the general methods for delivery of enzymatic RNA molecules. These protocols can be utilized for the delivery of virtually any nucleic acid molecule. Nucleic acid molecules can be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. Alternatively, the nucleic acid/vehicle combination is locally delivered by direct injection or by use of an

infusion pump. Other routes of delivery include, but are not limited to oral (tablet or pill form) and/or intrathecal delivery (Gold, 1997, Neuroscience, 76, 1153-1158). Other approaches include the use of various transport and carrier systems, for example, through the use of conjugates and biodegradable polymers. For a comprehensive review on drug delivery strategies including CNS delivery, see Ho *et al*., 1999, Curr. Opin. Mol. Ther., 1, 336-343 and Jain, Drug Delivery Systems: Technologies and Commercial Opportunities, Decision Resources, 1998 and Groothuis *et al*., 1997, J. NeuroVirol., 3, 387-400. More detailed descriptions of nucleic acid delivery and administration are provided in Sullivan *et al*., *supra*, Draper *et al*., PCT WO93/23569, Beigelman *et al*., PCT WO99/05094, and Klimuk *et al*., PCT WO99/04819.

The molecules of the instant invention can be used as pharmaceutical agents. Pharmaceutical agents prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, in certain embodiments all of the symptoms) of a disease state in a subject.

The negatively charged polynucleotides of the invention can be administered and introduced into a subject by any standard means, with or without stabilizers, buffers, and the like, to form a pharmaceutical composition. When it is desired to use a liposome delivery mechanism, standard protocols for formation of liposomes can be followed. The compositions of the present invention can also be formulated and used as tablets, capsules or elixirs for oral administration; suppositories for rectal administration; sterile solutions; suspensions for injectable administration; and the other compositions known in the art.

The present invention also includes pharmaceutically acceptable formulations of the compounds described. These formulations include salts of the above compounds, *e.g.*, acid addition salts, for example, salts of hydrochloric, hydrobromic, acetic acid, and benzene sulfonic acid.

A composition or formulation of the siRNA molecules of the present invention refers to a composition or formulation in a form suitable for

administration, *e.g.*, systemic administration, into a cell or subject, preferably a human. Suitable forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or by injection. Such forms should not prevent the composition or formulation from reaching a target cell. For example, pharmacological compositions injected into the blood stream should be soluble. Other factors are known in the art, and include considerations such as toxicity and forms which prevent the composition or formulation from exerting its effect.

By "systemic administration" is meant *in vivo* systemic absorption or accumulation of drugs in the blood stream followed by distribution throughout the entire body. Administration routes which lead to systemic absorption include, without limitations: intravenous, subcutaneous, intraperitoneal, inhalation, oral, intrapulmonary and intramuscular. Each of these administration routes exposes the desired negatively charged nucleic acids, to an accessible diseased tissue. The rate of entry of a drug into the circulation has been shown to be a function of molecular weight or size. The use of a liposome or other drug carrier comprising the compounds of the instant invention can potentially localize the drug, for example, in certain tissue types, such as the tissues of the reticular endothelial system (RES). A liposome formulation which can facilitate the association of drug with the surface of cells, such as, lymphocytes and macrophages is also useful. This approach can provide enhanced delivery of the drug to target cells by taking advantage of the specificity of macrophage and lymphocyte immune recognition of abnormal cells, such as cancer cells.

By pharmaceutically acceptable formulation is meant, a composition or formulation that allows for the effective distribution of the nucleic acid molecules of the instant invention in the physical location most suitable for their desired activity. Non-limiting examples of agents suitable for formulation with the nucleic acid molecules of the instant invention include: PEG conjugated nucleic acids, phospholipid conjugated nucleic acids, nucleic acids containing lipophilic moieties, phosphorothioates, P-glycoprotein inhibitors (such as

Pluronic P85) which can enhance entry of drugs into various tissues; biodegradable polymers, such as poly (DL-lactide-coglycolide) microspheres for sustained release delivery after implantation (Emerich, DF *et al.*, 1999, Cell Transplant, 8, 47-58) Alkermes, Inc. Cambridge, Mass.; and loaded nanoparticles, such as those made of polybutylcyanoacrylate, which can deliver drugs across the blood brain barrier and can alter neuronal uptake mechanisms (Prog Neuropsychopharmacol Biol Psychiatry, 23, 941-949, 1999).

The invention also features the use of the composition comprising surface-modified liposomes containing poly (ethylene glycol) lipids (PEG-modified, branched and unbranched or combinations thereof, or long-circulating liposomes or stealth liposomes). Nucleic acid molecules of the invention can also comprise covalently attached PEG molecules of various molecular weights. These formulations offer a method for increasing the accumulation of drugs in target tissues. This class of drug carriers resists opsonization and elimination by the mononuclear phagocytic system (MPS or RES), thereby enabling longer blood circulation times and enhanced tissue exposure for the encapsulated drug (Lasic *et al*. Chem. Rev. 1995, 95, 2601-2627; Ishiwata *et al*., Chem. Pharm. Bull. 1995, 43, 1005-1011). Such liposomes have been shown to accumulate selectively in tumors, presumably by extravasation and capture in the neovascularized target tissues (Lasic *et al*., Science 1995, 267, 1275-1276; Oku *et al*., 1995, Biochim. Biophys. Acta, 1238, 86-90). The long-circulating liposomes enhance the pharmacokinetics and pharmacodynamics of DNA and RNA, particularly compared to conventional cationic liposomes which are known to accumulate in tissues of the MPS (Liu *et al*., J. Biol. Chem. 1995, 42, 24864-24870; Choi *et al*., International PCT Publication No. WO 96/10391; Ansell *et al*., International PCT Publication No. WO 96/10390; Holland *et al*., International PCT Publication No. WO 96/10392). Long-circulating liposomes are also likely to protect drugs from nuclease degradation to a greater extent compared to cationic liposomes, based on their ability to avoid accumulation in metabolically aggressive MPS tissues such as the liver and spleen.

In a further embodiment, the present invention includes nucleic acid compositions, such as siRNA compositions, prepared as described in US 2003/0166601. In this regard, in one embodiment, the present invention provides a composition of the siRNA described herein comprising: 1) a core complex comprising the nucleic acid (*e.g.*, siRNA) and polyethyleneimine; and 2) an outer shell moiety comprising NHS-PEG-VS and a targeting moiety.

Thus, in certain embodiments, siRNA sequences are complexed through electrostatic bonds with a cationic polymer to form a RNAi/nanoplex structure. In certain embodiments, the cationic polymer facilitates cell internalization and endosomal release of its siRNA payload in the cytoplasm of a target cell. Further, in certain embodiments, a hydrophilic steric polymer can be added to the RNAi/cationic polymer nanoplex. In this regard, illustrative steric polymers include a Polyethylene Glycol (PEG) layer. Without being bound by theory, this component helps reduce non-specific tissue interaction, increase circulation time, and minimize immunogenic potential. PEG layers can also enhance siRNA distribution to tumor tissue through the phenomenon of Enhanced Permeability and Retention (EPR) in the often leaky tumor vasculature. Additionally, these complexes can be crosslinked to provide additional stability. This crosslinking can be done through coupling to the cationic polymers, hydrophilic steric polymers or both. Where a targeting moiety is used, the crosslinking can be done prior to or after the coupling of the crosslinking agents.

In a further embodiment, the present invention includes nucleic acid compositions prepared for delivery as described in US Patent Nos. 6,692,911, 7,163,695 and 7,070,807. In this regard, in one embodiment, the present invention provides a nucleic acid of the present invention in a composition comprising copolymers of lysine and histidine (HK) as described in US Patents 7,163,695, 7,070,807, and 6,692,911 either alone or in combination with PEG (*e.g.*, branched or unbranched PEG or a mixture of both), in combination with PEG and a targeting moiety or any of the foregoing in

combination with a crosslinking agent. In this regard, in certain embodiments, the present invention provides siRNA molecules in compositions comprising gluconic-acid-modified polyhistidine or gluconylated-polyhistidine/transferrin-polylysine.

In certain embodiments of the present invention a targeting moiety as described above is utilized to target the desired siRNA(s) to a cell of interest. In this regard, as would be recognized by the skilled artisan, targeting ligands are readily interchangeable depending on the disease and siRNA of interest to be delivered. In certain embodiments, the targeting moiety may include an RGD (Arginine, Glycine, Aspartic Acid) peptide ligand that binds to activated integrins on tumor vasculature endothelial cells, such as αvβ3 integrins.

Thus, in certain embodiments, compositions comprising the siRNA molecules of the present invention include at least one targeting moiety, such as a ligand for a cell surface receptor or other cell surface marker that permits highly specific interaction of the composition comprising the siRNA molecule (the "vector") with the target tissue or cell. More specifically, in one embodiment, the vector preferably will include an unshielded ligand or a shielded ligand. The vector may include two or more targeting moieties, depending on the cell type that is to be targeted. Use of multiple (two or more) targeting moieties can provide additional selectivity in cell targeting, and also can contribute to higher affinity and/or avidity of binding of the vector to the target cell. When more than one targeting moiety is present on the vector, the relative molar ratio of the targeting moieties may be varied to provide optimal targeting efficiency. Methods for optimizing cell binding and selectivity in this fashion are known in the art. The skilled artisan also will recognize that assays for measuring cell selectivity and affinity and efficiency of binding are known in the art and can be used to optimize the nature and quantity of the targeting ligand(s).

A variety of agents that direct compositions to particular cells are known in the art (see, for example, Cotten *et al*., Methods Enzym, 217: 618,

1993). Illustrative targeting agents include biocompounds, or portions thereof, that interact specifically with individual cells, small groups of cells, or large categories of cells. Examples of useful targeting agents include, but are in no way limited to, low-density lipoproteins (LDLs), transferrin, asiaglycoproteins, gp120 envelope protein of the human immunodeficiency virus (HIV), and diptheria toxin, antibodies, and carbohydrates.

Another example of a targeting moiety is sialyl-Lewis^x, where the composition is intended for treating a region of inflammation. Other peptide ligands may be identified using methods such as phage display (F. Bartoli et al., Isolation of peptide ligands for tissue-specific cell surface receptors, in Vector Targeting Strategies for Therapeutic Gene Delivery (Abstracts form Cold Spring Harbor Laboratory 1999 meeting), 1999, p4) and microbial display (Georgiou *et al.*, Ultra-High Affinity Antibodies from Libraries Displayed on the Surface of Microorganisms and Screened by FACS, in Vector Targeting Strategies for Therapeutic Gene Delivery (Abstracts form Cold Spring Harbor Laboratory 1999 meeting), 1999, p 3.). Ligands identified in this manner are suitable for use in the present invention.

Methods have been developed to create novel peptide sequences that elicit strong and selective binding for target tissues and cells such as “DNA Shuffling” (W. P. C. Stremmer, Directed Evolution of Enzymes and Pathways by DNA Shuffling, in Vector Targeting Strategies for Therapeutic Gene Delivery (Abstracts form Cold Spring Harbor Laboratory 1999 meeting), 1999, p.5.) and these novel sequence peptides are suitable ligands for the invention. Other chemical forms for ligands are suitable for the invention such as natural carbohydrates which exist in numerous forms and are a commonly used ligand by cells (Kraling *et al.*, Am. J. Path., 1997, 150, 1307) as well as novel chemical species, some of which may be analogues of natural ligands such as D-amino acids and peptidomimetics and others which are identifed through medicinal chemistry techniques such as combinatorial chemistry (P. D. Kassner *et al.*, Ligand Identification via Expression (LIVE.theta.): Direct selection of Targeting

Ligands from Combinatorial Libraries, in Vector Targeting Strategies for Therapeutic Gene Delivery (Abstracts form Cold Spring Harbor Laboratory 1999 meeting), 1999, p8.).

The present invention also includes compositions prepared for storage or administration which include a pharmaceutically effective amount of the desired compounds in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington: The Science and Practice of Pharmacy, 20th Edition. Baltimore, MD: Lippincott Williams & Wilkins, 2000. For example, preservatives, stabilizers, dyes and flavoring agents can be provided. These include sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid. In addition, antioxidants and suspending agents can be used.

A pharmaceutically effective dose is that dose required to prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, and in certain embodiments, all of the symptoms of) a disease state. The pharmaceutically effective dose depends on the type of disease, the composition used, the route of administration, the type of mammal being treated, the physical characteristics of the specific mammal under consideration, concurrent medication, and other factors which those skilled in the medical arts will recognize. Generally, an amount between 0.1 mg/kg and 100 mg/kg body weight/day of active ingredients is administered dependent upon potency of the negatively charged polymer.

The nucleic acid molecules of the invention and formulations thereof can be administered orally, topically, parenterally, by inhalation or spray or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes percutaneous, subcutaneous, intravascular (*e.g.*, intravenous), intramuscular, or intrathecal injection or infusion techniques and the like. In addition, there is provided a pharmaceutical formulation

comprising a nucleic acid molecule of the invention and a pharmaceutically acceptable carrier. One or more nucleic acid molecules of the invention can be present in association with one or more non-toxic pharmaceutically acceptable carriers and/or diluents and/or adjuvants, and if desired other active ingredients. The pharmaceutical compositions containing nucleic acid molecules of the invention can be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs.

The nucleic acid compositions of the invention can be used in combination with other nucleic acid compositions that target the same or different areas of the target gene (*e.g.*, Notch1), or that target other genes of interest. The nucleic acid compositions of the invention can also be used in combination with any of a variety of treatment modalities, such as chemotherapy, radiation therapy, or small molecule regimens.

Compositions intended for oral use can be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions can contain one or more such sweetening agents, flavoring agents, coloring agents or preservative agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients that are suitable for the manufacture of tablets. These excipients can be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets can be uncoated or they can be coated by known techniques. In some cases such coatings can be prepared by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer

period. For example, a time delay material such as glyceryl monosterate or glyceryl distearate can be employed.

Formulations for oral use can also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydropropyl-methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents can be a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions can also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions can be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions can contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents and flavoring agents can be added to provide palatable oral

preparations. These compositions can be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents or suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, can also be present.

Pharmaceutical compositions of the invention can also be in the form of oil-in-water emulsions. The oily phase can be a vegetable oil or a mineral oil or mixtures of these. Suitable emulsifying agents can be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions can also contain sweetening and flavoring agents.

Syrups and elixirs can be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol, glucose or sucrose. Such formulations can also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions can be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents that have been mentioned above. The sterile injectable preparation can also be a sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or

suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono-or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The nucleic acid molecules of the invention can also be administered in the form of suppositories, *e.g.*, for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient that is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include cocoa butter and polyethylene glycols.

Nucleic acid molecules of the invention can be administered parenterally in a sterile medium. The drug, depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local anesthetics, preservatives and buffering agents can be dissolved in the vehicle.

Dosage levels of the order of from about 0.01 mg to about 140 mg per kilogram of body weight per day are useful in the treatment of the disease conditions described herein (about 0.5 mg to about 7 g per patient or subject per day). The amount of active ingredient that can be combined with the carrier materials to produce a single dosage form varies depending upon the host treated and the particular mode of administration. Dosage unit forms generally contain between from about 1 mg to about 500 mg of an active ingredient.

It is understood that the specific dose level for any particular patient or subject depends upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

For administration to non-human animals, the composition can also be added to the animal feed or drinking water. It can be convenient to formulate the animal feed and drinking water compositions so that the animal takes in a therapeutically appropriate quantity of the composition along with its

diet. It can also be convenient to present the composition as a premix for addition to the feed or drinking water.

The nucleic acid molecules of the present invention can also be administered to a subject in combination with other therapeutic compounds to increase the overall therapeutic effect. The use of multiple compounds to treat an indication can increase the beneficial effects while reducing the presence of side effects.

The nucleic acid-based inhibitors of the invention are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells or tissues. The nucleic acid or nucleic acid complexes can be locally administered to relevant tissues *ex vivo*, or *in vivo* through injection or infusion pump, with or without their incorporation in biopolymers.

The siRNA molecules of the present invention can be used in a method for treating or preventing a Notch1 expressing disorder in a subject having or suspected of being at risk for having the disorder, comprising administering to the subject one or more siRNA molecules described herein, thereby treating or preventing the disorder. In this regard, the method provides for treating such diseases described herein, by administering 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more siRNA molecules as described herein, such as those provided in SEQ ID NOs:1-160, or a dsRNA thereof.

The nucleic acid molecules of the instant invention, individually, or in combination or in conjunction with other drugs, can be used to treat diseases or conditions associated with altered expression and/or activity of Notch1. Thus, the small nucleic acid molecules described herein are useful, for example, in providing compositions to prevent, inhibit, or reduce cardiovascular disorders such as aortic valve disease and atherosclerosis, certain inflammatory disorders and cancers including but not limited to T-ALL, breast, pancreatic cancer, colorectal cancer, gastric cancer, melanoma, leukemia, lymphoma, glioma, and lung cancer and/or other disease states, conditions, or

traits associated with Notch1 gene expression or activity in a subject or organism.

The nucleic acid molecules of the instant invention, individually, or in combination or in conjunction with other drugs, can also be used to prevent diseases or conditions associated with altered activity and/or expression of Notch1 in individuals that are suspected of being at risk for developing such a disease or condition. For example, to treat or prevent a disease or condition associated with the expression levels of Notch1, the subject having the disease or condition, or suspected of being at risk for developing the disease or condition, can be treated, or other appropriate cells can be treated, as is evident to those skilled in the art, individually or in combination with one or more drugs under conditions suitable for the treatment. Thus, the present invention provides methods for treating or preventing diseases or conditions which respond to the modulation of Notch1 expression comprising administering to a subject in need thereof an effective amount of a composition comprising one or more of the nucleic acid molecules of the invention, such as those set forth in SEQ ID NOs:1-160. In one embodiment, the present invention provides methods for treating or preventing diseases associated with expression of Notch1 comprising administering to a subject in need thereof an effective amount of any one or more of the nucleic acid molecules of the invention, such as those provided in SEQ ID NOs:1-160, such that the expression of Notch1 in the subject is down-regulated, thereby treating or preventing the disease associated with expression of Notch1. In this regard, the compositions of the invention can be used in methods for treating or preventing cardiovascular disorders such as aortic valve disease and atherosclerosis, certain inflammatory disorders and cancers including but not limited to T-ALL, breast, pancreatic cancer, colorectal cancer, gastric cancer, melanoma, leukemia, lymphoma, glioma, and lung cancer, or other conditions which respond to the modulation of Notch1 expression.

In a further embodiment, the nucleic acid molecules of the invention, such as isolated siRNA, antisense or ribozymes, can be used in combination with other known treatments to treat conditions or diseases discussed herein. For example, the described molecules can be used in combination with one or more known therapeutic agents to treat cardiovascular disorders such as aortic valve disease and atherosclerosis, certain inflammatory disorders and cancers including but not limited to T-ALL, breast, pancreatic cancer, colorectal cancer, gastric cancer, melanoma, leukemia, lymphoma, glioma, and lung cancer or other conditions which respond to the modulation of Notch1 expression. Such treatments include, but not limited to chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies or other antibody therapies, cytoxin, fludaribine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, cytokines, and irradiation. These drugs inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin). (Liu *et al.*, Cell 66:807-815, 1991; Henderson *et al.*, Immun. 73:316-321, 1991; Bierer *et al.*, Curr. Opin. Immun. 5:763-773, 1993). In a further embodiment, the RNA molecules of the present invention are administered to a patient in conjunction with (*e.g.*, before, simulataneously or following) bone marrow transplantation, T cell ablative therapy using either chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, or antibodies such as OKT3 or CAMPATH. In another embodiment, the cell compositions of the present invention are administered following B-cell ablative therapy such as agents that react with CD20, *e.g.*, Rituxan.

Compositions and methods are known in the art for identifying subjects having, or suspected of being at risk for having the diseases or disorders associated with expression of Notch1 as described herein.

Thus, the present invention provides a method for interfering with expression of a polypeptide, or variant thereof, comprising contacting a subject that comprises at least one cell which is capable of expressing the polypeptide with a siRNA polynucleotide for a time and under conditions sufficient to interfere with expression of the polypeptide.

EXAMPLES

EXAMPLE 1

siRNA Candidate Molecules For the Inhibition of Notch1 Expression

Notch1 siRNA molecules were designed using a tested algorithm and using the publicly available sequences for Notch1. There are two sequences for human Notch1 found in the NCBI database, NM_017617 (SEQ ID NO:161) and AF308602 (SEQ ID NO:162). Corresponding protein sequences are provided in SEQ ID NOs:163 and 164, respectively. Alignment of the two polynucleotide sequences reveals a very high degree of homology with only a few nucleotide differences between them. NM_017617 was used as the template for designing siRNA molecules as described in this example.

Notch1 candidate siRNA molecules are shown in Table 1 below and are set forth in SEQ ID NOs:1-160.

Table 1: Notch1 Candidate siRNA Molecules

Start Position	siRNA(sense strand/antisense strand)	GC%	SEQ ID NO:
775	5'-r(GACGAUUGUCCAGGAAACAACUGCA) -3' 3'- (CUGCUAACAGGUCCUUUGUUGACGU)r-5'	48	1 2
778	5'-r(GAUUGUCCAGGAAACAACUGCAAGA) -3' 3'- (CUAACAGGUCCUUUGUUGACGUUCU)r-5'	44	3 4
853	5'-r(CCAGAGUGGACAGGUCAGUACUGUA) -3' 3'- (GGUCUCACCUGUCCAGUCAUGACAU)r-5'	52	5 6
861	5'-r(GACAGGUCAGUACUGUACCGAGGAU) -3' 3'- (CUGUCCAGUCAUGACAUGGCUCCUA)r-5'	52	7 8
988	5'-r(GAGGACUGCAGCGAGAACAUUGAUG) -3' 3'- (CUCCUGACGUCGCUCUUGUAACUAC)r-5'	52	9 10
1511	5'-r(GCUGCCUGGACAAGAUCAAUGAGUU) -3' 3'- (CGACGGACCUGUUCUAGUUACUCAA)r-5'	48	11 12
1517	5'-r(UGGACAAGAUCAAUGAGUUCCAGUG) -3' 3'- (ACCUGUUCUAGUUACUCAAGGUCAC)r-5'	44	13 14
1519	5'-r(GACAAGAUCAAUGAGUUCCAGUGCG) -3' 3'- (CUGUUCUAGUUACUCAAGGUCACGC)r-5'	48	15 16

Start Position	siRNA(sense strand/antisense strand)	GC%	SEQ ID NO:
1521	5'-r(CAAGAUCAAUGAGUUCCAGUGCGAG) -3' 3'- (GUUCUAGUUACUCAAGGUCACGCUC)r-5'	48	17 18
1639	5'-r(CCCAACACUUACACCUGUGUGUGCA) -3' 3'- (GGGUUGUGAAUGUGGACACACACGU)r-5'	52	19 20
1644	5'-r(CACUUACACCUGUGUGUGCACGGAA) -3' 3'- (GUGAAUGUGGACACACACGUGCCUU)r-5'	52	21 22
1910	5'-r(ACUGCGAGAUCAACCUGGAUGACUG) -3' 3'- (UGACGCUCUAGUUGGACCUACUGAC)r-5'	52	23 24
1961	5'-r(CCUGUCUGGACAAGAUCGAUGGCUA) -3' 3'- (GGACAGACCUGUUCUAGCUACCGAU)r-5'	52	25 26
1969	5'-r(GACAAGAUCGAUGGCUACGAGUGUG) -3' 3'- (CUGUUCUAGCUACCGAUGCUCACAC)r-5'	52	27 28
2009	5'-r(ACACAGGGAGCAUGUGUAACAUCAA) -3' 3'- (UGUGUCCCUCGUACACAUUGUAGUU)r-5'	44	29 30
2012	5'-r(CAGGGAGCAUGUGUAACAUCAACAU) -3' 3'- (GUCCCUCGUACACAUUGUAGUUGUA)r-5'	44	31 32
2014	5'-r(GGGAGCAUGUGUAACAUCAACAUCG) -3' 3'- (CCCUCGUACACAUUGUAGUUGUAGC)r-5'	48	33 34
2015	5'-r(GGAGCAUGUGUAACAUCAACAUCGA) -3' 3'- (CCUCGUACACAUUGUAGUUGUAGCU)r-5'	44	35 36
2134	5'-r(ACCUGCCUGUCUGAGGUCAAUGAGU) -3' 3'- (UGGACGGACAGACUCCAGUUACUCA)r-5'	52	37 38
2143	5'-r(UCUGAGGUCAAUGAGUGCAACAGCA) -3' 3'- (AGACUCCAGUUACUCACGUUGUCGU)r-5'	48	39 40
2234	5'-r(GGAGUGGGACCAACUGUGACAUCAA) -3' 3'- (CCUCACCCUGGUUGACACUGUAGUU)r-5'	52	41 42
2239	5'-r(GGGACCAACUGUGACAUCAACAACA) -3' 3'- (CCCUGGUUGACACUGUAGUUGUUGU)r-5'	48	43 44
2260	5'-r(AACAAUGAGUGUGAAUCCAACCCUU) -3' 3'- (UUGUUACUCACACUUAGGUUGGGAA)r-5'	40	45 46
2539	5'-r(UCCGAGGACUAUGAGAGCUUCUCCU) -3' 3'- (AGGCUCCUGAUACUCUCGAAGAGGA)r-5'	52	47 48
2542	5'-r(GAGGACUAUGAGAGCUUCUCCUGUG) -3' 3'- (CUCCUGAUACUCUCGAAGAGGACAC)r-5'	52	49 50
2593	5'-r(ACCUGUGAGGUCGACAUCAACGAGU) -3' 3'- (UGGACACUCCAGCUGUAGUUGCUCA)r-5'	52	51 52

Start Position	siRNA(sense strand/antisense strand)	GC%	SEQ ID NO:
2600	5'-r(AGGUCGACAUCAACGAGUGCGUUCU) -3' 3'- (UCCAGCUGUAGUUGCUCACGCAAGA)r-5'	52	53 54
2603	5'-r(UCGACAUCAACGAGUGCGUUCUGAG) -3' 3'- (AGCUGUAGUUGCUCACGCAAGACUC)r-5'	52	55 56
2815	5'-r(GGCACUUUCUGUGAGGAGGACAUCA) -3' 3'- (CCGUGAAAGACACUCCUCCUGUAGU)r-5'	52	57 58
2816	5'-r(GCACUUUCUGUGAGGAGGACAUCAA) -3' 3'- (CGUGAAAGACACUCCUCCUGUAGUU)r-5'	48	59 60
3045	5'-r(CAGCUACUGCCAGCACGAUGUCAAU) -3' 3'- (GUCGAUGACGGUCGUGCUACAGUUA)r-5'	52	61 62
3055	5'-r(CAGCACGAUGUCAAUGAGUGCGACU) -3' 3'- (GUCGUGCUACAGUUACUCACGCUGA)r-5'	52	63 64
3059	5'-r(ACGAUGUCAAUGAGUGCGACUCACA) -3' 3'- (UGCUACAGUUACUCACGCUGAGUGU)r-5'	48	65 66
3060	5'-r(CGAUGUCAAUGAGUGCGACUCACAG) -3' 3'- (GCUACAGUUACUCACGCUGAGUGUC)r-5'	52	67 68
3169	5'-r(CAGAACCUUGUGCACUGGUGUGACU) -3' 3'- (GUCUUGGAACACGUGACCACACUGA)r-5'	52	69 70
3637	5'-r(ACUCAGGGUGUGCACUGUGAGAUCA) -3' 3'- (UGAGUCCCACACGUGACACUCUAGU)r-5'	52	71 72
3650	5'-r(ACUGUGAGAUCAACGUGGACGACUG) -3' 3'- (UGACACUCUAGUUGCACCUGCUGAC)r-5'	52	73 74
3654	5'-r(UGAGAUCAACGUGGACGACUGCAAU) -3' 3'- (ACUCUAGUUGCACCUGCUGACGUUA)r-5'	48	75 76
3655	5'-r(GAGAUCAACGUGGACGACUGCAAUC) -3' 3'- (CUCUAGUUGCACCUGCUGACGUUAG)r-5'	52	77 78
3706	5'-r(CCCAAGUGCUUUAACAACGGCACCU) -3' 3'- (GGGUUCACGAAAUUGUUGCCGUGGA)r-5'	52	79 80
3798	5'-r(GGAUGUCAACGAGUGCCUGUCCAAU) -3' 3'- (CCUACAGUUGCUCACGGACAGGUUA)r-5'	52	81 82
3912	5'-r(CGAGUCCGUCAUCAAUGGCUGCAAA) -3' 3'- (GCUCAGGCAGUAGUUACCGACGUUU)r-5'	52	83 84
3913	5'-r(GAGUCCGUCAUCAAUGGCUGCAAAG) -3' 3'- (CUCAGGCAGUAGUUACCGACGUUUC)r-5'	52	85 86
3916	5'-r(UCCGUCAUCAAUGGCUGCAAAGGCA) -3' 3'- (AGGCAGUAGUUACCGACGUUUCCGU)r-5'	52	87 88

Start Position	siRNA(sense strand/antisense strand)	GC%	SEQ ID NO:
3917	5'-r(CCGUCAUCAAUGGCUGCAAAGGCAA) -3' 3'- (GGCAGUAGUUACCGACGUUUCCGUU)r-5'	52	89 90
4253	5'-r(CCAAAUUCAACGGGCUCUUGUGCCA) -3' 3'- (GGUUUAAGUUGCCCGAGAACACGGU)r-5'	52	91 92
4429	5'-r(GGUGACUGCUCCCUCAACUUCAAUG) -3' 3'- (CCACUGACGAGGGAGUUGAAGUUAC)r-5'	52	93 94
4474	5'-r(CAGUCUCUGCAGUGCUGGAAGUACU) -3' 3'- (GUCAGAGACGUCACGACCUUCAUGA)r-5'	52	95 96
4475	5'-r(AGUCUCUGCAGUGCUGGAAGUACUU) -3' 3'- (UCAGAGACGUCACGACCUUCAUGAA)r-5'	48	97 98
4477	5'-r(UCUCUGCAGUGCUGGAAGUACUUCA) -3' 3'- (AGAGACGUCACGACCUUCAUGAAGU)r-5'	48	99 100
4595	5'-r(ACGACCAGUACUGCAAGGACCACUU) -3' 3'- (UGCUGGUCAUGACGUUCCUGGUGAA)r-5'	52	101 102
4597	5'-r(GACCAGUACUGCAAGGACCACUUCA) -3' 3'- (CUGGUCAUGACGUUCCUGGUGAAGU)r-5'	52	103 104
4598	5'-r(ACCAGUACUGCAAGGACCACUUCAG) -3' 3'- (UGGUCAUGACGUUCCUGGUGAAGUC)r-5'	52	105 106
4796	5'-r(UGCUGCACACCAACGUGGUCUUCAA) -3' 3'- (ACGACGUGUGGUUGCACCAGAAGUU)r-5'	52	107 108
5018	5'-r(GCUCCAUCGUCUACCUGGAGAUUGA) -3' 3'- (CGAGGUAGCAGAUGGACCUCUAACU)r-5'	52	109 110
5021	5'-r(CCAUCGUCUACCUGGAGAUUGACAA) -3' 3'- (GGUAGCAGAUGGACCUCUAACUGUU)r-5'	48	111 112
5225	5'-r(CCGCCUUUGUGCUUCUGUUCUUCGU) -3' 3'- (GGCGGAAACACGAAGACAAGAAGCA)r-5'	52	113 114
5227	5'-r(GCCUUUGUGCUUCUGUUCUUCGUGG) -3' 3'- (CGGAAACACGAAGACAAGAAGCACC)r-5'	52	115 116
5319	5'-r(CAAAGUGUCUGAGGCCAGCAAGAAG) -3' 3'- (GUUUCACAGACUCCGGUCGUUCUUC)r-5'	52	117 118
5411	5'-r(CCCUCAUGGACGACAACCAGAAUGA) -3' 3'- (GGGAGUACCUGCUGUUGGUCUUACU)r-5'	52	119 120
5414	5'-r(UCAUGGACGACAACCAGAAUGAGUG) -3' 3'- (AGUACCUGCUGUUGGUCUUACUCAC)r-5'	48	121 122
5450	5'-r(ACCUGGAGACCAAGAAGUUCCGGUU) -3' 3'- (UGGACCUCUGGUUCUUCAAGGCCAA)r-5'	52	123 124

Start Position	siRNA(sense strand/antisense strand)	GC%	SEQ ID NO:
5723	5'-r(CCGUCAUCUCCGACUUCAUCUACCA) -3' 3'- (GGCAGUAGAGGCUGAAGUAGAUGGU)r-5'	52	125 126
5855	5'-r(CAGAUGCCAACAUCCAGGACAACAU) -3' 3'- (GUCUACGGUUGUAGGUCCUGUUGUA)r-5'	48	127 128
5915	5'-r(ACGCACAAGGUGUCUUCCAGAUCCU) -3' 3'- (UGCGUGUUCCACAGAAGGUCUAGGA)r-5'	52	129 130
5917	5'-r(GCACAAGGUGUCUUCCAGAUCCUGA) -3' 3'- (CGUGUUCCACAGAAGGUCUAGGACU)r-5'	52	131 132
5918	5'-r(CACAAGGUGUCUUCCAGAUCCUGAU) -3' 3'- (GUGUUCCACAGAAGGUCUAGGACUA)r-5'	48	133 134
6111	5'-r(CGUGAACAAUGUGGAUGCCGCAGUU) -3' 3'- (GCACUUGUUACACCUACGGCGUCAA)r-5'	52	135 136
6114	5'-r(GAACAAUGUGGAUGCCGCAGUUGUG) -3' 3'- (CUUGUUACACCUACGGCGUCAACAC)r-5'	52	137 138
6152	5'-r(GGGCUAACAAAGAUAUGCAGAACAA) -3' 3'- (CCCGAUUGUUUCUAUACGUCUUGUU)r-5'	40	139 140
6154	5'-r(GCUAACAAAGAUAUGCAGAACAACA)-3' 3'- (CGAUUGUUUCUAUACGUCUUGUUGU)r-5'	36	141 142
6159	5'-r(CAAAGAUAUGCAGAACAACAGGGAG) -3' 3'- (GUUUCUAUACGUCUUGUUGUCCCUC)r-5'	44	143 144
6254	5'-r(CCAACCGGGACAUCACGGAUCAUAU) -3' 3'- (GGUUGGCCCUGUAGUGCCUAGUAUA)r-5'	52	145 146
6255	5'-r(CAACCGGGACAUCACGGAUCAUAUG) -3' 3'- (GUUGGCCCUGUAGUGCCUAGUAUAC)r-5'	52	147 148
6897	5'-r(CGGGUCCACCAGUUUGAAUGGUCAA) -3' 3'- (GCCCAGGUGGUCAAACUUACCAGUU)r-5'	52	149 150
6902	5'-r(CCACCAGUUUGAAUGGUCAAUGCGA) -3' 3'- (GGUGGUCAAACUUACCAGUUACGCU)r-5'	48	151 152
6903	5'-r(CACCAGUUUGAAUGGUCAAUGCGAG) -3' 3'- (GUGGUCAAACUUACCAGUUACGCUC)r-5'	48	153 154
7196	5'-r(CAGCAAACAUCCAGCAGCAGCAAAG) -3' 3'- (GUCGUUUGUAGGUCGUCGUCGUUUC)r-5	52	155 156
7199	5'-r(CAAACAUCCAGCAGCAGCAAAGCCU) -3' 3'- (GUUUGUAGGUCGUCGUCGUUUCGGA)r-5'	52	157 158
7645	5'-r(CGCAUUCCGGAGGCCUUCAAGUAAA) -3' 3'- (GCGUAAGGCCUCCGGAAGUUCAUUU)r-5'	52	159 160

The candidate siRNA molecules described in this Example can be used for inhibition of expression of Notch1 and are useful in a variety of therapeutic settings, for example, in the treatment of cardiovascular disorders such as aortic valve disease and cancers including but not limited to T-ALL, breast, pancreatic cancer, colorectal cancer, gastric cancer, melanoma, leukemia, lymphoma, glioma, and lung cancer and/or other disease states, conditions, or traits associated with Notch1 gene expression or activity in a subject or organism.

EXAMPLE 2

IN VITRO TESTING OF siRNA CANDIDATE MOLECULES FOR THE INHIBITION OF HUMAN NOTCH1 EXPRESSION

In this Example, 40 blunt-ended 25-mer siRNA that target human Notch1 were tested in the HepG2 tumor cell line for their potency in knockdown of Notch1 mRNA in the transfected cells.

The 40 human Notch1 siRNA molecules selected for *in vitro* testing are shown in Table 2 below.

Table 2: Blunt-ended 25-mer siRNA tested *in vitro* for knockdown of human Notch1 mRNA

siRNA No.	Start Position	siRNA(sense strand/antisense strand)	GC%	SEQ ID NO:
1	775	5'-r(GACGAUUGUCCAGGAAACAACUGCA) -3' 3'- (CUGCUAACAGGUCCUUUGUUGACGU)r-5'	48	1 2
2	778	5'-r(GAUUGUCCAGGAAACAACUGCAAGA) -3' 3'- (CUAACAGGUCCUUUGUUGACGUUCU)r-5'	44	3 4
3	853	5'-r(CCAGAGUGGACAGGUCAGUACUGUA) -3' 3'- (GGUCUCACCUGUCCAGUCAUGACAU)r-5'	52	5 6
4	1511	5'-r(GCUGCCUGGACAAGAUCAAUGAGUU) -3' 3'- (CGACGGACCUGUUCUAGUUACUCAA)r-5'	48	11 12

siRNA No.	Start Position	siRNA(sense strand/antisense strand)	GC%	SEQ ID NO:
5	1639	5'-r(CCCAACACUUACACCUGUGUGUGCA) -3' 3'- (GGGUUGUGAAUGUGGACACACACGU)r-5'	52	19 20
6	1644	5'-r(CACUUACACCUGUGUGUGCACGGAA) -3' 3'- (GUGAAUGUGGACACACACGUGCCUU)r-5'	52	21 22
7	1910	5'-r(ACUGCGAGAUCAACCUGGAUGACUG) -3' 3'- (UGACGCUCUAGUUGGACCUACUGAC)r-5'	52	23 24
8	1961	5'-r(CCUGUCUGGACAAGAUCGAUGGCUA) -3' 3'- (GGACAGACCUGUUCUAGCUACCGAU)r-5'	52	25 26
9	1969	5'-r(GACAAGAUCGAUGGCUACGAGUGUG) -3' 3'- (CUGUUCUAGCUACCGAUGCUCACAC)r-5'	52	27 28
10	2009	5'-r(ACACAGGGAGCAUGUGUAACAUCAA) -3' 3'- (UGUGUCCCUCGUACACAUUGUAGUU)r-5'	44	29 30
11	2012	5'-r(CAGGGAGCAUGUGUAACAUCAACAU) -3' 3'- (GUCCCUCGUACACAUUGUAGUUGUA)r-5'	44	31 32
12	2134	5'-r(ACCUGCCUGUCUGAGGUCAAUGAGU) -3' 3'- (UGGACGGACAGACUCCAGUUACUCA)r-5'	52	37 38
13	2143	5'-r(UCUGAGGUCAAUGAGUGCAACAGCA) -3' 3'- (AGACUCCAGUUACUCACGUUGUCGU)r-5'	48	39 40
14	2234	5'-r(GGAGUGGGACCAACUGUGACAUCAA) -3' 3'- (CCUCACCCUGGUUGACACUGUAGUU)r-5'	52	41 42
15	2239	5'-r(GGGACCAACUGUGACAUCAACAACA) -3' 3'- (CCCUGGUUGACACUGUAGUUGUUGU)r-5'	48	43 44
16	2260	5'-r(AACAAUGAGUGUGAAUCCAACCCUU) -3' 3'- (UUGUUACUCACACUUAGGUUGGGAA)r-5'	40	45 46
17	2539	5'-r(UCCGAGGACUAUGAGAGCUUCUCCU) -3' 3'- (AGGCUCCUGAUACUCUCGAAGAGGA)r-5'	52	47 48
18	2542	5'-r(GAGGACUAUGAGAGCUUCUCCUGUG) -3' 3'- (CUCCUGAUACUCUCGAAGAGGACAC)r-5'	52	49 50
19	2600	5'-r(AGGUCGACAUCAACGAGUGCGUUCU) -3' 3'- (UCCAGCUGUAGUUGCUCACGCAAGA)r-5'	52	53 54
20	2816	5'-r(GCACUUUCUGUGAGGAGGACAUCAA) -3' 3'- (CGUGAAAGACACUCCUCCUGUAGUU)r-5'	48	59 60
21	3045	5'-r(CAGCUACUGCCAGCACGAUGUCAAU) -3' 3'- (GUCGAUGACGGUCGUGCUACAGUUA)r-5'	52	61 62
22	3060	5'-r(CGAUGUCAAUGAGUGCGACUCACAG) -3' 3'- (GCUACAGUUACUCACGCUGAGUGUC)r-5'	52	67 68
23	3169	5'-r(CAGAACCUUGUGCACUGGUGUGACU) -3' 3'- (GUCUUGGAACACGUGACCACACUGA)r-5'	52	69 70
24	3912	5'-r(CGAGUCCGUCAUCAAUGGCUGCAAA) -3' 3'- (GCUCAGGCAGUAGUUACCGACGUUU)r'-5'	52	83 84
25	3917	5'-r(CCGUCAUCAAUGGCUGCAAAGGCAA) -3' 3'- (GGCAGUAGUUACCGACGUUUCCGUU)r-5'	52	89 90
26	4474	5'-r(CAGUCUCUGCAGUGCUGGAAGUACU) -3' 3'- (GUCAGAGACGUCACGACCUUCAUGA)r-5'	52	95 96

siRNA No.	Start Position	siRNA(sense strand/antisense strand)	GC%	SEQ ID NO:
27	4597	5'-r(GACCAGUACUGCAAGGACCACUUCA) -3' 3'- (CUGGUCAUGACGUUCCUGGUGAAGU)r-5'	52	103 104
28	4796	5'-r(UGCUGCACACCAACGUGGUCUUCAA) -3' 3'- (ACGACGUGUGGUUGCACCAGAAGUU)r-5'	52	107 108
29	5018	5'-r(GCUCCAUCGUCUACCUGGAGAUUGA) -3' 3'- (CGAGGUAGCAGAUGGACCUCUAACU)r-5'	52	109 110
30	5225	5'-r(CCGCCUUUGUGCUUCUGUUCUUCGU) -3' 3'- (GGCGGAAACACGAAGACAAGAAGCA)r-5'	52	113 114
31	5855	5'-r(CAGAUGCCAACAUCCAGGACAACAU) -3' 3'- (GUCUACGGUUGUAGGUCCUGUUGUA)r-5'	48	127 128
32	5918	5'-r(CACAAGGUGUCUUCCAGAUCCUGAU) -3' 3'- (GUGUUCCACAGAAGGUCUAGGACUA)r-5'	48	133 134
33	6111	5'-r(CGUGAACAAUGUGGAUGCCGCAGUU) -3' 3'- (GCACUUGUUACACCUACGGCGUCAA)r-5'	52	135 136
34	6114	5'-r(GAACAAUGUGGAUGCCGCAGUUGUG) -3' 3'- (CUUGUUACACCUACGGCGUCAACAC)r-5'	52	137 138
35	6152	5'-r(GGGCUAACAAAGAUAUGCAGAACAA) -3' 3'- (CCCGAUUGUUUCUAUACGUCUUGUU)r-5'	40	139 140
36	6254	5'-r(CCAACCGGGACAUCACGGAUCAUAU) -3' 3'- (GGUUGGCCCUGUAGUGCCUAGUAUA)r-5'	52	145 146
37	6897	5'-r(CGGGUCCACCAGUUUGAAUGGUCAA) -3' 3'- (GCCCAGGUGGUCAAACUUACCAGUU)r-5'	52	149 150
38	6902	5'-r(CCACCAGUUUGAAUGGUCAAUGCGA) -3' 3'- (GGUGGUCAAACUUACCAGUUACGCU)r-5'	48	151 152
39	7196	5'-r(CAGCAAACAUCCAGCAGCAGCAAAG) -3' 3'- (GUCGUUUGUAGGUCGUCGUCGUUUC)r-5'	52	155 156
40	7645	5'-r(CGCAUUCCGGAGGCCUUCAAGUAAA) -3' 3'- (GCGUAAGGCCUCCGGAAGUUCAUUU)r-5'	52	159 160

All siRNA transfections were carried out using a reverse-transfection protocol using Lipofectamine®RNAiMAX (Invitrogen, Carlsbad, CA) following vendor's instruction, except where indicated. At 72 hours post transfection, the transfected HepG2 cells were harvested and total RNA were prepared using Cell-to-Ct assay kit (ABI, Foster City, CA/Invitrogen, Carlsbad, CA). The relative levels of human Notch1 mRNA in the transfected cells were assessed using a QRT-PCT protocol and human Notch1 gene expression assay (ABI, Foster City, CA/Invitrogen, Carlsbad, CA). The relative levels of

Notch1 mRNA in each sample were calculated using a mock transfection control as 100%.

First round screening of the 40 Notch1 siRNA candidates was conducted at 10 nM (siRNA) concentration (Figure 1). The 12 most potent siRNA candidates from first round screening were subjected to a second round screening in which a 3 nM siRNA concentration was used (Figure 2). At least 5 siRNAs inhibit Notch1 gene expression by more than 75% at siRNA concentration of 3 nM in HepG2 cells at 72 hours post transfection. Among those 5 siRNAs, one siRNA (siRNA #8; SEQ ID NO : 25/26) reduces Notch1 gene expression by more than 90% in HepG2 cells transfected with 3 nM of siRNA (Figure 2).

Thus, this Example demonstrates that numerous siRNA molecules from Table 2 were effective for inhibiting expression of human Notch1 and may be used in therapeutic settings as described herein.

All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet, are incorporated herein by reference, in their entirety.

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

What is claimed is:

1. An isolated small interfering RNA (siRNA) polynucleotide, comprising at least one nucleotide sequence selected from the group consisting of SEQ ID NOs: 25, 26, 5, 6, 83, 84 and 135-138 and the complementary polynucleotide thereto.

2. An isolated small interfering RNA (siRNA) polynucleotide, comprising at least one nucleotide sequence selected from the group consisting of SEQ ID NOs:1-160.

3. The siRNA polynucleotide of claim 2 that comprises at least one nucleotide sequence selected from the group consisting of SEQ ID NOs:1-160 and the complementary polynucleotide thereto.

4. The small interfering RNA polynucleotide of either claim 2 or claim 3 that inhibits expression of a Notch1 polypeptide, wherein the Notch1 polypeptide comprises an amino acid sequence as set forth in SEQ ID NOs:163, or that is encoded by the polynucleotide as set forth in SEQ ID NO:161.

5. The siRNA polynucleotide of any one of claims 1-3 wherein the nucleotide sequence of the siRNA polynucleotide differs by one, two, three or four nucleotides at any position of a sequence selected from the group consisting of the sequences set forth in SEQ ID NOS: 1-160, or the complement thereof.

6. The siRNA polynucleotide of claim 3 wherein the nucleotide sequence of the siRNA polynucleotide differs by at least one mismatched base pair between a 5' end of an antisense strand and a 3' end of a sense strand of a sequence selected from the group consisting of the sequences set forth in SEQ ID NOS:1-160.

7. The siRNA polynucleotide of claim 6 wherein the mismatched base pair is selected from the group consisting of G:A, C:A, C:U, G:G, A:A, C:C, U:U, C:T, and U:T.

8. The siRNA polynucleotide of claim 6 wherein the mismatched base pair comprises a wobble base pair (G:U) between the 5' end of the antisense strand and the 3' end of the sense strand.

9. The siRNA polynucleotide of any one of claims 1-3 wherein the polynucleotide comprises at least one synthetic nucleotide analogue of a naturally occurring nucleotide.

10. The siRNA polynucleotide of any one of claims 1-3 wherein the polynucleotide is linked to a detectable label.

11. The siRNA polynucleotide of claim 10 wherein the detectable label is a reporter molecule.

12. The siRNA of claim 11 wherein the reporter molecule is selected from the group consisting of a dye, a radionuclide, a luminescent group, a fluorescent group, and biotin.

13. The siRNA polynucleotide of claim 12 wherein the detectable label is a magnetic particle.

14. An isolated siRNA molecule that inhibits expression of a Notch1 gene, wherein the siRNA molecule comprises a nucleic acid that targets the sequence provided in SEQ ID NOs: 161 or 162, or a variant thereof having Notch1 signaling activity.

15. The siRNA molecule of claim 14, wherein the siRNA comprises any one of the single stranded RNA sequences provided in SEQ ID NOs:1-160, or a double-stranded RNA thereof.

16. The siRNA molecule of claim 15 wherein the siRNA molecule down regulates expression of a Notch1 gene via RNA interference (RNAi).

17. A composition comprising one or more of the siRNA polynucleotides of any one of claims 1, 2, 3, 14 and 15, and a physiologically acceptable carrier.

18. The composition of claim 17 wherein the composition comprises a positively charged polypeptide.

19. The composition of claim 18 wherein the positively charged polypeptide comprises poly(Histidine-Lysine).

20. The composition of any one of claims 17-19 further comprising a targeting moiety.

21. A method for treating or preventing a cancer in a subject having or suspected of being at risk for having the cancer, comprising administering to the subject the composition of any one of claims 17-19, thereby treating or preventing the cancer.

22. A method for inhibiting the synthesis or expression of Notch1 comprising contacting a cell expressing Notch1 with any one or more siRNA molecules wherein the one or more siRNA molecules comprises a sequence selected from the sequences provided in SEQ ID NOs:1-160, or a double-stranded RNA thereof.

23. The method of claim 22 wherein a nucleic acid sequence encoding Notch1 comprises the sequence set forth in SEQ ID NO: 161 or 162.

24. A method for reducing the severity of a cancer in a subject, comprising administering to the subject the composition of any one of claims 17-19, thereby reducing the severity of the cancer.

25. A recombinant nucleic acid construct comprising a nucleic acid that is capable of directing transcription of a small interfering RNA (siRNA), the nucleic acid comprising:

(a) a first promoter; (b) a second promoter; and (c) at least one DNA polynucleotide segment comprising at least one polynucleotide that is selected from the group consisting of (i) a polynucleotide comprising the nucleotide sequence set forth in any one of SEQ ID NOs:1-160, and (ii) a polynucleotide of at least 18 nucleotides that is complementary to the polynucleotide of (i), wherein the DNA polynucleotide segment is operably linked to at least one of the first and second promoters, and wherein the promoters are oriented to direct transcription of the DNA polynucleotide segment and of the complement thereto.

26. The recombinant nucleic acid construct of claim 25, comprising at least one enhancer that is selected from a first enhancer operably linked to the first promoter and a second enhancer operably linked to the second promoter.

27. The recombinant nucleic acid construct of claim 25, comprising at least one transcriptional terminator that is selected from (i) a first transcriptional terminator that is positioned in the construct to terminate transcription directed by the first promoter and (ii) a second transcriptional terminator that is positioned in the construct to terminate transcription directed by the second promoter.

28. An isolated host cell transformed or transfected with the recombinant nucleic acid construct according to any one of claims 25-27.

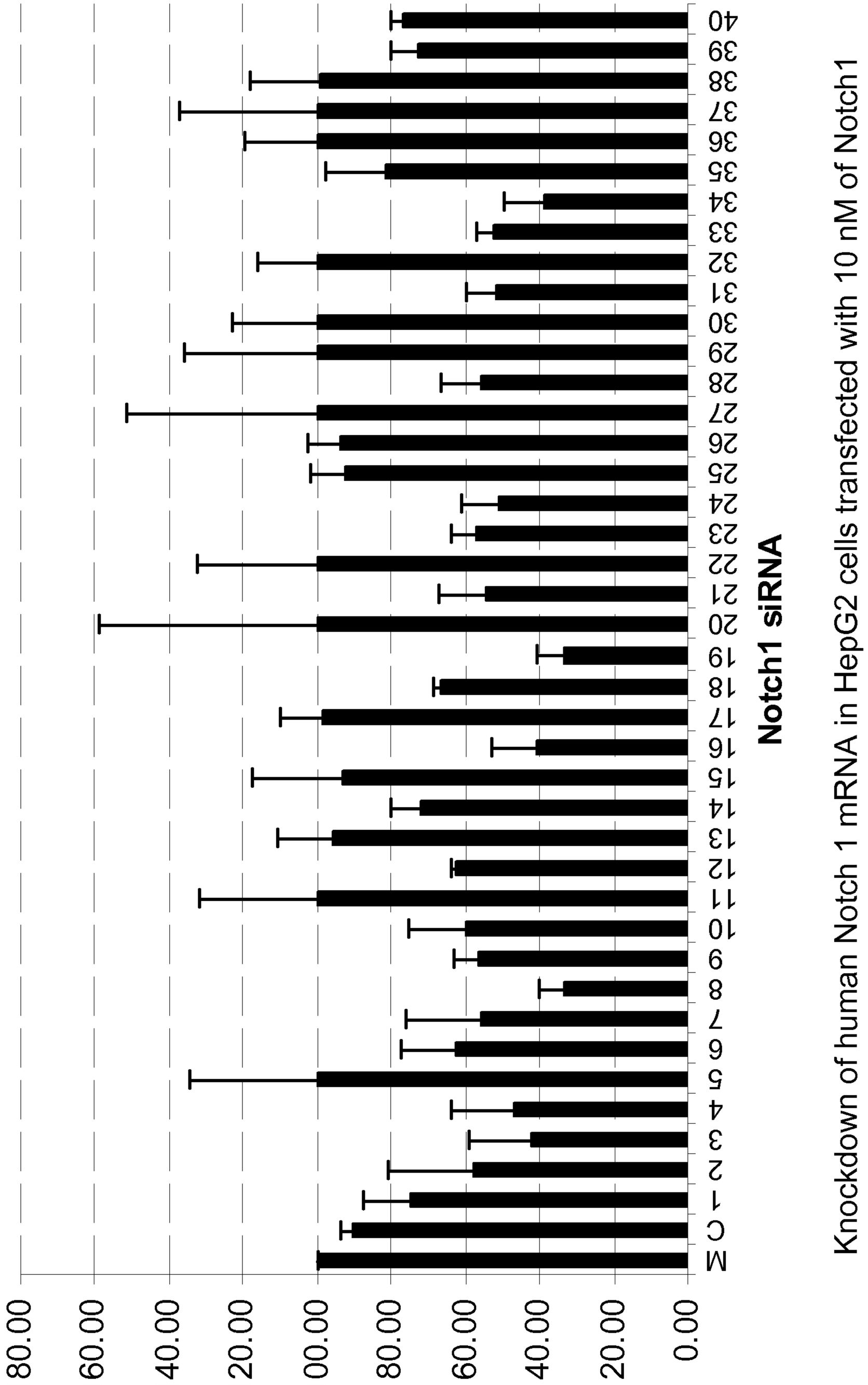
Knockdown of human Notch 1 mRNA in HepG2 cells transfected with 10 nM of
siRNA at 72 hours post-transfection.
Notch1 siRNA
Relative levels of Notch1 mRNA
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
M
C1
C2
1
2
3
4
5
6
7
8
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FIG. 1

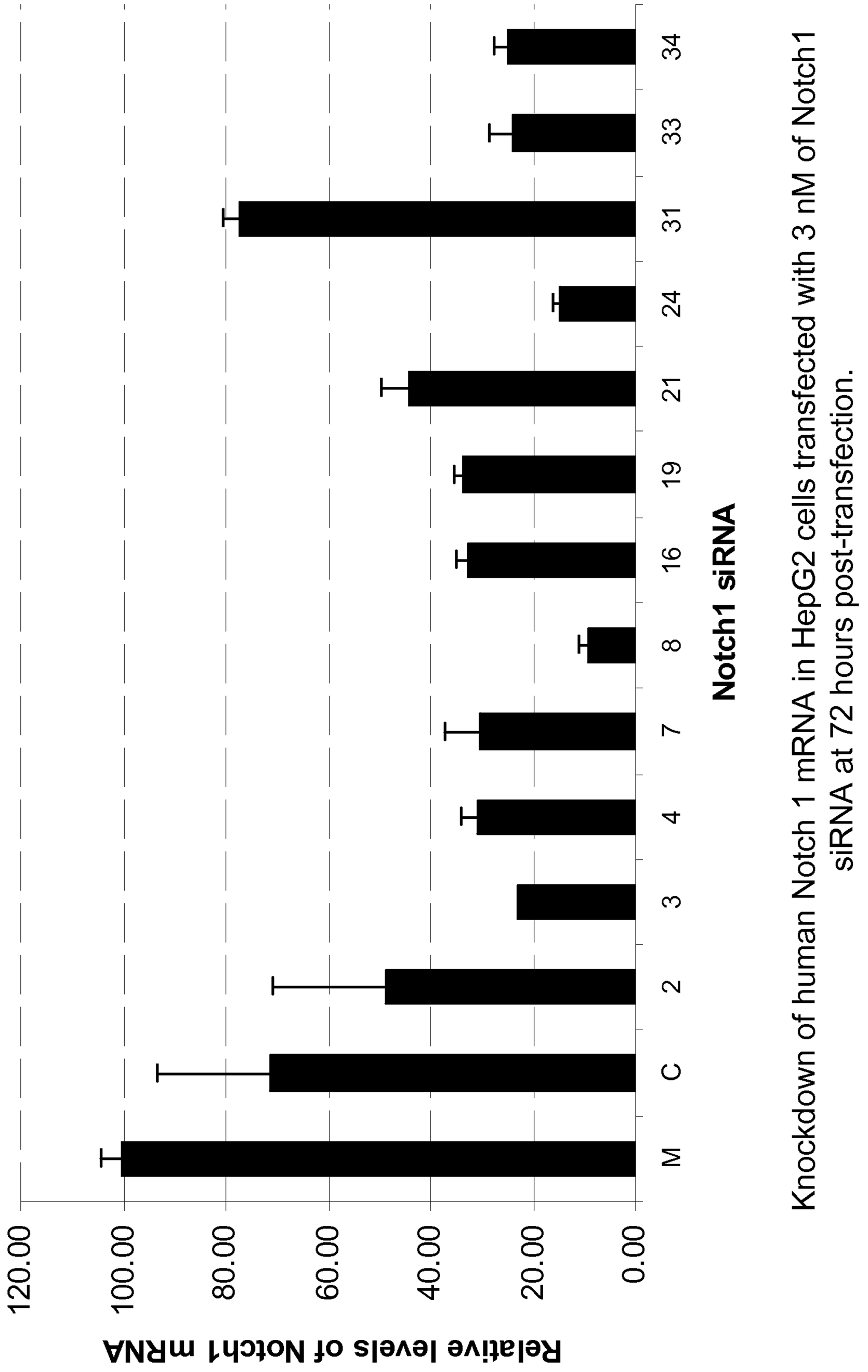
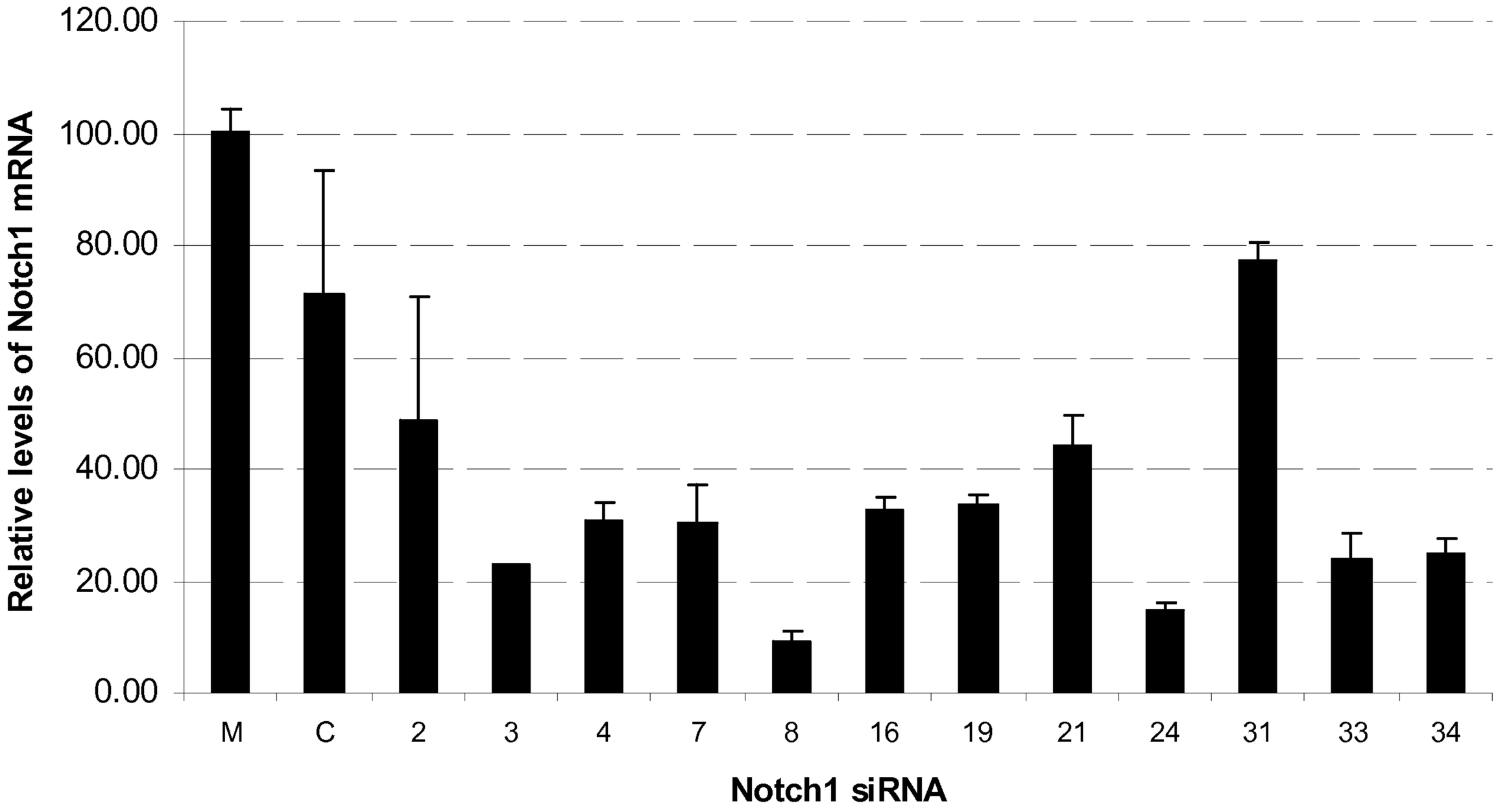

Knockdown of human Notch 1 mRNA in HepG2 cells transfected with 3 nM of
siRNA at 72 hours post-transfection.
Notch1 siRNA
M
C
2
3
4
7
8
16
19
21
24
31
33
34
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Relative levels of Notch1 mRNA

FIG. 2



Knockdown of human Notch 1 mRNA in HepG2 cells transfected with 3 nM of Notch1 siRNA at 72 hours post-transfection.

FIG. 2